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Program Number 1 Thyroid Hormone Metabolism

The Gene Coding for the Type 3 Iodothyronine Deiodinase Is Imprinted and Required for Normal Neonatal Growth and Survival

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The mouse Dio3 gene codes for the type 3 deiodinase (D3), a conserved selenocysteine-containing enzyme that inactivates thyroid hormones and is highly expressed in the pregnant uterus, the placenta and the fetus. The Dio3 gene and its human homolog, DIO3, map to chromosomal regions (12F1 and 14q32, respectively) which are known to include imprinted genes. Genomic imprinting refers to monoallelic gene expression and results from epigenetic mechanisms. Using homologous recombination in mouse embryonic stem (ES) cells we have introduced a critical mutation that renders the D3 inactive. No detectable D3 activity was found in the heads and bodies of fetuses (E14 to E18) that were homozygous (-/-Dio3) for the mutation. The D3 activities found in the heads, limbs, liver and bodies of +/-Dio3 fetuses were, respectively, 30%, 21%, 17%, and 22% of those found in +/+Dio3 fetuses when the mutation was inherited from the father. However, when the mutated allele was of maternal origin, D3 activities in the same tissues were essentially the same as those in the wild types, indicating preferential expression from the paternal allele. The weight of -/-Dio3 fetuses is normal during fetal life. However, mild growth retardation was observed in 2-day-old -/-Dio3 neonates (93% of the weight of wild type mice), and it became severe by 14 days of age (65%) and persists into adulthood (80%). Growth retardation was also observed in +/-Dio3 mice that inherited the mutation from the father, but not in those where inheritance came from the mother. Genotyping of the offspring of +/-Dio3 breedings identified 22% of fetuses (173 screened), but only 13% of 2-day-old neonates (205 screened) as -/-Dio3, rather than the expected 25% prevalence. We conclude that the Dio3 gene is preferentially expressed from the paternal allele and required for normal neonatal growth and survival.

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Program Number 2 Thyroid Diseases

NHANES III: Impact of TSH:TPOAb Relationships on Redefining the Serum TSH Normal Reference Range

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BACKGROUND: The conventional serum TSH reference range in euthyroid subjects approximates 0.4 to 4.0 m IU/L. However, several recent reports indicate that this range may be skewed to the high-end by the presence of occult autoimmune thyroid disease. As the NHANES III survey included sensitive TPOAb and TgAb measurements, it affords an opportunity to address this question (Hollowell JCEM 87:489,2002). STUDY DESIGN: 12,974 NHANES subjects [male (M) : female (F) ratio = 1.2:1] with normal total T4 (excluding a history of thyroid disease, thyromegaly, pregnancy, T4 or E2 Rx) were analyzed for TPOAb and TSH relationships. RESULTS: The prevalence of TPOAb for M+F was 11.2% (7.9%M/15.2%F). For males, TPOAb prevalence was lowest (4.9 ± 0.5 se %) in the TSH range 0.4 to 2.0 mIU/L (male reference) and rose progressively from 8.0 ± 1.5%, for the TSH 2.0-2.5 mIU/L interval to 94±4% for TSH>20mIU/L. For females, TPOAb prevalence was lowest (6.1±1.1%) in the TSH range 0.4 to 1.5 mIU/L (female reference) and rose progressively from 13.4±1.8% for the TSH 1.5-2.0 mIU/L interval ($p < 0.001$) to 98 ± 1% for TSH>20mIU/L. TgAb without TPOAb showed no association with TSH concentration. Serum TPOAb concentrations were increased for all TSH intervals above 2.5 mIU/L for males and above 1.5 mIU/L for females. CONCLUSIONS: (1) Occult thyroid disease is a common occurrence in normal reference populations. (2) Based on TPOAb prevalence, the new normal serum TSH adult reference range approximates 0.4 to 2.0mIU/L. (3) Pilot results of a highly selected normal sub-population (n=76) supports this new reference range (Spencer). (4) This new TSH reference range should be considered as the optimal end-point for T4 replacement Rx. (5) TPOAb measurement is indicated when the TSH value is outside this new range.

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ID: **Program Number** 3 Cancer

The Follicular Thyroid Carcinoma-associated PAX8/PPAR-γ-1 Fusion Gene Decreases the Rate of Apoptosis and Shortens the Doubling Time of Thyroid Cell Lines

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A translocation of chromosomes 3p25 and 2q13 is seen commonly in follicular thyroid carcinoma (FTC), resulting in the expression of a fusion protein including the first 9 exons of the thyroid-specific transcription factor PAX-8 with a full length peroxisome proliferator receptor gamma (PPAR-γ). Expression of the fusion protein (designated PPFP) is restricted to FTC, and is seen infrequently in follicular adenoma (FA) and in other thyroid carcinoma histotypes. PPFP is a putative oncogene, but direct evidence for its oncogenic action has not previously been reported. We assessed the impact of PPFP over-expression on an immortalized thyroid cell line (NT cells), following transient transfection. The PPFP gene was engineered into an expression vector (pCDNA3.1), which exhibits constitutive activity in mammalian cells. A normal thyroid cell line, transformed by incorporation of the SV40 large-T antigen (NT cell line), was transfected in triplicate either with PPFP-pCDNA3.1, the papillary thyroid carcinoma oncogene RET/PTC-1 incorporated into the same vector, or the control vector. Viable cell numbers were assessed by Coulter-counter after 12, 24, 48 and 72 hours, and the proportion of cells in early or late apoptosis was determined by flow cytometry after staining with Annexin and 7-AAD. Following transfection with a control vector, NT cells exhibited a doubling time of approximately 66 hours, which was essentially unchanged after transfection with the RET/PTC-1 oncogene. In contrast, after transfection with the PPFP gene, the doubling time was shortened to 41 hours, a decrease of 38% ($p<0.001$). Similarly, the rate of apoptosis in cells expressing PPFP was reduced by more than 65% at 72 hours, from 18.4% to only 6.3% ($p<0.002$). The FTC-associated PAX8-PPAR-γ fusion gene reduces apoptosis, enhances cell-survival and shortens the doubling time of thyroid cell lines. These data strongly support a role for PPFP as an important oncogene in follicular thyroid carcinoma.

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Program Number 4 Thyroid Diseases

T4 plus T3 Treatment for Hypothyroidism: A Double-Blind Comparison with Usual T4

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This study compares T4 versus a combination of T4 and T3 treatment in subjects with T4-treated hypothyroidism, with one or more persistent symptom of hypothyroidism. Subjects were euthyroid and had been on stable doses of T4 prior to entry. Subjects received T4 in their current dose or T4 plus T3 (in a physiological ratio of 15:1), in a double blind twice daily dosing over a 3- to 6-month period. Outcome was evaluated once TSH levels were in the normal range for two months, and using two primary outcome measures: the Sunnybrook Hypothyroid Symptom Severity scale (SHSS) and the Clinical Global Impression of improvement (CGI-I). 59 subjects completed the protocol. Using a 2 (group) by 2 (time) ANOVA for repeated measures, there was no main effect of group or a group by time interaction on the outcome measures. However, there was a significant positive main effect of time (SHSS: $F = 54.1$, $p < .0001$; CGI: $F = 22.2$, $p < .0001$). As expected the combination group had a significantly lower T4 level ($t = 4.0$, $p < .0001$) and a significantly higher T3 level ($t = 4.1$, $p < .0001$) by the final week as compared with the T4 alone group; however, there were no significant differences in TSH levels. Examination of all subjects showed that those subjects with the greatest reduction in T4 equivalent dosages had the greatest improvement on both outcome measures. Furthermore, improvement in hypothyroid symptoms was correlated with changes in cholesterol ($r = .36$, $p < .05$) and LDL-cholesterol ($r = .32$, $p < .05$). These data suggest that there is no overall advantage to the combination of T4 and T3 used in this study over T4 alone in hypothyroid patients already on T4 replacement, but that a reduction in overall T4 equivalent dose is associated with improvement in symptoms.

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Program Number 5 Thyroid Hormone Action

The S14 Knockout Mouse Shows Resistance to Diet-induced Obesity

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We have recently reported that the Spot 14 knockout mouse showed enhanced hepatic lipogenesis when maximally induced by carbohydrate feeding and T3 administration. The enhanced lipogenesis is consistent with a regulatory role for S14 in lipid metabolism. We have now followed the growth of the S14 knockout and wild type (WT) mice nursed by homozygous dams for over 200 days. These studies show that the knockout mouse is resistant to diet-induced obesity. Whereas the knockout mice weigh 19% less than the WT mice at weaning, they rapidly catch up when placed on a diet containing 11% calories from fat. However, by 200 days knockout mice weight 28% less than WT mice. Despite the slower weight gain, the knockout mice eat more than the WT (8.1 vs 6.8 gm/100gm BW/day, $p < 0.01$), and fecal fat excretion is the same. Total body DEXA scans show that the lower weight of the knockout animal is entirely due to a decrease in total body fat, while the lean body mass of the two genotypes are identical. Thus, the S14 knockout adult animal has a higher metabolic rate than the WT mouse. This higher metabolic rate was not due to enhanced thyroidal activity since the plasma T3 levels of both genotypes were the same (64 vs 68 ng/100ml, knockout vs WT). In contrast to pups nursed by homozygous dams, knockout and WT pups nursed on heterozygous dams exhibit no differences in weight gain. These data suggest that the differences in weight gain are maternally derived (Endocrine Society Abstract, 2002). The diminished neonatal growth of knockout pups nursed by knockout dams causes enhanced metabolism in adults, leading to resistance to diet-induced obesity. Thus, the S14 knockout mouse provides a unique model to study the effect of maternal milk deficiencies on adult metabolism.

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Program Number 6 Thyroid Hormone Action

Involvement of GATA2 in the T3-dependent Negative Regulation of the Thyrotropin Beta and Alpha Gene Promoters by Thyroid Hormone Receptor

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The DNA sequence immediately downstream to the transcription start site of the TSH beta gene promoter has been considered crucial for the negative regulation by T3 and its receptor (TR) as a negative regulatory element (NRE). The molecular mechanism of the regulation, however, has been unclear. We have recently established the assay system for the negative regulation of TSH beta gene with very high sensitivity in CV1 cells which are expressed with Pit1 and GATA2. When we deleted or mutated NRE in the TSH beta promoter, the basal transcriptional activity (BTA) was comparable to the wild type reporter gene and was fully suppressed by T3/TR, suggesting that NRE might not be the critical site for the negative regulation. Further deletion analysis revealed that the sequence between GATA2 binding site and TATA box has the function to repress BTA. The construct possessing only single Pit1 binding site and two GATA2 binding sites could mediate the negative regulation. The transcription of this construct was stimulated by expression of GATA2 alone and suppressed by T3/TR, but not by liganded other nuclear receptors, demonstrating the receptor specificity. On the other hand, TSH alpha gene promoter is stimulated by Ptx1, Lhx3a and GATA2 in CV1 cells. We found that the negative regulation by T3/TR was detected, only when GATA2, but not Ptx1 nor Lhx3a, was expressed. These results suggested that GATA2 might be a common important factor to mediate the T3 dependent negative regulation by TR in the TSH beta and alpha gene promoters.

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Program Number 7 Thyroid Hormone Action

Thyroid Hormone Thermogenesis in Transgenic Mitochondrial Glycerol 3-Phosphate Dehydrogenase (mGPD)-deficient Mice

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Known for over a century, thyroid hormone (TH) thermogenesis remains unexplained. TH stimulates mGPD—the rate-limiting enzyme in the glycerol-NADH shuttle$mdash;only in tissues and species where it stimulates oxygen consumption (QO2). The preferential use of this shuttle could reduce the efficiency of ATP synthesis. To investigate a role of mGPD in TH thermogenesis, we studied mice with deletion of the mGPD gene (mGPD-KO). Observable mGPD-KO phenotype is unremarkable. We measured QO2 and mRNAs of potential thermogenic genes by competitive RT-PCR assays, both in euthyroid (Eu) and hypothyroid (Hpo) males ±T3 treatment. Mice were acclimated at room temperature (22°C) as well as at thermoneutrality (32°C), to minimize brown adipose tissue (BAT) thermogenesis. QO2 [ml/(hx100g$^{0.7}$)] was decreased in Eu mGPD-KO at 22°C compared to wild type (WT) (278±14 vs 237±6; P<0.02). Hypothyroidism reduced QO2 (P<0.001) by 27% and 18% obliterating the difference between genotypes. In Eu at 32°C, QO2 was reduced (P<0.001) to the same level in both genotypes. Hypothyroidism still caused a reduction in QO2 (P<0.001) but this was greater in mGPD-KO (P<0.05). At 22°C, BAT of mGPD-KO showed signs of more activation than that of WT. Of all gene products analyzed, UCP3 mRNA was increased in tibialis and soleus muscles of Eu mGPD-KO (P<0.01) at both 22 and 32°C, while hypothyroidism reduced its levels and obliterated the genotype difference. Mild hyperthyroidism (T3 5x daily production rate) equally increased QO2 in both genotypes, but UCP3 responded more to T3 in mGPD-KO than WT (soleus P<0.05; tibialis P<0.01). Serum T4 (µg/dl) was higher in mGPD-KO than WT (3.5±0.2 vs 2.6±0.2; P<0.02) at 22°C, with T3 showing the same trend. There were no differences at 32°C. Conclusions: mGPD significantly contributes to the thermogenic effect of TH, as evidenced by a lower metabolic rate in Eu-mGPD-KO (22°C) and by the recruitment of potentially compensatory mechanisms (UCP3, thyroid stimulation).

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Program Number 8 Thyroid Hormone Action

Hyperthyroidism Induces Apoptosis in the Adult Cerebral Cortex: Direct Action of T3 on Mitochondria

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Differential thyroidal status is known to cause decrease in cell number and induces irreversible morphometric changes in adult brain resulting in different neuronal abnormalities. Whether the decrease in cell number is mediated by apoptosis is not known. We studied the effect of differential thyroidal status on adult rat cerebral cortex and cerebellum and direct action of T3 on cerebral cortex mitochondria to induce apoptosis. Altered thyroidal status induces DNA fragmentation in cerebral cortex and not in cerebellum. Enhanced caspase-3 and caspase-9 activity was observed in cerebral cortex whereas only basal level in cerebellum. The expression of Bcl-2, Bcl-xl and Bax proteins remained unaltered under differential thyroidal status in both cerebral cortex and cerebellum. Cytochrome c was localized in cytosol only in the hyperthyroid state both in cerebral cortex and cerebellum, but not under hypothyroid conditions. SMAC was absent in the adult cerebral cortex and cerebellum whereas altered thyroidal status not only induced expression but also its translocation to cytosol. In vitro experiments demonstrate that mitochondria from cerebral cortex treated with triiodothyronine (T3) do not induce PT. However, T3-induced released proteins cause nuclear condensation, protruding bodies and DNA fragmentation. Treatment with increasing concentration of T3 results in elevated levels of cytochrome c in supernatant. To our knowledge these results for the first time demonstrate that differential thyroidal status induces apoptosis in adult cerebral cortex. Triiodothyronine acts directly on cerebral cortex mitochondria and induces release of cytochrome c to induce apoptosis. Adult cerebellum seems to be less responsive to changes in thyroidal status.

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Program Number 9 Thyroid Hormone Action

Thyroxine-stimulated Mitogen-activated Protein Kinase Phosphorylation of the Thyroid Hormone Nuclear Receptor Requires a Docking Motif in the Receptor DNA-binding Domain

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L-Thyroxine (T_4) causes activation and nuclear translocation of mitogen-activated protein kinase (MAPK, ERK1/2). A consequence of MAPK activation is serine phosphorylation of the nuclear thyroid hormone receptor, TRB1(TR). We have provided evidence that these effects depend on the presence of the second zinc finger of the TR DNA-binding domain and that serine 142 of TR is the phosphorylation site for MAPK in T_4-treated cells. MAPK contains docking regions rich in acidic amino acids which interact with substrates containing corresponding basic amino acid sequences. We propose that the basic amino acid-enriched docking site on TR for activated MAPK is KGFFRR at residues 128-133. CV-1 cells were transfected with plasmids containing *wild-type (wt)* TR, or selected mutants *TR-K128A, -R132A* or *-R133A*. In studies with cells containing *wt* or mutant receptor, treatment with T_4, 10^{-7} M for 30 min, caused activation and nuclear translocation of MAPK. In T_4-treated cells with *wt* receptor, there was an increase in nuclear receptor content, co-immunoprecipitation of the receptor with activated MAPK, and serine phosphorylation of the *wt* receptor. Cells containing mutant receptors at residues 128, 132 or 133 showed no increase in nuclear receptor content, no co-immunoprecipitation of receptor with MAPK and no receptor serine phosphorylation after T_4 treatment. Immunoprecipitated from TR*wt*-transfected cells without T_4 treatment, the *wt* receptor was phosphorylated *in vitro* by constitutively activated MAPK. However, this MAPK did not phosphorylate mutant receptors with substitutions in the proposed docking domain. In summary, the TR docking site for T_4-activated MAPK includes residues 128-133 (KGFFRR), a basic amino acid- and phenylalanine-enriched motif that is novel for MAPK substrates. Similar sequences are found in other members of the superfamily of nuclear hormone receptors which are also subject to phosphorylation by activated MAPK, and for which MAPK docking sites have not previously been identified.

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Program Number 10 Thyroid and Development

Hypothyroidism Alters Mitochondrial Morphology and Induces Release of Apoptogenic Proteins during Development of Rat Cerebellum

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Mitochondria are the target organelles for thyroid hormone (TH) to maintain energy metabolism. Different signals also converge on mitochondria during apoptosis, to initiate release of proteins that activate proteolytic cascade through specific enzymes-caspases. Apoptosis is a central feature of neurogenesis. The decrease of cell numbers during cerebellar development under hypothyroid condition whether mediated through mitochondrial modulated apoptosis is not known. We studied the effect of hypothyroidism on alterations in mitochondrial structure and translocation of apoptogenic molecules during rat cerebellar development from P0 to P24 stage. Structural analysis of mitochondria was carried out using electron microscopy. The translocation of apoptogenic molecules were analyzed by Western blot. We report that normal circulating levels of TH maintain mitochondrial architecture and its deficiency leads to vacuolization, enlargement and decrease in number of cristae. Expression of Bax was detected at low level in mitochondria under euthyroid state, whereas expression of Bax was found to be increased under hypothyroid condition. Following this initial event, translocation of cytochrome c, AIF, and SMAC was detected primarily in early developmental stages under hypothyroid condition. Experimental evidence has been provided for the first time to show that TH maintains mitochondrial architecture and inhibits the release of apoptogenic molecules from mitochondria to prevent apoptosis during cerebellar development. Hypothyroidism leads to mitochondrial structural changes and results in translocation of apoptogenic proteins promoting apoptosis which may explain some of the deleterious effects seen during early neonatal brain development.

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Program Number 11 Autoimmunity

Immune Repertoire Shifting under the Influence of Apoptosis

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Apoptosis, programmed cell death, is known to enhance immune responses to antigen and may play an important role in autoimmune thyroid disease (AITD). We constructed two dual expression vectors: pcDNAhTSHRcsp2 is a dual expression vector expressing hTSHR and the murine caspase-2 gene under individual CMV promoters and pcDNAhTSHRcsp3|?9m expressing the hTSHR and a catalytically inactive caspase3 gene. The expression and function of these vectors were first studied on transiently transfected 293T cells. pcDNAhTSHRcsp2 expressed only low amounts of TSHR due to apoptotic cell loss, while cells with pcDNAhTSHRcsp3|?9m expressed equal amounts of TSHR compared to cells with wild type TSHR (pcDNAhTSHR). Six to 8 week old Balb/c mice were immunized with empty vector (Group 1, n=4), or wild type TSHR (Group 2, n=5), or with pcDNAhTSHRcsp2 (Group 3) or pcDNAhTSHRcsp3|?9m (Group 4, n=5). After 10 weeks of immunization, stimulating TSHR-Ab were detected in the 5 animals in Group 2 and in 2 of 5 in Group 4 and none in Group1 or 3. Blocking TSHR-Ab were seen after 20 weeks in all 5 animals in Group 3 and 4 of 5 in Group 4 and none in Group 1 or 2. Histologically, three animals in Group 3 showed severe thyroid atrophy associated with focal lymphocytic infiltration. These data indicated that apoptosis at the site of immunization changed the immune response against TSHR-Ag from antibody mediated to cell mediated and in addition shifted the humeral immune response against TSHR-Ag from stimulating TSHR-Ab to blocking TSHR-Ab. Apoptosis at the thyroid cell level may have a similar important role in determining the immune repertoire following TSHR immunization.

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Program Number 12 Autoimmunity

HLA and CTLA-4 Genes: Do They Interact in Graves' Disease?

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The HLA and CTLA-4 gene regions confer genetic susceptibility to Graves' disease (GD). Polymorphisms that confer susceptibility, which have evolved due to genetic drift and natural selection, are likely to be the same as those providing resistance to infection in the past. The complex nature by which these polymorphisms are contributing to the polygenic mode of inheritance is further complicated by gene interaction (epistasis) or independent effects (heterogeneity). In this study we genotyped 786 patients with GD, 621 control subjects, and 212 simplex families at the HLA DR and DQ loci by PCR-SSP. A significant increase in the DRB1*03-DQB1*02-DQA1*0501 haplotype was seen in patients with GD when compared with control subjects (chi-squared = 42.835; p <1 x 10-6) with DQA1*0501 being significantly increased in patients with GD in the absence of DRB1*03 (chi-squared = 10.275; p = 0.001). This result was confirmed in the independent family dataset with excess transmission from parents heterozygous for the DRB1*03-DQB1*02-DQA1*0501 haplotype to affected siblings (chi-squared = 25.94; p <1 x 10-6). Due to the close involvement of the HLA and CTLA-4 molecules in the development of the immune response, we examined the interaction between the associated HLA haplotype and an A/G SNP of the CTLA-4 gene (the G allele of which had previously been reported to be associated with GD in our population, chi-squared = 9.117; p = 0.0026). Using a logistic regression approach, we tested the global hypothesis of no interaction by performing a likelihood ratio test which yielded a chi-squared of 2.25; p = 0.69. From these data we conclude that both the HLA and CTLA-4 gene regions are conferring susceptibility to GD in our population but that no interaction (i.e., genetic heterogeneity) is occurring between the HLA susceptibility haplotype and the A/G SNP of the CTLA-4 gene.

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Program Number 13 Autoimmunity

Glycosaminoglycans Provide a Binding Site for Thyroglobulin in Orbital Tissues of Patients with Thyroid-associated Ophthalmopathy

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Intact thyroglobulin (Tg) is present in orbital tissues (OT) of patients with thyroid associated ophthalmopathy (TAO), supporting a role in its pathogenesis. Based on the knowledge that Tg is a heparin-binding protein, we studied its binding to glycosaminoglycans (GAGs) known to be abundantly present in OT: chondroitin sulfate B (CSB), chondroitin sulfate C (CSC) and hyaluronic acid (HA). In solid phase assays both rat Tg (rTg) and human Tg (hTg) bound to all GAGs tested, especially to CSB. Binding of rTg to immobilized GAGs was reduced by co-incubation with soluble GAGs themselves or heparin. hTg bound to GAGs with lower affinity than rTg, as predicted from differences in the sequence of a heparin/GAG binding site (2489-2503): rTg, RELPSRRLKRPLPVK; hTg, REPPARALKRSLWVE. In contrast with rTg, hTg binding to immobilized GAGs was markedly increased by soluble GAGs or heparin. In addition, an antibody against the hTg sequence 2489-2503 also increased binding, suggesting that occupation of 2489-2503 determines a conformational change in hTg with exposure of additional binding sites. We obtained indirect evidence that the Tg in OT of TAO patients is complexed with GAGs. Thus, following salt-dissociation from binding sites, Tg in OT extracts from TAO patients acquired the ability to bind to immobilized CSB. Heparin enhanced binding of the Tg in OT extracts to CSB, resembling its effect on binding of purified hTg to immobilized GAGs. In conclusion, both rTg and hTg bind to GAGs present in OT of TAO patients. Different binding mechanisms between rTg and hTg, possibly related to their sequences and conformational modes, may explain the absence of TAO in rodents. The accumulation of Tg in OT and the peculiar binding mechanism of hTg may be involved in the development or worsening of TAO.

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Program Number 14 Autoimmunity

Pathogenic T Cell Epitopes Predicted from Human Thyroglobulin Can Generate Cytotoxic T Cells and Serve as Target Antigens in an H2A^{-}E^{+} Transgenic Model Susceptible Only to Heterologous Thyroglobulin

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Recently we described a novel *H2E* transgenic model of murine experimental autoimmune thyroiditis (EAT) that precludes self reactivity to mouse (m) thyroglobulin (Tg) while susceptible to heterologous human (h) and porcine (p) Tg. In conventional, susceptible strains, EAT is inducible with mTg, hTg and pTg, due to conserved thyroiditogenic epitopes. In contrast, after the introduction of an *H2Ea*^{k} transgene into class II-negative B10.Ab^{0} mice [without surface IA (mutant Aß chain) or surface IE (nonfunctional *Ea* gene) molecules], the sole expression of H2E^{b} molecules permits EAT induction by hTg or pTg, but not mTg. Moreover, unlike conventional strains, cells from hTg- or pTg-immunized mice do not crossreact with mTg. To delineate the unique capacity of E^{+} B10.Ab^{0} mice to distinguish self from nonself Tg epitopes, we synthesized 20 15-20mer peptides according to predicted E^{b}-binding motifs on hTg. As presented elsewhere, 3 of the 20 were thyroiditogenic and unique to hTg. Here, we determined if 2 of the 3, hTg410 (410-424; QSQQFSVSENLLKEA) and hTg2344 (2344-2359; LTWVQTHIRGFGGDPR), could generate cytotoxic T cells (Tc) and serve as target antigens when loaded onto indicator cells (EL-4 lymphoma, H2^{b}). First, cells from hTg-immunized E^{+} B10.Ab^{0} mice were cultured with hTg. Tc were generated not only against targets loaded with hTg, but also hTg410 or hTg2344, using an 18-hr, Cr-51 release assay. No Tc against targets loaded with mTg or nonpathogenic peptide were observed. Second, hTg-primed cells cultured with hTg410 or hTg2344 were cytotoxic for targets labeled with the respective peptide. Third, cells from mice primed and cultured with hTg 410 or hTg2344 were cytotoxic only for itself. Specific lysis was blocked by either anti-CD8 or anti-class I, D^{b}. Thus, one pathogenic mechanism in this novel model is the generation of Tc. (Supported by NIH DK45960 and St. John Hosp. & Med. Ctr.)

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Program Number 15 Autoimmunity

Localization of the Thyroid Peroxidase Autoantibody Immunodominant Region to a Junctional Region Containing Portions of the Domains Homologous to Complement Control Protein and Myeloperoxidase

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Thyroid peroxidase (TPO) autoantibody epitopes are restricted to an immunodominant region (IDR) on the extracellular region of the native antigen. Localization of the IDR has been a longstanding and difficult goal. The TPO extracellular region comprises a large myeloperoxidase (MPO)-like domain, linked to the plasma membrane by two smaller domains homologous to complement control protein (CCP) and epidermal growth factor (EGF), respectively. Recent studies have focused on the CCP- and EGF-like domains as the putative location of the TPO autoantibody IDR. To address this issue, we modified TPO cDNA to delete the CCP- and EGF-like domains, together or individually. We used four human monoclonal autoantibodies that define the TPO IDR, as well as polyclonal TPO autoantibodies in patients' sera, to detect by flow cytometry the TPO mutants expressed on the surface of transfected COS-7 cells. As a control to determine the efficacy of TPO mutant trafficking to the cell surface, we used a mouse polyclonal antiserum with epitopes extending outside the autoantibody IDR. The combined CCP/EGF-like domain deletion did not produce a signal with TPO autoantibodies but did not traffic to the cell surface. In contrast, both monoclonal and polyclonal autoantibodies recognized equally well TPO with the juxtamembrane EGF-like domain deleted and wild-type TPO. Although TPO with the CCP-like domain deleted was also expressed on the cell surface, this TPO mutant was recognized very poorly by both the human monoclonal autoantibodies and by polyclonal autoantibodies in patients' sera. In conclusion, we have excluded the juxta-membrane EGF-like domain as being part of the IDR. In contrast, a facet in the CCP-like domain does contribute to the IDR. These observations, together with data from other studies, localize the TPO autoantibody IDR to the junction of the CCP- and MPO-like domains on TPO.

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Program Number 16 Autoimmunity

Relative Expression of Preadipocyte Factor-1 (Pref-1) and Thyrotropin Receptor (TSHr) Genes in Orbital Adipose Tissues and Cell Cultures from Patients with Graves' Ophthalmopathy

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TSHr is thought to be an important orbital autoantigen in Graves' ophthalmopathy (GO). The increased volume of orbital fat seen in GO may result from de novo stimulation of adipogenesis. We studied the relative expressions of TSHr and Pref-1, a gene in preadipocyte fibroblasts whose expression is markedly decreased following differentiation. We examined orbital adipose tissue samples from patients with GO (n=10) and normal individuals (n=6), and cultures of GO preadipocyte fibroblasts (n=3). Cells were grown under conditions known to stimulate adipocyte differentiation. Preadipocyte cultures were treated during differentiation with interleukin-6 (IL-6; 1 ng/mL), a cytokine elevated in the serum of patients with Graves' disease and a potent stimulator of TSHr expression in orbital fibroblasts, and rosiglitazone (10 microM), a PPAR-gamma agonist that stimulates both adipogenesis and TSHr expression in these cells. We examined relative gene expression using real-time PCR and gene-specific fluorescent probes and primers. We found significantly decreased Pref-1 expression (range 38-91%) following differentiation. There was no further decline in expression with IL-6 and rosiglitazone treatment. In contrast, TSHr expression increased (2.5 to 9.8 fold) with differentiation and there was further enhancement (10 to 29 fold) following treatment with these compounds. Results of studies examining relative TSHr and Pref-1 gene expression in uncultured orbital adipose tissue samples showed wide variability both within and between GO and control tissues. In summary, differentiation of orbital preadipocytes resulted in decreased expression of Pref-1 and increased TSHr gene expression. Further increase in TSHr expression following IL-6 and rosiglitazone treatment was not accompanied by further decrement in Pref-1 expression. We conclude that stimulation of TSHr expression in orbital cultures is associated with, but does not require, de novo differentiation of preadipocytes into adipocytes. These and other data suggest that novel cytokine or PPAR-gamma antagonists may be useful in the treatment of GO.

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Program Number 17 Cell Biology

Regulation of the PI3K, Akt/PKB, FRAP/mTOR, and S6K1 Signaling Pathways by Thyroid Stimulating Hormone and Stimulating-type TSH Receptor Antibodies in the Thyroid Gland

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The thyroid stimulating hormone receptor (TSHR) regulates the life cycle of thyrocytes and hormone production in the thyroid gland through diverse signaling pathways. This study addresses how TSH, the stimulating-type TSHR antibody, and insulin activate PI3K, FRAP/mTOR, and S6K1 in thyroid cells in vitro and in vivo. PI3K inhibitors (wortmannin, LY294002) and a FRAP/mTOR inhibitor (rapamycin) prevented TSH-induced progression of the cell cycle in FRTL-5 thyroid cells, showing that the TSH pathway acts through PI3K and FRAP/mTOR. In immunoprecipitates, the TSHR interacted with the p85 regulatory subunit of PI3K, and TSH and cAMP up-regulated TSHR-associated PI3K activity. A mutant p85 regulatory subunit of PI3K that does not bind to PI3K p110 lowered S6K1 phosphorylation in response to TSH. Downstream of PI3K, TSH caused PI3K-dependent membrane translocation of PDK-1, which resulted in the activation of S6K1 and phosphorylation of the ribosomal S6 protein, but not in phosphorylation of Akt/PKB. In contrast, insulin induced both S6k1 and Akt/PKB in thyroid cells. TSHR antibodies (IgG) from patients with Graves' disease also induced phosphorylation of S6K1 and its target protein, ribosomal S6 protein, whereas blocking-type TSHR antibodies from patients with primary myxedema inhibited TSH- but not insulin-induced S6K1 phosphorylation. Rapamycin inhibited both TSH/cAMP- and insulin-mediated phosphorylation of S6K1, suggesting that FRAP/mTOR is a central controller for thyrocyte proliferation. Rapamycin treatment in an MMI-induced thyroid hypertrophy model showed decreased TSH-mediated thyroid follicular activity, including follicle hyperplasia and colloid filling. These results indicate that PI3-kinase and FRAP/mTOR are required for the activation of PDK-1 and S6K1 for TSH-mediated thyrocyte proliferation in vitro and for TSH action in the thyroid gland in vivo.

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Program Number 18 Cell Biology

Thyroglobulin (Tg) Can Increase the Growth of FRTL-5 Thyrocytes by an Akt-driven Mechanism Distinct from TSH, Insulin, or IGF-1

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Tg accumulated in the follicular lumen is a potent regulator of thyroid-specific gene expression and thyroid function in individual follicles, counteracting the action of TSH/cAMP. In this report we show that Tg can stimulate thyroid cell growth in addition to altering function. FRTL-5 cells maintained without TSH or insulin and in only 0.2% calf serum (4H/0.2CS) for 4 days, in order to achieve G1 arrest, were exposed to Tg in the absence or presence of TSH, insulin, or 5% serum. Tg increased thymidine incorporation into DNA 42-fold more than the 4H/0.2%CS control, 5-fold more than TSH alone, 8-fold more than insulin alone, and 1.4± 0.1-fold more than TSH plus insulin plus 5% CS. Similar results were obtained when cell number was measured; and the Tg growth effect was not insulin-dependent. The Tg effect on growth was concentration dependent, being maximal at 5 mg/ml. When Tg was separated into 27, 19, and 12S fractions, the effect was measured mostly in the 27S fraction. Removal of Tg by antibody-specific immunoprecipitation proportionately decreased the growth effect. The Tg did not increase cAMP production and the specific kinase A inhibitor, H-89, had no effect on Tg-induced growth. Tg increased Akt kinase activity to the same level as insulin plus TSH plus 5% CS and LY294002, a PI3 kinase inhibitor, abolished Tg-induced growth. An IGF-1 or IGF-2 neutralizing antibody had no effect on the Tg-induced growth. We conclude that Tg has a potent thyroid growth stimulating effect involving a PI3 kinase/Akt signal and is independent of insulin/IGF-1 or A kinase signals. Unlike TSH, bFGF, EGF, or TPA, the effect is not synergized by or dependent on insulin/IGF-1. Tg may therefore be an autocrine regulator of thyroid growth allowing adjustments in follicular size to accomodate Tg accumulating in the follicular lumen while suppressing thyroid function.

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Program Number 19 Cell Biology

Expression of Functional Growth Hormone (GH) Receptors and Direct Effects of GH on Thyroid Cells

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We have previously revealed that 99mTc thyroidal uptake is suppressed in active acromegalic patients and that IGF-I suppressed TSH induced iodide uptake as well as Na/I symporter gene expression in rat FRTL-5 cells. The inhibitory effects of IGF-I partially account for the decrease of 99mTc thyroidal uptake in the patients. However, GH has various metabolic effects that are not mediated by IGF-I. We therefore studied effects of GH on rat FRTL-5 in order to clarify the possible roles of GH on thyroid functions. FRTL-5 cells were cultured as reported. Recombinant human GH was used in all experiments. Iodide uptake and DNA synthesis were determined by the standard methods. Messenger RNA levels were determined by RT-PCR or Northern blot hybridization. First we studied expression of functional GH receptor gene by RT-PCR and revealed that the transcript for full length GH receptor gene was expressed in the thyroid cells. Then we investigated effects of GH on early responsive gene in the cells. GH stimulation increased c-fos mRNA levels after 30 to 60 minutes in 4H medium with 0.2% calf serum. Next we studied the effect of GH on iodide uptake and NIS mRNA expression. GH treatment transiently potentiated NIS mRNA increase by TSH within 24 hour but iodide uptake stimulated by TSH was decreased by 24-hour-GH treatment. However, the treatment did not affect DNA synthesis determined by thymidine incorporation in both 4H basal and TSH-stimulated conditions. We next studied the effect on TSH-stimulated cAMP production. GH treatment significantly potentiated TSH-induced cAMP production. The observation is compatible with the fact that GH treatment potentiated TSH-induced NIS mRNA increase. The longer incubation with GH inhibited TSH-induced NIS mRNA expression as well as iodide uptake similar to IGF-I treatment. Thus GH may elicit unique effects such as potentiation of TSH but the exact mechanism and the role of the direct effects of GH in thyroid cells should be elucidated by further studies.

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Program Number 20 Thyroid Hormone Action

Activated by Thyroid Hormone, Mitogen-activated Protein Kinase Phosphorylates Nuclear Estrogen Receptor (ER) in HeLa Cells

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L-Thyroxine (T_4)-activated MAPK rapidly serine phosphorylates the nuclear human thyroid hormone receptor TRB1 (TR)(JBC 275:38032, 2000). Kato *et al.* (Science 270:1491, 1995) have shown that 17B-estradiol (E_2) nongenomically activates MAPK, causing phosphorylation of Ser-118 of estrogen receptor-A (ER) and activation of the receptor. Several laboratories have reported that thyroid hormone has estrogen-like effects in several cell models. We examined the possibility that T_4-directed MAPK may phosphorylate ER. HeLa cells containing ER, but little or no functional TR, were incubated with T_4 (total concentration, 10^{-7} M) and activated MAPK (pERK1/2) and Ser-118-phosphorylated ER were quantitated by immunoblot of nuclear fractions. T_4 activated MAPK at 5-15 min and T_4 treatment of cells was associated with Ser-118 phosphorylation of nuclear ER within 15 min. The effect on ER decayed by 60 min. PD 98059, an inhibitor of the MAPK signal transduction cascade at MAPK kinase (MEK), blocked the T_4 effect on ER phosphorylation. 17B-Estradiol (E_2, 10^{-10} to 10^{-8} M) also caused MAPK-dependent Ser-118 phosphorylation of ERA, reproducing the report of Kato *et al.*. This ER contains a MAPK docking site in the DNA-binding domain that is similar to the MAPK docking motif we have described on TR, and which may be relevant to Ser-118 phosphorylation by MAPK. Thus, T_4 nongenomically promotes Ser-118 phosphorylation of ERA, mimicking the biochemical action of physiological concentrations of E_2. This action of thyroid hormone may contribute to estrogen-like effects of iodothyronines obtained in certain cellular models.

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Program Number 21 Cell Biology

Quantifying TSH Regulation of Cleavage at the Human Thyrotropin Receptor

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The TSH receptor (TSHR) undergoes cleavage and forms two subunits which remain disulfide-linked. When followed by reduction of disulfide bridges(s), there is shedding of the large extracellular domain (the α or A subunit). Since the TSHR is the major autoantigen in Graves' disease we have begun to quantify the cleavage phenomenon and to study its regulation. We used, two monoclonal TSHR antibodies (B. Rees Smith, UK): #4 recognizing amino acids 322-341, an epitope within the cleaved region, and #1 recognizing amino acids 381-385, an epitope on the N–terminus of the β or B subunit of the receptor. On cleavage, of the receptor, the epitope recognized by #4 is removed whereas the receptor residency recognized by antibody #1 remains intact and unaltered. When tested by flow cytometry using CHO cells stably transfected with human TSHR (JP09), a 75% positivity for Mab #1 and a 70% positivity was seen with Mab #4. The specificity of MAb#4 was confirmed using CHO cells expressing a non-cleavable receptor (vector B. Rapoport, CA) where amino acids 316-377 had been deleted. In the presence of 1% serum we observed a 12% increase in cleavage which increased by >70% in the presence of TSH. There was a dose -response relationship with increasing concentrations of TSH first observed with 10 μU/ml. This increase in cleavage was not secondary to autophagocytosis of the receptor or apoptosis of cells as demonstrated by retention of binding by Mab #1 to the β or B subunit across all serum conditions used. This enhancement of cleavage by TSH was not due to the blockade or steric-hinderance of the epitope for Mab #4 nor due to the agumented endocytosis of receptors by the agonist. These data confirm the TSH regulation of TSHR cleavage which is likely to be followed by receptor shedding into the circulation.

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Program Number 22 Iodine Uptake and Metabolism

Activation of the Human Sodium/Iodide Symporter Upstream Enhancer cAMP Response Element-like Sequence by PKA-dependent and PKA-independent Pathways in Normal Thyroid and Thyroid Cancer Cells

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We recently identified a thyroid-specific far-upstream enhancer (-9847 to -8968) in the human sodium/iodide symporter (hNIS) gene, which is active in normal thyroid cells but not in human papillary thyroid cancer cells (BHP cells). The hNIS upstream enhancer (hNUE) has a cAMP response element (CRE)-like sequence which is activated via protein kinase A (PKA)-dependent and PKA-independent pathways. We utilized pharmacologic inhibitors and binding assays to characterize these pathways and transcription factors binding to the CRE-like sequence.
It is known that TSH activates mitogen-activated protein kinases (MAPKs) in thyroid cells. To determine whether MAPKs are involved in the PKA-independent pathway, the hNUE was inserted upstream of an SV40 heterologous promoter in pGL3-promoter vector and was transiently transfected into FRTL-5 cells cultured without TSH for 7 days. H-89, an inhibitor of PKA, inhibited forskolin-induced enhancer activity about 50%. Moreover, forskolin-induced enhancer activity was also decreased approximately 50% by PD98059, an inhibitor of upstream extracellular signal-regulated kinase (ERK)1/2-MAPK. These results indicate that the hNIS gene expression is mediated by both PKA-dependent and PKA-independent pathways and that the ERK cascade is, at least in part, involved in the PKA-independent pathway for activation of the hNUE.
We characterized nuclear proteins bound to the CRE-like sequence of the hNUE utilizing EMSAs. Multiple shifted bands were observed in nuclear extracts prepared from FRTL-5 cells and BHP2-7 cells. One of the bands was identified as CREB-1, ATF-1 and/or CREM that is present in both FRTL-5 and BHP2-7 cells, and one of the others was an unknown factor(s) that is absent in BHP2-7 cells. The unknown transcription factor(s) was upregulated by TSH/forskolin in FRTL-5 cells but not in BHP2-7 cells. Deficient CRE-like sequence binding protein(s) that bind to the hNUE in normal thyroid cells may be responsible for reduced hNIS gene expression in some thyroid carcinomas.

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Program Number 23 Cancer

Ultrasonographic Parameters Predictive of Malignancy in Thyroid Nodules with Indeterminate Cytologic Pattern

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Fine needle aspiration biopsy (FNAB) is clearly the best method for separating benign from malignant thyroid nodules. The problem with FNAB is the cytologic finding of an indeterminate pattern. The objective of our study was to determine whether thyroid ultrasonographic evaluation could be useful in predicting malignancy in indeterminate cytologic patterns. Since 1997, we examined 2460 patients with thyroid nodules referred to the Thyroid Unit of São Paulo University School of Medicine. In all of them, an ultrasonographic examination followed by ultrasound-guided fine-needle aspiration biopsy (US-FNAB) was carried out. Thyroid nodules were classified ultrasonographically into four grades, with increasing score numbers (1-4) indicating progression to malignant lesions. Grade I: a benign lesion consisting in a round anechoic area that is suggestive of a thyroid cyst. Grade II: isoechoic or hyperechoic solid nodules with or without cystic change and coarse calcifications are suggestive of adenomatous goiter. Grade III: hypoechoic solid nodule with regular border or cystic nodule with a solid mass arising from the cystic wall are considered uncertain. Grade IV: hypoechoic solid nodule with an irregular border and with the presence of micro-calcifications is suspicious for malignancy. Two hundred and sixty-eight patients underwent thyrodectomy. Ninety-seven patients, 87 females and 10 males, had an indeterminate cytologic pattern. Of this group, benign histology was reported in 83 (85.5%) and malignancy was found in 14 (14.5%). Nevertheless, malignant lesions are distinctly distributed among the different grades of ultrasonographic classification: Grade I 0/2, Grade II 1/39, 2.5%, Grade III 8/48, 16.7% and Grade IV 5/8, 62.5%. We concluded that nodules with indeterminate cytologic pattern with non-suspicious ultrasonographic findings (grade I and II) can be safely followed-up with periodic sonography. On the other hand, nodules with ultrasonographic characteristics suspicious for malignancy (Grade III and Grade IV) should be considered to harbor risk factors for cancer.

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Program Number 24 Cancer

Recombinant Human TSH Stimulation of Undetectable Serum Thyroglobulin Levels on Adequate Thyroxine Suppressive Therapy Seldom Reveals New Evidence of Recurrent Disease in Patients with Follicular Cell-derived Thyroid Cancer

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In the postoperative surveillance of patients with follicular cell-derived thyroid carcinoma (FCTC), recombinant human TSH (rhTSH) has been used to enable whole body scanning (WBS), and to stimulate thyroglobulin (Tg) secretion. Our aim was to determine if rhTSH-stimulated WBS and Tg measurements significantly alter management in FCTC patients with undetectable Tg levels on thyroxine suppressive therapy (TST). Of 93 FCTC patients undergoing rhTSH-stimulated testing from July 1999 through September 2001, 31 patients (mean age 47 years; females 68%) had undetectable basal Tg (<0.5 ng/ml) on TST, negative Tg-autoantibodies, a stimulated Tg level, a WBS and adequate follow-up. Twenty-one (68%) had papillary cancer; 10 (32%) had either follicular or Hurthle cell cancer; 45% were TNM stage 1 at thyroidectomy, 36% stage 2, and 19% stage 3. Ninety-seven percent had received prior ablation. Using a stimulated Tg >2ng/ml as a surrogate for thyroid remnant or recurrent cancer, 5 patients had abnormal results. WBS showed uptake in 7 patients: 4 in remnant tissue and 3 in possible recurrent neck disease. Only one patient had both an elevated Tg and neck uptake; 10 patients had either a Tg increment >2ng/ml or an abnormal WBS. Clinical management was altered only in three patients. The one patient with a positive Tg (3.5 ng/ml) and WBS received further radioiodine to ablate a neck uptake of only 0.06%. Two patients with stimulated Tg levels of 4.2 and 13.5 ng/ml underwent PET, CT and MRI scanning, which showed no clear evidence of disease. None of these 31 patients (after a mean follow-up of 21 months) have definitive radiologic evidence of recurrence. Conclusion: FCTC patients with negative Tg autoantibodies and serum Tg <0.5ng/ml on TST do not significantly benefit from rhTSH-stimulated Tg measurement and concomitant WBS. In this setting, the measurement of rhTSH-stimulated Tg levels appears to be of questionable clinical utility.

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Program Number 25 Cancer

Novel Type of ret/PTC Rearrangement in Radiation-associated Papillary Thyroid Carcinoma

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Molecular analysis of the expression of the ret gene in a case of papillary thyroid carcinoma developed in an externally irradiated patient revealed a strong imbalance between the abundance of extracellular and tyrosine kinase (TK) domain mRNA level suggestive of a structural mutation moving apart portions of the ret gene. Using 5'-RACE, a novel rearrangement involving the 5' portion of an rfp gene juxtaposed upstream of the fragment encoding for the TK domain of the ret gene was identified in the tumor tissue. At the genomic level, formation of a fusion gene occurred as a result of balanced chromosomal translocation between short arm of chromosome 6 and long arm of chromosome 10 confirmed by interphase FISH. Sequencing of breakpoints in respective introns of direct and reciprocal chimeric genes revealed the presence of small deletions, at that no sequence homology was found between the flanking fragments. Functional analysis of the product of full-length cDNA of the chimeric rfp/ret gene cloned into the expression vector demonstrated that in spite of 6 amino acid deletion at the c-terminal portion of coiled-coil domain of rfp, chimeric protein retained the propensity to form homodimers as assessed by yeast two-hybrid system. This confers the condition for sustained TK activity of ret and implies the oncogenicity of the fusion protein. Stably transfected NIH 3T3 fibroblasts inoculated into immunocompromised mice yielded rapidly growing fibrosarcomas indicative of high tumorigenic potential of the rfp/ret. Resembling the original tumor, immunohistochemical staining of the transfectant cell lines and mouse tumors revealed cytoplasmic localization of the protein consistent with the absence of transmembrane domain from the fusion protein.
Thus, the finding provides additional line of evidence of facilitated susceptibility of the ret gene to structural mutations eventually leading to the formation of activated oncogene in irradiated human thyroid cells.

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Program Number 26 Thyroid Diseases

A Novel Germline Point Mutation in RET Exon 8 in Familial Medullary Thyroid Carcinoma

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Familial medullary thyroid carcinoma (FMTC) is a dominantly inherited disease caused by activating germline mutations of RET oncogene. Until recently, the majority of described patients carried a germline point mutation in RET extracellular domain affecting cysteine residues encoded by exons 10 or 11, whereas few FMTC families have point mutations in RET intracellular domains involving noncysteine codons in exons 13, 14, or 15. New reports have changed this picture, showing many more patients with mutations in intracellular domains and, also, one family showing a 9-bp duplication in the extracellular domain of exon 8. We now report a RET screening mutation in a large five-generation Caucasian family with 92 members residing in Brazil, whose ancestors emigrated from Spain about 100 years ago. In 43/92 members, sequence analysis of PCR-amplified products, using specific primers to exons 7 to 19 of the RET oncogene, revealed a new germline RET mutation in exon 8 (Gly533Cys). Twenty-six of those 43 patients had already confirmed the diagnosis of MTC by presenting thyroid nodules or elevated serum calcitonin and histological examination of their thyroid tumor tissues after total thyroidectomy. Many of remaining patients with this new RET mutation (17/43), particularly the older ones, already present thyroid nodules or elevated serum calcitonin and will be submitted to thyroidectomy. No family-member has evidence of MEN. In addition, we found a previously described G1296A single nucleotide polymorphism in exon 7 that does not segregate with the disease. In summary, we report a new germline point mutation in RET extracellular domain involving exon 8 in a five-generation family with MTC. This report and the previous account of a 9-bp duplication in exon 8 suggest that the extension of RET analysis to exon 8 should become a routine procedure for DNA testing in patients with FMTC.

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Program Number 27 Cancer

An Approach to Therapy for Anaplastic Carcinoma of the Thyroid

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Anaplastic thyroid carcinoma (ATC) is a thyroid-derived tumor that has lost differentiated features. It is one of the most aggressive solid tumors in humans and does not respond to most therapies. Although multimodality treatments have been extensively used to control the disease and enhance survival, no successful treatment protocol has been established. Our current study demonstrated that that IFN-gamma combined with tumor necrosis factor alpha-related apoptosis-inducing ligand (TRAIL) or 7-hydroxystaurosporine (UCN-01) treatment is much more effective than these agents alone in killing thyroid anaplastic carcinoma cells (ARO cells). To further study the mechanism by which IFN-gamma sensitizes thyroid carcinoma cells to apoptosis, we used cDNA microarray to screen for gene alterations in thyroid carcinoma cells treated with IFN-gamma. Our results demonstrated that expression of the pro-apoptotic gene, Bak, was up-regulated four fold by IFN-gamma. These findings were confirmed by RNase protection assay. Western blot analysis further showed an increase in Bak at the protein level. Accompanying the increase in Bak, caspase-10 isoforms (a, c and d) were all elevated by IFN-gamma treatment at both the mRNA and protein level. To test the role of increases in BAK on the facilitation of apoptosis in thyroid cancer cells, transfecting ARO cells with a pRC/CMV vector containing antisense for Bak significantly blocked the sensitizing effect of IFN-gamma. This documented that increases in Bak are important in mediating IFN-gamma induced alterations in apoptotic pathways. Of interest, the sensitizing effect of IFN-gamma on apoptosis was only evident in thyroid cancer cells and not seen in normal thyroid cells. Our findings clearly indicated that IFN-gamma increases the susceptibility of human thyroid cancer cells to apoptosis in a manner that does not harm normal human thyroid cells, and this may be of therapeutic significance.

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Program Number 28 Cell Biology

Inverse Correlation between Heparan Sulfate Deposition and Heparanase-1 Gene Expression in Thyroid Papillary Carcinomas: A Potential Role in Tumor Metastasis

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Heparanase-1 (HPR1) is an endoglycosidase that degrades the side chains of heparan sulfate proteoglycan (HSPG), a key component in the extracellular matrix (ECM) and the basement membrane (BM). In the present study, we first evaluated HPR1 expression in thyroid neoplasms using in situ hybridization (ISH) and immunofluorescence (IF). ISH analysis of 81 tumor samples (62 papillary carcinomas and 19 follicular adenomas) revealed that HPR1 was expressed at a much higher frequency in papillary thyroid carcinomas (PTC) than in follicular adenomas ($P<0.05$). RT-PCR analyses of fresh tumor tissues revealed that HPR1 mRNA could be detected in primary and metastatic PTC. HPR1 expression was further confirmed at the protein level by using IF staining with an HPR1-specific mAb. To test whether HPR1 expressed in thyroid neoplasm had a functional role in degrading HSPG, we conducted IF staining with a heparan sulfate (HS)-specific mAb to evaluate the HS deposition in the BM. Our results show that lack of HS deposition in PTC inversely correlated with HPR1 expression. Clinicopathological data analyses revealed that PTC with local and distant metastasis scored HPR1 positive at a significantly higher frequency than the non-metastatic thyroid cancers ($p=0.02$). To further explore the role of HPR1 in tumor metastases, we characterized HPR1 expression in 10 thyroid tumor cell lines using RT-PCR and Western blot, and measured HPR1 enzymatic activity using a novel ELISA. We found that HPR1 was differentially expressed in different types of cell lines; overexpression of HPR1 in two tumor cell lines led to a dramatic increase of their invasive potential in vitro in the artificial BM. These results collectively suggest that HPR1 expressed in PTC is functional and that HPR1 expression is associated with thyroid tumor malignancy and may significantly contribute to thyroid tumor metastases.

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Program Number 29 Thyroid Diseases

Involvement of Coactivators in the Dominant Negative Potency of the Mutant TRs in RTH: Analysis of a Novel Mutant, F455S

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We have recently found a sporadic case of an 11-year-old girl with RTH who showed several typical manifestations including a goiter, tachycardia, and low weight (BMI 14.9). She was heterozygous for a novel single nucleotide substitution (1364T to C) resulting in a phenylalanine to serine mutation at codon 455 (F455S). It has recently been proposed that the impaired dissociation of corepressors from mutant TRs may reflect the dominant negative potency of the mutant TRs. In the present study, we investigated how a corepressor, NcoR, and a coactivator, SRC-1, worked in this novel F455S mutant and compared it with those of an artificial mutant, E457A. Although F455S showed comparable binding of T3 to wild type TR, its ability to activate transcription via DR4 and PAL TRE was mildly impaired. In contrast, the E457A mutant showed almost no activation. Furthermore, F455S demonstrated relatively weak dominant negative effect on wild-type TR compared to the E457A mutant. GST pull down analysis showed that F455S exhibit strong interaction with NcoR similar to the wild-type, but E457A showed only 50% of binding. Surprisingly, the dissociation of NcoR from TR induced by addition of T3 was significantly impaired in the F455S mutant, while the E457A mutant showed approximately 50% of the dissociation of the wild-type. Similar results were also observed in EMSA studies. In contrast, the hormone-dependent association with SRC-1 was significantly impaired in the E457A mutant compared to F455S in both GST-pull down assay and EMSA. These findings demonstrated that the dominant negative potency of the mutant TR in RTH may be involved not only in the impairment of the dissociation of NcoR but also in that of the association to SRC-1.

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Program Number 30 Thyroid Hormone Action

Effects of the Thyroid Hormone Receptor Beta (TRb)-selective Compound GC-1 on Bone Development of Wistar Rats

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We investigated the effects of GC-1, a TRb-selective thyromimetic, on the epiphyseal growth plate (EGP) and bone mineral density (BMD) of developing rats. In this study, 21-day-old female rats (n=5-6 in each group) were made hypothyroid and treated for 32 days with 0.3 ug/100 g BW/day of T3 (1xT3), 5xT3, or with equimolar doses of GC-1 (1xGC-1 and 5xGC-1). As expected, hypothyroidism impaired longitudinal body growth (LBG), delayed the ossification of epiphyses (OE), reduced the number of hypertrophic chondrocytes (HC) and caused disorganization in the columns and layers of chondrocytes in the EGPs of vertebra and distal femur. Serum IGF-I was reduced in hypothyroid rats [-67% vs. euthyroid (EUT) rats, $p<0.001$]. We showed that hypothyroidism significantly impaired BMD gain (final BMD-basal BMD) in the lumbar spine, femur, tibia and hind body (hind limbs+pelvis+lumbar spine+first four caudal vertebrae) in a range of -71% to -75% vs. EUT ($p<0.001$, for all bone regions). T3 completely or partially normalized these changes while GC-1 had selective effects. GC-1 treatment resulted in organized columns and layers of chondrocytes in the EGPs with similar number of HCs as compared with EUT rats, but was unable to normalize the LBG, OE and serum IGF-I. The 1xGC-1 and 5xGC-1 groups had higher BMD gain in the tibia when compared with hypothyroid rats (44% and 50%, respectively, $p<0.05$ for both), but lower BMD gain, when compared with T3-treated rats (-28%, $p<0.05$, and -29%, $p<0.01$, respectively). Our findings suggest that TRb mediates the effects of T3 on EGP chondrocyte organization and HC differentiation, but not on OE. T3 has anabolic effects on bone mass that are both dependent and independent of its action on the GH/IGF-I axis, since GC-1 increases the BMD without increasing serum IGF-I and does it in a lower magnitude than T3.

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Program Number 31 Thyroid Hormone Action

Thyroid Status and T3 Receptor Isoforms Differentially Regulate the Pacemaker Ion Channels HCN2 And HCN4

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T3 markedly influences the electrophysiological function of the heart. Mice with deletion of thyroid hormone receptor alpha (TR-alpha KO) or expressing a mutant TR-beta receptor (PV-mutant) have a decreased heart rate. Thus both TR-alpha and TR-beta are expressed in cells that generate heart rate. Patch clamping of sino-atrial pacemaker cells in TR-alpha KO mice shows that receptor inactivation leads to a slowing of If channel kinetics, an observation that may explain bradycardia in this strain. We explored T3 and TR isoform influences on HCN2 and HCN4 gene expression by determining mRNA levels in the atrium and ventricle from wt, TR-alpha KO, TR-beta KO and PV-mutant mice of different thyroid states. In the wt ventricle HCN2 mRNA increases 300%, and in the atrium 350% by hyperthyroidism. In contrast, HCN4 is almost unchanged in the atria of wt hypo- and hyperthyroid mice, but decreased by 30% in hypothyroid and increased by 40% in hyperthyroid ventricles. Significant differences in the levels of HCN2 and HCN4 were noted in the hearts of different TR knockout mice. For example, HCN2 was markedly lower in atria of TR-alpha KO mice but increased in TR-beta KO mice. Atrial HCN4 levels were normal in TR-alpha KO mice but diminished in euthyroid TR-beta KO mice. A specific TR-beta effect on HCN4 expression is also evident in euthyroid TR-beta PV-mutant mice where HCN4 mRNA is significantly lower but HCN2 mRNA remains unchanged in atria and ventricles. T3 increases HCN2 mRNA levels but we found a lack of promoter responsiveness and interestingly a T3 mediated increase of mRNA stability. Conclusion : 1. Bradycardia in the TR-alpha KO correlates with slowed If channel kinetics in the sinus node. 2. Cardiac pacemaker channels HCN2 and HCN4 show a regional TR isoform differential response. 3. T3 increases HCN2 in part by stabilizing its mRNA.

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Program Number 32 Thyroid Hormone Action

Autoregulation of Expression of Thyroid Hormone Receptor Isoforms and Coactivators in Liver and Heart by Thyroid Hormone

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Autoregulation of thyroid hormone (TH) receptors (TRs) allows cells to regulate responsiveness to TH. Nuclear coactivators (NCoAs) modulate TH action and may also regulate TR expression. We have determined the effect of TH withdrawal and treatment on expression of TR isoforms and NCoAs SRC-1, TIF-2 and SRC-3 using quantitative RT-PCR. To identify the effects that each TR isoform exerts over expression of another, NCoA and TR transcripts were measured in liver and heart from wild type mice or mice with disruption of either *TR* subtype or *SRC-1* genes. In WT mice, TH deprivation resulted in 5.4±1.6- and 2.9±0.5-fold up-regulation of TRα2 and TRβ1, respectively, compared to untreated mice, while TH treatment resulted in a 66.6±9.6- and 39.4±5.2%-fold down-regulation of TRα2 and TRβ1, respectively. Contrary to liver, in heart of WT mice, TH deprivation resulted in a 53.8±4.3%-fold decrease in TRα2 and no significant change in TRα1 or TRβ1. TH treatment resulted in 25.3±10.9%-fold decrease in TRα1 and 1.4±0.2- and 2.2±0.3-fold increase in TRα2 and TRβ1, respectively. Changes in TRβ1 in both liver and heart with TH tx were blunted in $TR\alpha^{0/0}$ mice, suggesting that TRβ expression is, in part, controlled by TRα. In the liver of WT mice, TH deprivation resulted in a 2.2±0.3- and 3.2±0.2-fold increase in TIF-2 and SRC-3, respectively, without any change in SRC-1 expression. TH treatment resulted in no change in SRC-1 or TIF-2, but a 33.2±5.1%-fold decrease in SRC-3 expression. The increase in TIF-2 was absent in $TR\beta^{-/-}$, $TR\alpha^{0/0}$ and $SRC\text{-}1^{-/-}$ mice, suggesting that these transcription factors may be important for TH regulation of TIF-2 expression. NCoAs are, in general, increased in response to hypothyroidism or in states of TH resistance, but SRC-1 specifically does not regulate TR isoform expression. We have demonstrated that TR isoforms and NCoAs are autoregulated transcription factors with tissue specificity.

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Program Number 33 Thyroid Hormone Action

Thyroid Hormone Receptor Subtype-specific Interaction with SRC-1 Mediates Thyroid Hormone-dependent Gene Expression in Mouse Liver

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Isoforms of the thyroid hormone (TH) receptor (*TR*)*α* and *TRβ* genes mediate TH action both centrally and peripherally. How TR isoforms modulate tissue-specific TH action remains largely unknown. The steroid receptor coactivator-1 (SRC-1) is among a group of transcriptional coactivator proteins that bind to TRs, and modulates gene activity. Mice deficient in SRC-1 or TRβ have resistance to thyroid hormone (RTH), while TRα-deficient mice are hypersensitive to thyroid hormone (HTH). We investigated whether SRC-1-mediated activation of TH-regulated gene transcription in the liver is TR subtype-specific. To investigate this, we have generated mice deficient in both TRα and SRC-1 ($TR\alpha^{0/0}SRC\text{-}1^{-/-}$), as well as in both TRβ and SRC-1 ($TR\beta^{-/-}SRC\text{-}1^{-/-}$), and compared their responses to TH withdrawal and treatment (Tx) with those of WT mice and mice with disruption of the individual genes. Quantitative RTPCR was performed on TH-responsive liver genes identified by microarray analysis. Treatment of WT and $SRC\text{-}1^{-/-}$ mice resulted in 30±8%- and 30±5%-fold increase in 5'deiodinase (5'DI) expression, respectively. $TR\beta^{-/-}$ mice demonstrated minimal response to TH treatment (+1%) and the $TR\beta^{-/-}SRC\text{-}1^{-/-}$ responded even less (<1%). On the other hand, mice deficient in TRα had the highest response to TH-induced 5'DI expression, 242±35%-fold increase, which was abrogated in $TR\alpha^{0/0}SRC\text{-}1^{-/-}$ mice (only a 33±14%-fold increase). This suggests that the presence of SRC-1 is necessary for the HTH phenotype. Glutathione-S-transferase mRNA decreased between 69% and 78% with TH Tx in all genotypes except in $TR\beta^{-/-}$. Osteopontin mRNA, on the other hand, was up-regulated in response to TH treatment only in WT and $SRC\text{-}1^{-/-}$ mice but not in $TR\beta^{-/-}$ or $TR\alpha^{0/0}$ mice, suggesting a role for both TR subtypes in its regulation. We conclude that (1) SRC-1 mediates the HTH seen in $TR\alpha^{0/0}$ mice and (2) we have identified two new TRβ-dependent markers of TH action; osteopontin (up-regulated) and glutathione-S-transferase (down-regulated).

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Program Number 34 Cell Biology

Thyroid Hormone Receptor α2 Is an RNA Binding Protein Localized to the Nucleus and Cytoplasm

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Thyroid hormone receptor α2 is an alternative splice product of the TRα gene whose unique C terminus does not bind T3 or activate transcription. In transfection assays, TRα2 is a weak inhibitor of T3 induced transactivation. However, the physiological function of TRα2 is unknown. We have tested whether TRα2 might be an RNA-binding protein. A purified fusion protein consisting of GST followed by TRα2 and 6 histidines was used to bind RNA; GST followed by 6 histidines served as a control. GST-TRα2-6His bound to a random sequence 32P-labeled RNA probe 6-10 fold higher than GST-6His. Protein truncation studies revealed that the RNA binding domain of TRα2 is located between the second zinc finger and the ligand binding domain. Since TRα2 contains multiple casein kinase II phosphorylation sites in its unique C-terminus, we tested whether its RNA binding is regulated by phosphorylation. CK-II treatment of TRα2 eliminated its RNA binding, whereas CK-II only slightly inhibited the RNA binding of TRα2 with its C-terminal CK-II sites mutated. TRα2 bound single stranded but not double stranded RNA. To determine the specificity of RNA binding, the 32P-RNA probe was competed with increasing doses of polyribonucleotides. Poly (C) and poly (U) competed well, but poly (A) did not. To provide further clues as to the function of TRα2, confocal microscopy was used to examine the subcellular localization of green fluorescent protein tagged TRα1 and TRα2 transfected into CV-1 and JEG cells. Although GFP-TRα1 was almost entirely nuclear, GFP-TRα2 localized to both the nucleus and cytoplasm. This difference was confirmed by Western blots of nuclear and cytoplasmic extracts of GFP- and Flag-fusion proteins in CV-1 cells. We speculate that TRα2 may function in the post-transcriptional processing or translation of RNA, or other RNA functions.

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Program Number 35 Iodine Uptake and Metabolism

Potential Sources of Excess Dietary Iodine in 2002: Milk and Bread

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The most recent national survey of iodine (I) nutrition (NHANES III, 1988-1994, JCEM 1998) in the US demonstrated an approximate 50% reduction in mean dietary I intake (mean urinary I, 26.5mcg/dl) compared to values reported in NHANES I, 1971-1974 (mean urinary I, 45.2mcg/dl). This reduction was ascribed, in part, to a possible reduction in I content in dairy products and to decreased use of I as a dough conditioner in commercial bread production. Children in both surveys had urinary iodine excretion that was higher than that of adults. We measured urinary I excretion in 100 children, ages 6-12, residing in Boston, by the Sandell-Kolthoff reaction. Mean I excretion was 34.2 mcg/dl, which is slightly higher than that reported in NHANES III for children ages 6-11 (30.5mcg/dl). To determine the primary sources of dietary I, we measured I content in 20 varieties of bread, 20 brands of milk, and 8 different infant formulas sold locally, and in samples of breast milk from two iodine-sufficient volunteers. All brands of milk had >88mcg I per 250ml (8oz., 1 cup), ranging from 88mcg to 168mcg (116.0 +/- 22.1mcg/250ml, mean +/- SD). I content in the 2 breast milk samples were 18.9 and 22.0mcg/250ml. Values for infant formulas ranged from 27.4mcg to 96.0mcg I per 8 oz. serving (39.7 +/- 23.1 mcg/8oz., mean +/- SD), often far higher than labeled. Three varieties of bread contained >313mcg I per slice (313.5 to 587.4mcg). I content in the other 17 brands ranged from 2.2 to 54mcg I per slice (mean 10.1 +/- 13.2mcg I/slice). Conclusion: These findings strongly suggest that milk continues to be a major source of US dietary iodine, especially in children, and that some brands of bread and some brands of milk-based infant formula contain an inordinate amount of I.

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Program Number 36 Cancer

Radioiodine Therapy of Colon Cancer following CEA Promoter-driven Expression of the Sodium Iodide Symporter

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Colorectal carcinoma is the second leading cause of malignancy in Western countries with a significant number of patients dying from recurrent and metastatic disease. We therefore investigated the feasibility of radioiodine therapy in carcinoembryonic antigen (CEA)-producing colon carcinoma cells (HCT116) following tissue-specific expression of the human sodium iodide symporter (hNIS) using the CEA promoter. For this purpose, HCT116 cells were stably transfected with an expression vector, in which hNIS cDNA has been coupled to the CEA promoter (fragment of 365 bp). This promoter is responsible for tissue-specific expression of CEA in gastrointestinal tract epithelium, and has been shown to target therapeutic genes to colorectal cancer cells expressing high levels of CEA. The stably transfected HCT116 cells showed perchlorate-sensitive iodide uptake, concentrating 125-I about 10-fold *in vitro*. In contrast, transfection of control cancer cells without CEA expression (osteosarcoma and fibrosarcoma cells) did not result in NIS-specific iodide accumulation. NIS protein expression was confirmed by Western blot analysis using a monoclonal hNIS-specific antibody, which revealed a band of a molecular weight of approximately 90 kDa. In addition, immunostaining of stably transfected HCT116 cells revealed hNIS-specific immunoreactivity, which was primarily membrane-associated. In an *in vitro* clonogenic assay approximately 95% of HCT116 cells stably expressing NIS under the control of the CEA promoter were killed by exposure to 131-I, while only about 5% of NIS-negative control cells were killed. These data clearly show that the amount of accumulated 131-I is sufficiently high to selectively kill NIS-transfected HCT116 cells. In conclusion, a therapeutic effect of 131-I has been demonstrated in colon carcinoma cells following induction of tissue-specific iodide uptake activity by CEA promoter-directed NIS expression *in vitro*. This study demonstrates the potential of NIS as a therapeutic gene allowing radioiodine therapy of colon cancer following tissue-specific NIS gene transfer.

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Program Number 37 Iodine Uptake and Metabolism

Systemic Retinoic Acid Treatment Induces Radioiodide Uptake and Sodium/Iodide Symporter mRNA Expression in Mouse Breast Cancer Models

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Lactating breast tissue and some breast cancers express sodium/iodide symporter (NIS) and can concentrate iodide. Selective stimulation of NIS expression in breast tumors offers the possibility of diagnostic and therapeutic use of radioiodide for breast cancer. We recently demonstrated that all-trans retinoic acid (tRA) induces both NIS gene expression and iodide accumulation in vitro in well-differentiated human breast cancer cells (MCF-7). We first investigated the in vivo efficacy and specificity of tRA-stimulated iodide accumulation into MCF-7 xenograft tumors on SCID mice. These animals were treated with tRA pellets (0.8 to 16.0 μmol/day) for 7 days and then injected (i.p.) with a single dose of radioiodide-125. Animals were euthanized 3 hours after the administration of radioiodide, tissue was harvested, and radioactivity was counted. Treatment with tRA increased iodide accumulation in MCF-7 xenograft tumors in a dose-dependent manner. The highest dose induced accumulation 3.4- to 3.9-fold compared with non-stimulated tumors. Iodide accumulation in other organs was not significantly increased by the tRA treatment. Northern blot analysis indicated significant stimulation of NIS mRNA expression in tumors after the tRA treatment. Next, we tested transgenic mice with neu oncogene-mediated breast cancer. These animals were treated with tRA (0.8 μmol/day, i.p. injection) for 2 days. Iodide accumulation in some transgenic mouse tumors (2 of 4) was also increased by tRA treatment (1.8- to 2.8-fold) relative to non-stimulated tumors. These data demonstrate selective induction of NIS in some breast cancers by tRA. Treatment with short-term systemic retinoic acid followed by radioiodide administration might be useful for diagnosis or treatment of some differentiated breast cancer. The schedule and dose of retinoid treatment needs to be adjusted to optimize NIS expression and specific tumor uptake.

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Program Number 38 Cancer

Restoration of Na+/I- Symporter (hNIS) Gene Expression in Dedifferentiated Human Thyroid Carcinoma Cells Is Associated with Enhanced Histone Acetylation at Its Promoter

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Dedifferentiated thyroid carcinomas may lose iodide-concentrating ability rendering them unresponsive to radioiodine therapy. Restoration of this function may permit the successful treatment of these cancers. Using human papillary (NPA'87) and follicular (KAK-1) thyroid cancer cell lines as models, we previously demonstrated that sodium butyrate (NaB), a histone deacetylase inhibitor and 5-azacytidine (azaC), an inhibitor of cytosine DNA methylation, are capable of restoring hNIS mRNA expression as well as functional iodide transport. Herein, we find that separate and combined treatment of NaB and azaC, restores and enhances hNIS mRNA expression which is lacking under basal conditions with TSH. However, trichostatin A, a different inhibitor of histone deacetylase, was unable to induce this effect. NaB and azaC may bring about changes in the chromatin structure restoring transcriptional competence. Such changes, affecting expression of hNIS were evaluated by Chromatin ImmunoPrecipitation (ChIP) assays using antibodies specific for acetylated histones. PCR amplification of immunoprecipitated DNA revealed acetylated histone H4 to be increased at the hNIS promoter following NaB treatment in both the cell lines. The tendency for increased acetylation of histones associated with the hNIS promoter was also observed in phenylbutyrate and azaC treated cells. These findings further support the concept of epigenetic regulation, and loss of function in tumor dedifferentiation, suggesting histone acetylation status as a target for restoring hNIS expression.

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Program Number 39 Cancer

Use of Probasin Promoter ARR2PB to Express NIS Gene in Prostate Cancer Cell Lines

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Background & Purpose: Prostate cancer is a one of the most promising candidates for sodium iodide symporter mediated gene (NIS) therapy. Adenovirus-mediated expression of NIS that is driven by prostate specific promoters induces radio-iodide accumulation in prostate cancer cells that can be utilized for radioactive iodine therapy. We have recently developed a replication deficient adenovirus carrying the human NIS gene linked to prostate specific expression promoter, the probasin promoter (ARR2PB), which was modified to contain two androgen response elements (a gift of Dr. R. J. Matusik, Vanderbilt). Here we have examined the efficiency and specificity NIS gene expression with this promoter construct. Method & Material: ARR2PB-NIS and CMVp-NIS constructs were cloned into a shuttle vector containing the adenovirus backbone (Ad5k-NpA, ViraQuest Inc.). Transient transfection in androgen receptor positive (LNcaP) and negative (PC-3, DU-145) prostate cancer cell lines, and non-prostate origin tumor cell lines (HCT-116, MCF-7, and SUN445) was performed. After 24-48 hours incubation in 10% CS-FBS containing growth medium with or without 3.2 nM mibolerone, perchlorate sensitive I^{125} uptake was examined. Results: Androgen-dependent iodide uptake activity was observed in LNcaP cells transfected with Ad5k-NpA-ARR2PB-NIScDNA (with and without mibolerone; 1194.5±86.95 and 495.1±21.5 cpm). The construct containing CMV promoter showed no androgen dependency. The promoter activity of ARR2PB was equal to that of CMV promoter (1280.9±81.3 cpm). These findings were not seen in the other cell lines examined indicating that the activity is specific for prostate tissue. Conclusion: These results suggest that the probasin promoter can be utilized to achieve high magnitude and tissue specific expression of hNIS in prostate cancer cells. This promoter may be useful for construction of prostate specific adenoviral constructs for gene therapy of prostate cancer using radioiodine.

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Program Number 40 Cancer

The Altered mRNA Expression Levels of the Sodium Iodide Symporter Can Help in the Identification of Thyroid Tumors with Aggressive Behavior

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Sodium iodide symporter (NIS) mediates active iodide uptake and is fundamental in the evaluation, diagnosis, and treatment of thyroid disease. NIS expression forms the molecular basis of using radioiodine for a scintigraphic diagnosis of recurrence and/or metastasis and therapeutic agent for iodide uptake in the management of differentiated thyroid carcinomas. In order to investigate the correlation between the NIS mRNA expression levels and clinical outcome of the patients, total RNA was extracted from 3 follicular (FC) and 9 papillary (PC) carcinomas, 2 Graves' and 2 Hashimoto's thyroid tissues; 20 normal thyroid tissues were used as reference. The 12 thyroid carcinoma patients were submitted to a surgery complemented by radioiodine ablation and had blood samples collected after 6, 12, and 24 months of follow-up, under levothyroxine continued suppressive therapy. A fluorescent probe was designed for the NIS gene, and real-time RT-PCR was used to measure the NIS mRNA expression levels. We normalized the mRNA expression by using the GAPDH gene and the values are expressed as the number of cDNA copies of NIS divided by the number of cDNA copies of GAPDH. NIS mRNA expression, expressed as X+/-SD, was significantly decreased in all thyroid carcinomas (19.63+/-39.81 pg Eq) and increased in autoimune thyroid disease (181.76+/-92.20 pg Eq) compared to normal tissues (75.71+/-25.68 pg Eq) ($p<0.01$). PC presented levels 4-fold higher (22.68+/-43.5 pg Eq) than FC (5.42+/-3.36 pg Eq). According to their histology features and clinical outcome, we could characterize a group A (N=6 cases) of PC patients with non-aggressive carcinomas, whose NIS levels were more than 100-fold higher than a group B (N=6 cases) with aggressive carcinomas (121.58+/-32.18 versus 0.79+/-0.74, $p<0.0001$). We suggest that the quantification of NIS mRNA expression may predict poor outcome and be, therefore, useful to indicate more aggressive therapy in a subset of PC patients.

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Program Number 41 Autoimmunity

Graves' Dermopathy and Acropachy Are Markers of Severe Graves' Ophthalmopathy

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Background: It is generally considered that thyroid dermopathy and acropachy are almost always associated with thyroid associated ophthalmopathy (TAO). It is also believed that the two manifestations are indicators of severe autoimmune disease, hence more severe TAO. However, documentation of these clinical impression is needed. Methods: In the present study we analyzed the presence of optic neuropathy frequency of transantral orbital decompression in 40 patients with acropachy and dermopathy. We also studied 138 patients with Graves' dermopathy alone. We compared the data with 114 control patients from a cohort from Olmsted County Minnesota who had ophthalmopathy without dermopathy and acropachy. We used the frequency of transantral orbital decompression (TAOD) and presence of optic neuropathy (ON) as indicators of severity of ophthalmopathy. Results: Frequency of TAOD was significantly higher in the acropachy group compared to dermopathy alone, also cohort control group with TAO alone ($p<0.001$). Dermopathy alone group had more TAOD compared to TAO alone ($p<0.004$). Occurrence of ON was higher in the acropachy group compared to TAO alone ($p<0.08$). There were more ON patients in the dermopathy-alone group than TAO cohort group, but this did not achieve statistical significance. A similar trend was noted for ON in the acropachy group compared to the dermopathy group. There were also unusual cases. We encountered 5 patients without clinically obvious ophthalmopathy who had definite dermopathy of Graves' Disease. Conclusion: Dermopathy and acropachy are markers of more severe ophthalmopathy. However, in rare cases Graves' dermopathy can occur in the absence of clinically obvious ophthalmopathy.

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Program Number 42 Autoimmunity

Analysis of Age at Onset of Familial Graves' Disease Reveals Evidence for Gender Effects but No Evidence for Genetic Anticipation

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It is widely accepted that genetic predisposition is an important factor in the development of Graves' disease (GD). A strong genetic influence in GD may give rise to an earlier age of onset (AO) of disease. Our aims were to examine the effect of certain genetic parameters on the AO of GD. We collected a dataset of 70 multiplex GD families and 173 sporadic GD patients and examined the influence of the following parameters on AO: (1) familiality (being an offspring of a GD patient; i.e., genetic anticipation) and (2) gender. Twenty-eight parent-offspring pairs with GD were available for analysis of genetic anticipation. The average AO for parents was 38.8 years and for offspring, 23.8 ($p=0.0003$, applying the t-test). However, this difference may be a truncation effect; i.e., since parents are older than their children, the observed AO distribution in children will be lower compared to their parents. Therefore, we applied a correction developed by Huang & Vieland [Am J Hum Genet 62:1212, 1998] correcting for this truncation effect. The corrected estimates of mean AO were 46.1 years for parents, and 46.9 years for children (p-value = 0.54). One hundred seventy-seven female and 41 male GD patients were analyzed for gender effects on AO. There was a statistically significant difference between the AO of females and males (39.7 years and 44.4 years, respectively, $p=0.039$). Conclusions: (1) There was no evidence of genetic anticipation in our GD families; the observed difference in AO between parent and offspring resulted from truncation bias; (2) female GD patients had an earlier AO, possibly due to modulating factors such as estrogen.

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Program Number 43 Autoimmunity

Lack of Association of IDDM8 with Graves' Disease in the United Kingdom

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Both genetic and environmental factors contribute to the development of autoimmune disorders such as Graves' disease (GD), type 1 diabetes and rheumatoid arthritis (RA). These diseases not only share many histological and immunological features but also cluster within families indicating shared causal genetic factors. The role of shared genetic loci conferring susceptibility to an autoimmune process is supported by the overlapping of chromosomal regions of linkage and association with different autoimmune diseases in humans. The HLA and CTLA-4 gene regions have been consistently reported to be associated with a number of autoimmune diseases including GD and type 1 diabetes. Chromosome 6q27 has been linked to, and associated with type 1 diabetes (IDDM8) and was also recently reported to be linked to and associated with both RA and systemic lupus erythematosus (SLE). A number of genes including programmed cell death 2 (PDCD2), have been mapped to this chromosomal region making IDDM8 a candidate gene region for GD. We, therefore, performed a case-control association study on 502 subjects with GD and 513 controls using two IDDM8 polymorphic dinucleotide repeat microsatellite markers, D6S446 and M122K genotyped using fluorescence-based technology. There were no differences in allele frequencies for the 11 alleles of M122K, (size range 175-195 bp) or 7 alleles of D6S446, (size range 195-215 bp) between patients with GD and controls, chi-square = 1.89, p = 0.76 and chi-square = 3.12, p = 0.54 respectively. Specifically, there was no increase in the 205 bp allele of D6S446 (GD = 7.4%, controls 8.4%), previously reported to be associated with RA and SLE. These data suggest, therefore, that IDDM8 is not contributing to the observed clustering of GD with other autoimmune disorders and is not a susceptibility locus for GD in the UK.

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Program Number 44 Autoimmunity

Thyroid Involvement in HCV-related Mixed Cryoglobulinemic Patients

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Objective: Mixed cryoglobulinemia (MC) is a systemic vasculitis associated with hepatitis C virus (HCV) infection in over 90% of cases and frequently complicated by multiple organ involvement. We first investigated the prevalence and characteristics of thyroid disorders in an unselected series of HCV-associated MC patients (HCV+MC-p).
Design: A case-control study has been conducted, in the 1999-2001 period, matching HCV+MC-p with two different control groups. All HCV+MC-p were recruited in the Rheumatology Unit of the University of Pisa.
Patients: Ninety-three HCV+MC-p (17 males and 76 females, mean age 63.4±10.2SD years, mean disease duration 14.1±7.4SD years) were studied and each one was randomly matched by sex and age (±2 years), with: 1) one subject of the general population from the same area; 2) one patient with type C chronic hepatitis (CH) without CM.The prevalences of hypo- or hyperthyroidism, thyroid autoantibodies, thyroid nodules and cancer were evaluated.
Results: The prevalence of the following thyroid abnormalities was significantly higher in HCV+MC-p compared to the other groups: serum antithyroperoxidase autoantibody (AbTPO) (28.2% vs 8.8% and 14.1%, respectively; $p<0.0022$); the presence of AbTPO and/or antithyroglobulin autoantibody (30.6% vs 12.2% and 18.7%, respectively, $p<0.0098$); subclinical hypothyroidism (10.8% vs 2.2% and 7.5%, respectively; $p<0.042$).Thyroid papillary cancer was observed in 2 HCV+MC-p and in none of the controls. Thirty-three out of 93 MC patients had been treated with alfa-IFN (MC/IFN+) since 6 to 84 months (mean 43) before the study. In MC/IFN+ patients the prevalence of thyroid autoimmune phenomena and preclinical hypothyroidism was comparable to that found in untreated patients.
Conclusion: The present study firstly demonstrated a significantly high prevalence of different thyroid disorders in HCV-related MC. Because of the clinical relevance of this complications, in particular of thyroid cancer, a careful thyroid surveillance is advisable in these patients.

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Program Number 45 Autoimmunity

Thyroid and Pancreatic Beta Cells' Function and Autoimmunity in Children with Vitiligo

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The frequent association of vitiligo (V) with autoimmune thyroiditis (AT) and diabetes mellitus presents a special interest. The aim of this study is to examine the function and autoimmunity of the thyroid and pancreatic beta cells of children with V. Sixty one children with V, 35 girls and 26 boys, aged 1.2-16.2 yrs have been studied. Goiter was found in 31 children (53.4%), thyroid dysfunction (T4,TSH) in 8 children with V (13.8%), hyperthyroidism in 3 and subclinical hypothyroidism in 5; hypothyroid answer(TRH test) in 6 (27.3%). Antithyroglobulin (ATA) and antimicrosomal antibodies (AMA abs) were with elevated titer in 29 children (50%), TBII in 5 (15.6%). Ultrasound examination showed picture typical or suspected for AT in 15 children with V (34.1%) and typical for Graves' disease (GD) in 2 (4.5%). Autoimmune thyroid disease has been diagnosed in 32 children with V (52.5%-AT:GD=29:3). Elevated insulin secretion during OGTT (60 and 120 min., $p<0.01$ and 0.05) was found in children with V and normal blood sugar curves (n=24) and in V and AT (60 and 120 min., $p<0.05$), (n=15). There was no correlation between changes of IRI and thyroid function. GAD 65 abs were with elevated titer in 6 children (18.8%) and ICA 512 abs in 2 (7.4%). All except one were with normal blood sugar and insulin secretion. Our study showed increased thyroid dysfunction and autoimmunity as well as increased stimulated insulin secretion and islet cells autoimmunity in children with vitiligo. Thyroid and beta cells function and autoimmunity should be checked in children with V at diagnosis and also at least twice a year thereafter.

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Program Number 46 Autoimmunity

Can Early Postpartum Thyroglobulin and Thyroid Ultrasound Echogenicity Enhance the Predictive Value of Thyroid Peroxidase Antibody Measurements in Early Pregnancy?

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Thyroid peroxidase antibodies (TPOAb) in early pregnancy are important in the prediction of postpartum thyroid dysfunction (PPTD). However, their usefulness is limited as only about 50% of TPOAb positive women develop PPTD. To increase the predictive value of TPOAb, we have measured serum thyroglobulin (Tg) and thyroid ultrasound echogenicity in the early postpartum period, in a group of subjects from a cohort of 258 TPOAb +ve [121 with PPTD (Group A) and 137 without PPTD (Group B)] and 271 TPOAb -ve subjects (none developed PPTD). Median TPOAb in early pregnancy was higher in Group A [$p<0.001$] compared to Group B. The relative risk of developing PPTD was 2.5 when TPOAb was moderately elevated (>58.2 kIU/l) and sensitivity nearly 100 %. Mean postpartum integrated TPOAb levels (measured during the first 26 weeks postpartum) was also higher in Group A compared to Group B [$p<0.001$]. We measured early postpartum (mean 6.8 ±1.3 weeks) Tg and ultrasound thyroid echogenicity in a small subset of TPOAb +ve subjects (28 with PPTD [Group 1] and 98 without PPTD [Group 2]). Although Tg was elevated in 12/28 (43%) Group 1 vs. 27/98 (28%) Group 2 subjects [$p<0.05$], there was no significant difference in Tg concentration between them. Ultrasound thyroid echogenicity was abnormal in 13/28 (46 %) Group 1 vs. 24/74 (32 %) Group 2 subjects [$p< 0.05$] with no significant difference in thyroid volume and no correlation with Tg concentration. We conclude that TPOAb in early pregnancy is a sensitive predictor of PPTD in a population where TPOAb –ve subjects do not develop PPTD. Early postpartum serum Tg and thyroid echogenicity may be useful in improving the prediction of PPTD in those women where these test results are abnormal.

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Program Number 47 Autoimmunity

Correlation of Anti-inflammatory Therapy in Graves' Ophthalmopathy and Autoantibodies to Thyroidal Antigens

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Objective: Autoimmune thyroid disease is characterized by the presence of autoantibodies to the TSH receptor (measured here as TBII), thyroid peroxidase (TPOAb), and thyroglobulin (TgAb). Several independent reports indicated also a pathophysiological relevance of these antigens for Graves' ophthalmopathy (GO). We evaluated the association between autoantibody levels and the clinical outcome of anti inflammatory GO therapy. Patients and Methods: TBII, TPOAb and TgAb were measured with commercial kits (LUMItest, BRAHMS AG) in 239 patients with GO after steroid therapy and, if indicated, orbital radiation. The antibody levels of all patients were correlated with the Clinical activity score (CAS) and the severity of GO (modified NOSPECS score). Results: After therapy TBII titres were positive in 91% in the non responsive group (CAS>6) compared to 44% in post therapeutic inactive GO (CAS<2, $p<0.001$). For TPOAb or TgAb the difference was not significant. TBII but not TPOAb or TgAb medians increased with CAS score. The CAS was positively correlated to TBII ($r=0.39$, $p<0.001$), and negatively to TgAb ($r=-0.20$, $p<0.01$) but not to TPOAb. A similar result was seen between TBII levels and the NOSPECS score, which were positive in 78% of the patients with NOSPECS >6 compared to 22% with NOSPECS 1-2 ($p<0.001$, n.s. for TPOAb/TgAb). Only the TBII median increased significantly with the NOSPECS score ($p<0.001$), the TPOAb and TgAb medians decreased. There was a positive correlation between TBII and GO severity ($r = 0.41$, $p<0.001$), but a negative correlation between TPOAb and GO severity ($r=-0.23$, $p<0.001$) and TgAb and GO severity ($r = -0.29$, $p<0.001$). Conclusion: We find a positive association between TBII and the activity and severity of GO after anti-inflammatory therapy. The severity of GO was also associated with the absence of TPOAb or TgAb. Thyroid autoantibodies, especially TBII, may be helpful in the disease management of GO.

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Program Number 48 Autoimmunity

Two Models for Variations in the Age- and Sex-related Distribution of Anti-thyroid Peroxidase (ATA) and Anti-thyroglobulin (ATG) Antibodies

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Community-based studies on the prevalence of circulating ATA and ATG have typically demonstrated shifts to higher levels with age, especially in women. It remains an open question, however, whether normal reference limits for ATA and ATG should be treated as age- and/or sex-dependent. Draft guidelines from the National Academy of Clinical Biochemistry (www.NACB.org/Thyroid_LMPG.stm) recommend restricting the reference group to men under 30 years with TSH between 0.5 and 2.0 mIU/L. This suggests an alternative model: an age- and sex-dependent frequency of abnormally elevated antibody levels superimposed on a single underlying distribution of normal reference values. If, in addition, abnormal values tend to be highly elevated (negligible overlap with the common normal range), then truncation based on centiles determined for the NACB reference group should produce similar distributions, independent of sex and age, even for older women. We tested this using cross-sectional data from Busselton (Western Australia), wherein 2108 samples from the 1981 cohort were analyzed for TSH, free T4, ATA, and ATG on the IMMULITE 2000 (Diagnostic Products Corp., Los Angeles, CA). The 123 men meeting the NACB selection criteria for age and TSH yielded central 90% limits of 5.6 to 23.2 and 3.3 to 20.8 kU/L for ATA and ATG, respectively, after eliminating two men with outlying values (ATA 93, ATG 55). Of 1958 apparently euthyroid subjects, 1302 had both ATA and ATG results within these limits. Broken down into 6 age brackets, and also by sex, with 80 to 134 subjects per group, the ATA and ATG results were analyzed as if they represented the central 90% of 12 separate reference groups. Consistent with the second model, representative inner centiles (25th, 50th, and 75th) showed no clear trend as a function of age for either sex.
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Program Number 49 Autoimmunity

Association of Antibodies to Double-stranded DNA and to Single-stranded DNA in the Serum of Patients with Hashimoto's Thyroiditis and Graves' Disease

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The importance and prevalence of antibodies to double-stranded DNA (anti-dsDNA) and to single-stranded DNA (anti-ssDNA) in patients with Hashimoto´s thyroiditis (HT) and Graves` disease (GD) is not well established.We report the detection of antinuclear antibody (ANA), rheumatoid factor (RF), antibody to smooth muscle (anti-SM), anti-dsDNA and anti-ssDNA in sera from 12 euthyroid HT and 39 untreated hyperthyroid GD patients. Serum concentrations of anti-SM, anti-dsDNA and anti-ssDNA were measured using specific and sensitive ELISA. The coefficient of variations was less than 8%. The euthyroid sera of 25 health subjects without prior autoimmune diseases were the normal control. The mean + SD of anti-dsDNA and anti-ssDNA were 32.8+15 IU/mL (mean+2SD was 2.8-62.8 IU/mL) and 53+4.0 IU/mL (mean+2SD: 41.2-61.2 IU/mL), respectively. In HT patients the mean anti-dsDNA was 69.9+40 IU/mL ($p<0.01$ compared with controls) and anti-ssDNA was 75.2+17.6 IU/mL ($p<0.001$). In GD patients the mean anti-dsDNA was 136.7+86.9 IU/mL ($p<0.001$) and anti-ssDNA was 70.3+7.2 IU/mL ($p<0.001$). Both anti-dsDNA and anti-ssDNA had the same prevalence in HT and GD - 75% (9/12) for anti-dsDNA and 94.8% (37/39) for anti-ssDNA ($p=0.0001$ by Fischer's exact test). Futhermore, in the control subjects ANA, anti-SM and RF were negative but we find these antibodies in three, two and two patients with HT and GD, respectively. Using Spearman's correlation test serum anti-dsDNA values associated ($rs = 0.49$-0.59; $p=0.001$) with serum T4, T3, TPOAb, and TSH but not with TgAb. The serum anti-ssDNA values correlated ($rs=0.51$-0.66, $p=0$ .0001) with serum T3, TSH and TPOAb but not with T4 or TgAb. However, stepwise multiple regression analysis showed that anti-dsDNA had a close association ($p=0.0001$) with TPOAb and anti-ssDNA with both TgAb and TPOAb. In conclusion, our results suggest that association of various types of autoantibodies occurs in HT and GD, so the immunological spectrum of these diseases may be wider.

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Program Number 50 Autoimmunity

Thyroid Autoimmunity Induced by Radioiodine (RAI) Treatment of Patients with Multinodular Goiter

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Treatment of patients with multinodular goiter with radioiodine, as an alternative therapeutic option to surgery, is becoming more widely employed mostly in elderly patients with cardiac diseases. About one third of those patients may have subclinical or overt hyperthyroidism due to exposure to a relatively high iodine intake (iodized salt or iodine containing medication). In this study we have analyzed the course of thyroid autoantibodies (anti-TPO, anti-Tg and TRAb) before and after the RAI therapeutic dosis (for 12 months). The patients were divided in two groups: Group I patients (n=14) received only RAI and group II patients (n=17) were pretreated with hrTSH (Thyrogen, 0.45 mg 24 hours before 131I dosis). In both groups the presence of anti-TPO (2/31), anti-Tg (3/31), and TRAb (1/31) was similar before RAI. After the ablation we noticed that the surge of autoantibodies was significantly higher in the hrTSH-treated group II (8/17, 47%) as compared with group I (2/14, 14,2%). The presence of anti-TSH receptor Ab (TRAb) was more commonly found in group II patients (7/17, %). Overall there was a significant correlation of TRAb-positive and the concentrations of serum Tg levels ($r=0.43$, $p<0.01$). We conclude that the surge of thyroid autoimmunity is frequently observed in patients treated with RAI, possibly due to a higher serum concentration of thyroid antigens released from the thyroid gland exposed to radiation.

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Program Number 51 Autoimmunity

Diagnostic Value of Thyroid Antibodies in Different Thyroid Diseases

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Objective: To determinate diagnostic value of different thyroid autoantibodies in patients with autoimmune thyroid diseases (AITD). Subjects: 339 patients were included. Graves' disease (GD), chronic Hashimoto's thyroiditis (CHT), subacute thyroiditis (ST), miscellaneous thyroid disease and control patients (CP). Intervention: Anti-thyroglobulin antibody (ATG), anti-thyroid peroxidase antibody (ATPO), thyrotropin binding inhibiting immunoglobulin (TBII), thyroid stimulating immunoglobulin (TSI), thyroid growth stimulating immunoglobulin (TGSI) and thyrotropin blocking antibody (TBAB). Antibodies were measured by Quest Labs, San Juan Capistrano, Cal. Methods: We determined diagnostic value of each antibody, patients were diagnosed by history, physical examination and hormonal profile. Results: 339 patients were included: 141 with GD, 100 with CHT, 55 with TS, 20 with miscellaneous diseases and 23 as CP. The most frequent antibodies in all diseases were ATPO and ATG. In GD TSI and TGSI had specificities of 92% and 93% respectively. 78% of patients with positive TSI and TGSI had GD and 85% of patients with positive TSI and TBII had GD as well. In CHT patients, ATPO and ATG had the most sensitivity (62%) and positive predictive value (79 %). In ST sensitivity and specificity was low in all antibodies. In patients with 3 or more positive antibodies, probability of GD was higher. All antibodies had low sensitivity but high specificity in different diseases. Conclusion: TSI and TGSI have high specificity for GD; ATPO and ATG have high sensitivity for CHT. Number of positive antibodies is an important factor for GD diagnosis. All antibodies have low sensitivity but high specificity in different diseases.

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Program Number 52 Autoimmunity

Mapping of the Immunodominant Region of Thyroid Peroxidase

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Thyroid peroxidase (TPO), previously called the "thyroid microsomal antigen", is a membrane-bound enzyme expressed at the apical pole of thyrocytes. TPO generates the functional form of thyroglobulin by iodination and coupling of tyrosine residues. During autoimmune thyroid diseases (AITD), TPO is one of the major targets for the immune system. Indeed, high titer autoantibodies (aAbs) directed against TPO are detected in the sera from most patients suffering from Hashimoto's thyroiditis or Graves' disease. Nowadays, there is no doubt as to the existence of a highly conformational immunodominant region (IDR) on human TPO; but because of the difficulty in mapping discontinuous epitopes, this region on the TPO molecule has not yet been precisely located. However, this information is crucial to improve our understanding of TPO recognition by the immune system, including antigen presentation by TPO-specific cells. We used a recombinant human monoclonal anti-TPO aAb (aAb T13) directed against the IDR to select peptides (mimotopes) which mimic the epitope of aAb T13 by phage-displayed peptide technology. Using multiple peptide synthesis, we identified by alanine scanning critical motifs for the interaction between the mimotopes and aAb T13. Then, we localized by sequence alignments of these critical motifs and the human TPO primary sequence four discontinous regions defining the conformational immunodominant binding surface on native TPO and comprising amino acids in both the myeloperoxidase- and the complement control protein-like domains. Finally, we precisely demonstrated by guided mutagenesis studies that these regions localize within the IDR and are recognized by the majority of TPO aAbs from patients with AITD.

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Program Number 53 Autoimmunity

Dendritic Cells Infected with Adenovirus Expressing the TSH Receptor Induce Graves' Hyperthyroidism in BALB/c Mice

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Dendritic cells (DCs) are the most potent antigen-presenting cells and a prerequisite for the initiation of primary immune response. This study was performed to investigate the contribution of DCs to the initiation of Graves' hyperthyroidism, an organ-specific autoimmune disease in which the TSH receptor (TSHR) is the major autoantigen. DCs were prepared from bone marrow precursor cells of BALB/c mice by culturing with granulocyte macrophage-colony stimulating factor and interleukin-4. Subcutaneous injections of DCs infected with recombinant adenovirus expressing the TSHR (but not b-galactosidase) in syngeneic female mice induced Graves'-like hyperthyroidism (8% and 35% of mice after 2 and 3 injections, respectively) characterized by stimulating TSHR antibodies, elevated serum thyroxine levels, and diffuse hyperplasitc goiter. TSHR antibodies determined by ELISA were of both IgG1 (Th2-type) and IgG2a (Th1-type) subclasses. Surprisingly, induction of antibodies and disease was completely suppressed by co-administration of alum/pertussis toxin, a Th2-dominant adjuvant, whereas with polyriboinosinic polyribocytidylic acid, a Th1-inducer, the incidence of disease was ~50%. These observations demonstrate that DCs efficiently present the TSHR to naive T cells to induce TSHR antibodies and Graves'-like hyperthyroidism in mice. In addition, our results challenge the previous concept of Th2 dominance in Graves' hyperthyroidism and provide support for the role of Th1 immune response in disease pathogenesis.

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Program Number 54 Autoimmunity

Fas Signaling in Human Thyroid Epithelial Cells

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Fas-mediated apoptosis has been proposed to play an important role in the pathogenesis of Hashimoto's thyroiditis. Normal thyroid cells are resistant to Fas-mediated apoptosis in vitro but can be sensitized by the combination of IFNg and IL-1b. We sought to examine the mechanism of this sensitization and apoptosis signaling in primary human thyroid cells. IFNg/IL-1b treatment resulted in the up-regulation of Fas mRNA expression and increased the Fas protein cell surface expression. The concentration of procaspase 7, 8 and 10 were enhanced by the presence of the cytokines. A dramatic increase in Bid mRNA and protein levels was observed in response to IFNg/IL-1b. Other Bcl-2 family members, Bcl-X and Bak were also up regulated while Bcl-2 and Bax were unchanged. In the course of Fas signaling, the proximal caspases, including caspase-8 and caspase-10, were cleaved without cytokine pretreatment but this did not lead to the activation of caspase-7 and caspase-3. The cleavage of Bid and activation of mitochondria and effector caspases in response to anti-Fas antibody occurred only after cytokine pretreatment and resulted in the commitment to apoptosis. p42/44 MAPK (Erk) was activated through the Fas signaling and the MEK inhibitor (U0126) sensitized the thyroid cells to the anti-Fas antibody. This suggests that the activation of p42/44 MAPK provides a protective signal. In conclusion, the IFNg/IL-1b pretreatment sensitizes human thyroid cells to Fas-mediated apoptosis in a complex manner involving the expression of Fas receptor, caspases and Bcl-2 family members. In the course of Fas-mediated apoptosis, Bid and caspases 8, 10, 7 and 3 were cleaved and mitochondria were activated. Clarifying the regulation of apoptosis signaling provides an opportunity to influence disease progression in autoimmune thyroiditis.

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Program Number 55 Autoimmunity

Thyroglobulin-Thyroperoxidase (TGPO) Autoantibodies Are Polyreactive, Not Bi-specific: Analysis Using Human Monoclonal Autoantibodies

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Autoantibodies to thyroglobulin (TgAb) and thyroid peroxidase (TPOAb) are reported to share common epitopes and it has been suggested that bispecific autoantibodies, "TGPOAb", distinguish between different clinical presentations of thyroid autoimmunity. Because human monoclonal autoantibodies are powerful tools for investigating disease-associated epitopes, we attempted to clone TGPOAb. A Graves' patient with high TgAb and TPOAb titers also had TGPOAb, namely ^{125}I-TPO binding by autoantibodies captured on Tg-coated ELISA wells (1773 ± 52 cpm vs 34 ± 4 cpm with normal serum). Availability of thyroid tissue from this patient allowed us to construct an immunoglobulin gene combinatorial library in the phage display vector pComb3H. We screened this library by alternating panning on Tg and TPO. Intriguingly, after 4 rounds of panning, phage binding increased to both Tg and TPO (ELISA OD 0.07 before panning, versus 0.71 and 0.79 on Tg- and TPO-coated wells, respectively). Of 526 clones tested for expressed autoantibodies, most were negative; 3 clones recognized Tg (but not TPO) and 5 clones recognized TPO (but not Tg). Moreover, the H and L chains of these Tg- or TPO-specific clones were the same as those obtained by panning on Tg or TPO alone. A single clone with a unique non-Tg, non-TPO H chain produced antibody that bound equally well to Tg and TPO (TGPO activity). However, this antibody was non-specific, being polyreactive with numerous non-thyroid antigens. In conclusion, alternate panning on Tg and TPO did lead to phage enriched for Tg- and TPO-binding. However, this enrichment was attributable to a mixture of phage specific for either Tg or TPO. The only "TGPOAb" obtained was polyreactive and non-specific. These findings do not support previous observations of "TGPOAb" prepared by affinity chromatography from patients' sera. Overall, our data provide powerful evidence against shared, cross-reactive epitopes on two major thyroid autoantigens.

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Program Number 56 Autoimmunity

Persistent Suppression of IL-4 Prevents Development of Experimental Autoimmune Graves' Disease

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In autoimmune Graves' disease, autoantibodies bind to the thyrotropin receptor (TSHR) and cause hyperthyroidism. We studied the effects of Flt3-L or GM-CSF treatment on the development of experimental autoimmune GD (EAGD) induced by immunization with TSHR; a slowly progressing antibody-mediated organ-specific autoimmune disease in mice. Flt3-L and GM-CSF treatment resulted in up-regulation of CD8a+ and CD8a- DCs, and skewing of early cytokine and immune responses to TSHR in favor of Th1 and Th2 respectively. However, this initial skewing neither persisted nor influenced the course or severity of the disease. To determine the effects of persistent absence of IL-4 and IFN-gamma on the development of EAGD, we immunized wild type, IFN-gamma $^{-/-}$ and IL-4 $^{-/-}$ Balb/C mice with TSHR. Nearly 100% of the wild type and IFN-gamma $^{-/-}$ mice developed EAGD, while IL-4 $^{-/-}$ mice completely resisted disease development. These data showed that early skewing of immune responses to TSHR induced by cytokine modulators, in favor of either Th1 or Th2, did not persist and thus failed to alter the outcome of the disease, while a persistent deficiency of IL-4, as in IL-4 $^{-/-}$ mice, prevented development of EAGD. These data showed that prolonged suppression of IL-4 may have therapeutic value for GD.

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Program Number 57 Autoimmunity

Alpha-Fodrin as Candidate Autoantigen in Graves' Ophthalmopathy

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Alpha-fodrin, an intracellular organspecific cytoskeleton protein is a recently identified autoantigen associated with both Sicca- and Sjoegren's syndromes (SS). Keratoconjunctivitis, dry eyes and mouth due to lymphocyte infiltration of the lacrimal and salivary glands characterize these autoimmune disorders. Sicca syndrome frequently affects patients with Graves' ophthalmopathy (GO). We therefore have cloned and expressed the human recombinant 120-kDa fodrin-fragment and subsequently developed an ELISA employing human alpha-fodrin as antigen. Sera from 144 patients with GO and 1200 blood donors were screened for the presence of anti-alpha-fodrin IgA and IgG antibodies. In contrast to blood donors (< 1% IgA only), alpha-fodrin autoantibodies were detected in 22% of patients with GO ($n = 32$, $p < 0.01$). IgA and IgG antibodies were present in 21 (15 %) and 14 (10%) GO subjects, respectively. In comparison, antinuclear antibodies against the ribonucleoprotein (SS-A) and the La antigen (SS-B) were less prevalent (21 of 144 or 15%). Six GO patients (4%) with specific clinical signs of SS showed both SS- as well as high titers of alpha-fodrin-autoantibodies. These six subjects complained of abnormal lacrimal excretion ability. A total of 45 patients with GO (31%) had at least one alpha-fodrin or SS autoantibody. Since previous studies have shown that various IgA antibodies are produced in the lacrimal gland of patients with SS, production of IgA alpha-fodrin autoantibodies may indicate an inflammation of the lacrimal glands in GO patients more specifically. Furthermore, the fodrin alpha subunit, which seems critical for the initiation of exocrinopathy, is cleaved in association with apoptosis and has binding sites for autoreactive T cells. Apoptosis-mediated alteration of alpha-fodrin structure may lead to antibody production against glandular and orbital target cells. Thus, this autoantigen could play a role in the immunopathogenesis of GO and its associated Sicca syndrome.

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Program Number 58 Autoimmunity

Evidence to Support a Role for CD4+ Helper T-cells in Active Thyroid-associated Orbitopathy

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Background: The pathogenesis of thyroid-associated orbitopathy (TAO) remains to be defined but it is thought to be a T-cell mediated autoimmune disorder. Some previous studies, but not all, have found elevated circulating CD4+ helper T-cells in TAO patients. These contradictory findings reflect the differences in TAO activity in the patients studied. Therefore, we have analysed the expression of the phenotype of T-cell subtypes in the peripheral blood from patients with active TAO. **Methods:** We studied 26 patients (23 females, 3 males) with active TAO (Clinical Activity Score 3 or higher, NOSPECS class 2a-4c), and 19 healthy controls (17 females, 2 males) matched for age, sex and smoking status. Flow cytometry was performed on peripheral blood samples to characterise different T-cell subtypes including CD4, CD8, CD45RO, CD45RO on CD4, CD45RA, CD45RA on CD4, CD69, CD69 on CD4, CD29, CD29 on CD4, CD16, CD3, CD11, CD11 on CD3. The relative percentages of T-cell subtypes in TAO patients and controls were compared by student t-test. **Results:** CD4+ helper T-cells in TAO patients were significantly increased compared to controls (49.1% +/- 1.6 vs 34.6% +/- 2.0, $p<0.0001$). The CD4+/CD8+ ratio was higher in the TAO patients than in controls (3.0 +/- 0.3 vs 1.8 +/- 0.3, $p=0.01$). The naïve cells CD45RA+ ($p=0.02$) and CD45RA+CD4+ ($p=0.004$) were significantly increased while the memory cells CD45RO+ ($p<0.0001$), CD45RO+CD4+ ($p=0.02$), CD29+ ($p<0.0001$) and CD29+CD4+ ($p<0.0001$) were significantly decreased in TAO patients. The CD3+ ($p=0.02$) and CD11+ ($p<0.001$) cells were also significantly decreased in TAO patients. **Conclusions:** Our study confirms that, in patients with active TAO, there is a relative increase in circulating CD4+ helper T-cells. This study also shows an increase in naïve and decrease in memory helper T-cells in these patients. Together, these results support a role for CD4+ helper T-cells in the pathogenesis of TAO.

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Program Number 59 Autoimmunity

The Effects of Alpha Interferon on the Development of Autoimmune Thyroiditis in the NOD H2h4 Mouse

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INTRODUCTION: Alpha interferon (αIFN) therapy is known to induce thyroid autoimmunity in up to 40% of patients. The mechanisms are presumed to be Th1-mediated, since αIFN is a known potent inducer of Th1 switching. However, other non-Th1 mechanisms have also been proposed. AIM: The aim of our study was to examine whether the pathogenesis of aIFN induced thyroiditis (AIIT) involves αIFN-mediated Th1 switching in genetically susceptible individuals, or whether non-Th1 mediated mechanisms are also contributing. We took advantage of NOD-H2h4, a genetically susceptible mouse model, which develops thyroiditis when fed with a high iodine diet. Unlike in humans, αIFN does not induce Th1 switching in mice since the mouse αIFN receptor does not activate STAT4. Therefore, if αIFN causes thyroiditis by inducing Th1 differentiation of T-cells, αIFN should not accelerate the thyroiditis in NOD-H2h4 mice. However, if other mechanisms are operating, αIFN could accelerate the course of thyroiditis in these mice. METHODS AND RESULTS: 6- to 8-week-old male NOD-H2h4 mice were injected with mouse αIFN (200U x 3/week) or saline, for 8 weeks. All mice drank iodinated water. After 8 weeks mouse thyroids were examined for histology, and T4 and thyroglobulin antibody levels were measured. In the IFN-injected group, 6/13 mice (46.2%) developed thyroiditis and/or thyroglobulin antibodies while in the controls, 4/13 (30.8%) developed thyroiditis ($p=0.4$). The grade of thyroiditis was similar in the two groups. CONCLUSIONS: Our results showed that αIFN treatment did not accelerate thyroiditis in this mouse model. The lack of acceleration of spontaneous thyroiditis in genetically susceptible mice by αIFN may be due to the fact that αIFN does not induce Th1 differentiation in mice. Therefore, it seems that induction of Th1 switching is central to the initiation of αIFN-induced thyroiditis.

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Program Number 60 Autoimmunity

No Evidence for CD40 as the Susceptibility Gene for Graves' Disease on Chromosome 20q11 (GD2)

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Graves' disease (GD) is a complex multigenic disorder, with several chromosomal regions of linkage having been identified in recent years. One such region of linkage, on chromosome 20q11, has significant evidence for a GD locus (termed GD2), but the disease susceptibility gene has not been identified. In our UK family cohort evidence to favour linkage at 20q11 was found particularly in families with dominant transmission of GD. One candidate gene within this large interval for linkage on chromosomal 20q11 is CD40 (gene symbol *TNFRSF5*). CD40 is expressed on the surface of B lymphocytes and has a key role in B cell activation and differentiation following their interaction with helper T cells. We have examined two single nucleotide polymorphisms (SNPs) within the CD40 gene for allelic association in a cohort of 308 unrelated Caucasian subjects with GD and an ethnically matched local control group (n=307). The SNPs within the CD40 promoter (C to T, position -1), and within intron 4 (C to T, position +4434), were PCR genotyped from genomic DNA followed by restriction enzyme digestion with *NcoI* and *TfiI*, respectively. Statistical analysis was carried out by Chi^2 testing. The T allele at the CD40 promoter SNP was found in 145 of the 616 alleles from GD probands (23.5%), compared to 149 of 614 control alleles (24.3%); p=0.76, NS. The C allele of the intron 4 SNP was found in 15 of 616 GD alleles (2.4%) compared to 23 of 614 (3.7%) control alleles; p=0.18, NS. Subgroup analysis of probands with dominant transmission of GD, or those from families with evidence for linkage at 20q11, showed no significant differences in allele frequency at either SNP. We can find no evidence to support a role for CD40 as the GD2 susceptibility gene in our Graves' disease population.

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Program Number 61 Autoimmunity

TSH Receptor Gene Mutation and Pathogenesis of Autoimmune Thyroid Disease

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Objective: To investigate the association between the mutation of thyroid stimulating hormone (TSH) receptor gene and the pathogenesis of autoimmune thyroid disease. Methods: The whole extracellular domain of the TSH receptor gene from thyroid tissues of 50 patients with Graves' disease(GD) was analyzed by using reverse transcription-polymerase chain reaction-single strand conformation polymorphism (RT-PCR-SSCP) and twenty-one normal thyroid tissues were analyzed as control. The exon 1 of TSH receptor gene in white blood cells from 146 patients with Graves dsease and 30 patients with Hashimoto thyroiditis was also analysed with PCR-SSCP. Results: It was showed from the analysis of thyroid tissues that there was abnormal mobility shift of SSCP in 11 GD patients and in 2 controls, and the abnormal mobility shift was same in GD patients and in control, the nucleotide sequence analysis showed the nucleotide position 660 (the third position of codon 187) has the T/C polymorphism. The codon is AAT or AAC separately, and both code Asn. No abnormal migration was found in the analysis of exon 1 of TSH receptor gene in white blood cells for both groups of Graves' disease and Hashimoto thyroiditis. Conclusion: It is suggested from our study that the gene mutation of the TSH receptor is possibly not associated with the occurrence of autoimmune thyroid disease.

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Program Number 62 Thyroid Diseases

Four Years' Experience with the First Prospective Incidence Study of Overt Thyroid Dysfunction: The Danish Register of Hyper- and Hypothyroidism

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In Denmark the natural dietary iodine intake has been below recommended levels for many years. Epidemiological studies demonstrated that the major consequences were high occurrences of goitre and of hyperthyroidism due to multifocal thyroid autonomy in middle-aged and elderly subjects. To prevent goitre and hyperthyroidism a national program of iodine supplementation was initiated in 1998 by voluntary use of iodized salt. As the market share was low compulsory use of iodized household salt and salt in bread (13 ppm iodine with expected increase in iodine intake of 50 microg/day) was introduced in 2000. A system of monitoring (DanThyr) was developed including a register of new cases of hyper- and hypothyroidism in the populations of two areas with different iodine intake levels due to differences in water iodine content (Aalborg (AAL), median urinary iodine excretion 45 microg/l, population cohort investigated n = 310,124; and Copenhagen (CPH), 61 microg/l, n = 225, 734). Data has now been accumulated for the year before iodine supplementation (1), the 2 years with voluntary iodization (2 and 3) and the first year after compulsory iodization (4). Incidence rates per 100,000/year were: Hyperthyroidism AAL: 119.7, 129.9, 149.5, 166.6; CPH: 82,2, 81.3, 84.3, 99.8. Hypothyroidism AAL: 29.0, 35.0, 31.4, 44.3; CPH: 50.3, 51.0, 52.0, 56.8. Conclusion: The register gave consistent results over 4 years. Hyperthyroidism rates were higher in AAL with the lowest iodine intake, and hypothyroidism rates higher in CPH with higher iodine intake ($p < 0.01$), demonstrating that even small long-term differences in iodine intake level are associated with considerable differences in risk of overt thyroid dysfunction. Although trends were observed, the cautious iodine supplementation program did, so far, not induce statistically significant short term shifts in incidence rates in any of the areas.

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Program Number 63 Thyroid Diseases

Heart Rate Variability in Mild Exogenous Thyrotoxicosis during Thyrotropin Suppressive Therapy

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Heart rate variability (HRV), a sensitive marker of cardiac sympathetic activity, has been shown to be a predictive factor of heart disease. Our goal was to determine whether thyroxine suppressive therapy affects HRV. Nineteen patients being treated with suppressive doses of thyroxine for epithelial thyroid cancer and nineteen age-matched controls were enrolled. Subjects with known heart disease or those taking medication affecting heart rate were excluded. Circulating TSH, and 1-minute HRV were measured in all subjects and results compared between the groups. The 1-minute HRV was defined as the difference in beats/minute (BPM) between the shortest and the longest heart rate interval, measured by ECG recording during forced inspiratory and expiratory cycles. Free thyroxine and free triiodothyronine were measured in thyroxine treated subjects. The mean TSH was significantly lower in the study group (0.09±0.05 mIU/L (mean ± SD)) than in the control group (1.75±0.9 mIU/L; $p<0.005$). TSH did not vary by age among study subjects, but was significantly higher among healthy subjects older than 40 years (2.1±1.01 mIU/L) than among the younger subjects (1.38±0.49 mIU/L; $p<0.05$). The HRV was significantly lower among thyroxine treated patients compared to healthy controls (25.6±10.2 BPM vs. 34.3±12.4; $p<0.05$). This difference was attributed solely to subjects older than 40 years (n=11) (20.7±8.7 BPM vs. 32.8±15.4; $p<0.05$). No difference was detected between the groups of younger subjects (n=8) (32.4±8.2 BPM vs. 36.5±5.7; $p=0.2$). No difference was found between either minimal or maximal heart rates of thyroxine treated patients and healthy controls. Thyroxine therapy administered in clinically relevant doses for epithelial thyroid cancer significantly affects HRV, especially in older patients. The 1-minute HRV test may be used bedside to determine HRV in thyrotoxic individuals.

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Program Number 64 Thyroid Diseases

Optic Neuropathy of Graves' Disease: Results of Transantral Orbital Decompression and Long-term Follow-up of 215 Patients

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Background: Optic neuropathy occurs in 5% of patients with Graves' ophthalmopathy. We reviewed the demographic characteristics, clinical features, and long-term outcomes of patients with Graves' optic neuropathy following transantral orbital decompression at a tertiary care academic medical center. **Design:** Retrospective analysis of noncomparative interventional case series and long-term follow-up by questionnaire. **Main outcome measures:** Demographic characteristics and preoperative and postoperative assessments of visual acuity, visual fields, exophthalmometry, and disk edema. Questionnaire responses obtained from patients on 2 occasions 10 years apart. **Results:** Between November 1969 and May 1989, 215 patients (428 eyes) underwent transantral orbital decompression for Graves' optic neuropathy. The median age of the 152 women and 63 men was 56 years. In the 314 eyes that had had visual acuity worse than 20/20 before decompression and were examined within 6 months after decompression, visual acuity improved by 1 Snellen line or more in 230 (73%) ($p<0.001$). In the 205 eyes with visual acuity of 20/40 or worse before decompression, visual acuity improved by 3 Snellen lines or more in 110 (54%) ($p<0.001$). Visual field defects resolved in 93 of 194 patients (48%)assessed and improved in 84 (43%) ($p<0.001$). Proptosis was reduced in 350 eyes by 4.4±2.3 mm (mean±SD) ($p<0.001$) in the early postoperative period. Papilledema resolved in 72 of 104 eyes (69%). Although diplopia was present in 172 of 197 patients (88%) in the early postoperative period compared with 147 of 211 (70%) preoperatively, only 19 of 181 (10%) had constant diplopia on later follow-up. Responses to questionnaires mailed to patients in 1990 and 2000 showed that 76% and 88% of respondents, respectively, were subjectively satisfied with the results of orbital decompression. **Conclusions:** Transantral orbital decompression appears to be effective in treating optic neuropathy of Graves' disease. Patient satisfaction with the operation was high in 10-year and 20-year follow-up.

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The Adverse Effect of Hyperthyroidism on Right Ventricular Function and Pulmonary Hypertension: Rapid Reversal following Normalization of the Serum T3

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The effects of hyperthyroidism on left ventricular function have been well described, but there is little data related to right ventricular function and pulmonary hypertension in hyperthyroidism. We present three women with Graves' hyperthyroidism (ages 21, 28 and 51 years) in which there was associated pulmonary hypertension and right ventricular dysfunction with normal left ventricular function. These right sided heart abnormalities resolved with treatment of the hyperthyroidism. Right ventricular function and pulmonary artery pressure were determined by pulmonary artery catheterization and/or transthoracic echocardiogram. All three patients were treated with combinations of iopanoic acid, dexamethasone, beta-blockers and methimazole. Within one week, serum concentrations of T3 were markedly reduced. There was significant improvement in right ventricular function in two of the patients after one week of treatment with antithyroid therapy. In the third patient there was persistent, but decreased pulmonary hypertension after one week of treatment with antithyroid therapy ascribed to concomitant hypertensive cardiovascular disease. Euthyroidism was maintained in this 51 year old woman with methimazole for three additional months at which time repeat echocardiography revealed marked improvement in the pulmonary hypertension and right ventricular dysfunction. Conclusion: Possible etiologies for pulmonary hypertension in hyperthyroidism have been postulated including an autoimmune process and pulmonary vascular dysfunction. However, it seems likely that the etiology is due to a direct toxic effect of elevated concentrations of T3 on the myocardium and/or pulmonary vasculature since in two of our patients there was significant and rapid improvement in right ventricular function following a rapid decrease in serum T3 levels.

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Serum C-Reactive Protein Levels in the Diagnosis of Thyroid Disease

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Serum C-reactive protein (CRP) levels have not been routinely used in the diagnosis of thyroid disease, although many thyroid conditions involve inflammatory processes. Thus, we hypothesized that CRP levels might help to distinguish between Type 1 (iodine-induced) amiodarone-induced thyrotoxicosis (AIT) and Type 2 AIT (destructive thyroiditis). We measured CRP levels in patients with 14 different thyroid conditions and in 93 non-goitrous, euthyroid controls (C). In the C group, 4.3% had CRP levels >10mg/L. No subjects with non-toxic multinodular goiter (n=34), untreated toxic nodular goiter (n=23), or toxic diffuse goiter, either untreated (n=23) or euthyroid on methimazole (n=33), had CRP >10mg/L. Of 35 euthyroid patients with Hashimoto's thyroiditis, 8.6% had CRP levels >10mg/L (NS vs. C). Only one of 38 patients with short-term hypothyroidism due to L-T4 withdrawal after thyroidectomy (2.6%) had serum CRP >10mg/L. Six of 7 patients (86%) with untreated subacute thyroiditis had CRP levels >10mg/L ($p<0.00001$ vs. C). One of 27 hypothyroid women (4%), 3 of 34 euthyroid women (9%), and 2 of 9 hyperthyroid women (22%) with postpartum thyroiditis had CRP levels >10mg/L; these individual groups did not differ from C. One of 7 euthyroid patients treated with amiodarone (14%) had CRP levels >10mg/L; 6 of 31 patients with Type I AIT (19%); 4 of 33 patients with Type II AIT (12%) had CRP levels >10mg/L. The occurrence of positive CRP values did not differ significantly between the 3 groups of amiodarone-treated patients. Type 1 patients did differ significantly from C ($p= 0.02$), but Type 2 patients did not. Conclusions: Elevated CRP levels were significantly more prevalent in patients with subacute thyroiditis than in euthyroid control subjects, but are not useful in distinguishing between Type1 and Type 2 AIT. CRP levels were not significantly increased in the other thyroid disorders studied.

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Program Number 67 Thyroid Diseases

Thyroid Function Affects Exercise Capacity in Congestive Heart Failure

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Peak oxygen consumption (VO2) and the ventilatory equivalent for carbon dioxide (VE/VCO2) are established prognostic indices in patients with chronic heart failure (CHF). The role of low levels of biologically active thyroid hormone (T3, with metabolic, inotropic and vasodilator actions)in the absence of thyroid disease, has been recently underlined as a determinant of clinical status in CHF. This study was designed to evaluate the relation between abnormalities in thyroid hormone levels and exercise capacity in patients with severe CHF. Forty-six patients (mean age 62±1 yrs, mean±SEM), with either ischemic (25) or idiopathic (21) cardiomyopathy were enrolled in the study. Mean left ventricular ejection fraction (LVEF) was 27.1±1.3%. Nine patients were in NYHA class II, 26 in III and 10 in IV. All patients were on stable (i.e., at least 3weeks) three/four drug regimen (frusemide, ACE-inhibitor, carvedilol, spironolactone). In the same day blood was sampled at 8 a.m. for thyroid hormones (fT3 and fT4) and TSH, and a maximal cardiopulmonary stress test (Vmax 229, Sensormedics) was performed in all patients. Plasma fT3 values were 2.3±0.1 pg/mL (reference: 2.1-4.2), fT4 13.4±0.5 pg/mL (reference: 7.1-18.5) and TSH 1.5±0.2 mUI/mL (reference: 0.3-3.8). The fT3/fT4 ratio was 0.18 (reference:0.16-0.36). Mean peak oxygen consumption was 0.91±0.05 l/min (11.7±0.6 ml/min/kg) and peak VE/VCO2 was 51±2. A significant correlation were found between peak VO2/kg and 1) fT3 (r= 0.29, p=0.046), 2) fT3/fT4 ratio (r=0.36, p=0.014), and 3) TSH (r=-0.34, p=0.022). Moreover, a relation was observed between VE/VCO2 and 1) fT3 (r=-0.30, p=0.04) and 2) TSH (r=0.38, p=0.01). No correlation was found among thyroid hormones/TSH and LVEF or NYHA class. In conclusion, functional exercise capacity and ventilatory efficiency in severe CHF, may be influenced by low levels of triiodothyronine (related to a deficit in peripheral T4 conversion).

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Program Number 68 Thyroid Diseases

The Clinical Diagnosis of Heart Failure Is Predicted by Neurohumoral and Immune Derangement: A Role for Thyroid Dysfunction

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Overexpression of vasoconstrictor neurohormonal agents, and of inflammatory/immune markers, as well as the deactivation action of vasodilator natriuretic molecules have been described in chronic heart failure (CHF). A comprehensive neuroendocrine characterisation of out/inpatients has been prospectively performed since January 2000 in our Institution (106 patients, 78 males, age 67±1 years, left ventricular ejection fraction 29±1% (m±SD), 42% with ischaemic, 38% with idiopathic cardiomyopathy, and 20% with HF secondary to other causes, 37% NYHA class I-II, 63% class III-IV), and in 47 sex-/age-matched control healthy subjects. To assess the relationship between "CHF status" and the continuous variables, logistic regression has been used. Multiple logistic regression with forward stepwise selection (Wald) was used to test the independent relation between "CHF status" and the covariates found to be significant at the univariate logistic regression. The diseased condition was predicted by 1) creatinine level, $p < 0.001$, 95% CI for odds ratio (OR) 7.7883-188.4538; GGT activity, p= 0.0120, OR 1.0057-1.0463), 2) TNF-alpha, p= 0.0128, OR 1.0274-1.2579), 3) neurohormonal indices: a) plasma renin activity: p= 0.0005,OR 2.2746-18.4472, angiotensin II: p= 0.0103, OR 1.0037-1.0140, aldosterone: p= 0.0007, OR 1.0037-1.0140, b) cortisol as a stress marker (p=0.0145, OR 1.0013-1.0119), c) norepinephrine levels ($p < 0.0001$, OR 1.0045-1.0100); d) thyroid hypofunction (free T3 p= 0.0139, OR 0.2772-0.8649¸ ft3/ft4 ratio -index of abnormal peripheral metabolism of thyroid hormone-, $p < 0.0001$, OR 0.8312-0.9365), e) elevation of ANP and BNP, $p<0.0001$; OR 1.0471-1.108, and 1.036-1.0936, respectively). Increasing values of GGT, ANP, BNP, TNF-alpha and plasma renin activity, angiotensin II, aldosterone, cortisol, norepinephrine significantly augmented the probability of HF, which was decreased by increasing values of thyroid hormone and clearance of creatinine. In conclusion, these findings confirm that thyroid function plays a key role within the complex neurohormonal derangement part of HF syndrome.

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Program Number 69 Thyroid Diseases

Assessment of Disease Activity in Graves' Ophthalmopathy (GO) by Serum Hyaluronic Acid (HA) and Urinary Glycosaminoglycans (GAGs)

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GAGs are complex polysaccharides that participate on the pathogenesis of GO. Once deposited on extraocular matrix, these compounds, particularly HA and chondroitin sulfate, attract water, resulting in swelling of connective tissue and enlargement of extraocular muscles. Attempts to correlate the local increase of GAGs to serum HA or urinary GAGs in GO patients have been made for several years, but there are controversies whether serum HA is really increased during disease activity; on the other hand, urinary GAG is increased, but its technique is laborious, time-consuming and difficult for routine use. In this study we analyze serum HA and urinary GAGs in 140 patients with GO using, respectively, a fluorassay for serum HA and a microelectrophoresis for urinary GAGs developed in our laboratory. Patients were classified according to the Clinical Activity Score (CAS) and compared to 138 normal subjects. Accordingly, patients with inactive disease (CAS $\leq$ 2) had serum HA (10.5 ± 7.0 µg/L) and urinary GAGs (4.1 ± 1.3 µg/mg/creatinine) that did not differ from normal subjects (13.9 9.6 µg/L and 3.1 ± 1.1 µg/mg/creatinine). Differently, patients with active eye disease (CAS $\geq$ 3) had serum HA (32.3 ± 18.1 µg/L) and urinary GAGs (8.4 ± 2.7 µg/mg/creatinine) 2-3 times higher than those with inactive disease. Using a cut-off of 22.5 µg/L for serum HA and 6.0 µg/mg/creatinine for urinary GAGs we found, respectively, 87.0% and 81.5% of sensitivity and 92.5% and 91.3% of specificity. The positive and negative predictive values were 76.9% and 96.1% for serum HA, and 71% and 96% for urinary GAGs. Our data show that either serum HA or urinary GAGs measurements are very useful to discriminate patients with active from inactive eye disease, and can be used in combination to CAS and/or other parameters for disease activity to decide the better therapy for GO.

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Program Number 70 Thyroid Diseases

Effect of Thyroxine Therapy on Serum Lipid Levels in Mild Thyroid Failure (TSH 5.1-10 mIU/L) in a Clinical Practice Setting

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There is evidence for lipid lowering effect of thyroxine therapy in subclinical hypothyroidism. However, for the subgroup with TSH levels 5.1-10 mIU/L more information is needed. We retrospectively studied the effect of thyroxine therapy in this group. **Methods and Patients:** Inclusion: living within 120 miles of institution, serum TSH levels 5.1-10 mIU/L (January 1,1995-December 30, 1996). Exclusion: less than 2 TSH values, abnormal free thyroxine, previous abnormal TSH, prior thyroid disease. For the remaining 2655 patents we applied a computer based random selection of a stratified sample of 538 patients. Records of these patients were reviewed in detail. Further exclusions were for lipid lowering therapy and confounding factors such as corticosteroid therapy and severe illness. Only those with normalized serum TSH in the treated group (n=49) and sustained elevated TSH (n=26) in the untreated group were analyzed. Mean thyroxine dose was 0.07 mg/day. We compared the initial and final lipid levels of two groups. Length of tracking from therapy was 5 years, mean 3.9. **Results:** Serum LDL, total cholesterol (TC), triglyceride (TG) and HDL levels did not change significantly ($p>0.20$) in the untreated group (n=26, LDL; 122±6.4, TC; 203±7.7,TG; 158±13.9, HDL; 50±3.8 mg/dl, follow-up levels; 122±7.4, 208±8.0,182±13.8, 51±3.7 mg/dl; Mean ±SEM) and the treated group (n=49, LDL; 128±5.6, TC; 209±6.3, TG; 130±9.1, HDL; 55±2.1; follow-up levels 126±4.5, 208±5.1, 142±10.4, 53.8±2.2 mg/dl). **Conclusion:** In a clinical practice setting, in patients with serum TSH levels in the range of 5.1-10 mIU/L, thyroxine therapy and normalization of serum TSH does not lower serum total cholesterol, triglycerides and LDL and change HDL levels. Randomized studies in this subgroup are needed for definitive conclusion.

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Program Number 71 Thyroid Diseases

Screening for Hyperthyroidism in Early Pregnancy

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Thyroid dysfunction potentially affects pregnancy and fetal outcomes. We have been performing a screening program for hyper- and hypothyroidism using FT4 and TSH concentrations in early pregnancy among women in Tokyo who want to have the test. We also use MCHA and hCG as an indicator of autoimmune and non-autoimmune thyroid diseases. The cost is about □□23. Blood samples obtained on filter paper are used, conforming with the screening test for congenital hypothyroidism. In the present study, we report the results of screening for hyperthyroidism performed from April 1995 to March 1999. Women whose FT4 concentrations were 4.0 ng/dl or higher were advised to consult a doctor promptly. In the other women, FT4 levels were examined again when their initial FT4 was above normal, and they were recommended to see a doctor when levels again rose above normal. 75,934 women were screened during this period. We obtained information on the diagnosis and treatment in 78% (204 of 262) of the overall total who were advised to consult a doctor. Of these women, 64 were diagnosed as having Graves' disease (GD) and 133 as having transient hyperthyroidism due to hCG (GTH), on the bases of TBII, hCG, and/or duration untreated hyperthyroidism. In GD patients, spontaneous abortion and premature delivery occurred in 3.4% and 3.4%, respectively; the prevalence of small- or light-for-date infants was 6.8%. Pediatricians were informed of possible influences of maternal diseases before delivery, and infants were closely followed after birth if necessary. Consequently, they were able to avoid overt thyroid dysfunction. These findings suggest that, although it may not be very useful in avoiding abortion due to hyperthyroidism, screening for hyperthyroidism during the early stages of pregnancy may be beneficial, because both the mother and the child can avoid serious morbidity due to maternal GD.

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Program Number 73 Thyroid Diseases

Genetic Markers in Prediction of Outcome in Patients with Graves' Disease after Antithyroid Drug Treatment

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Many parameters, such as initial thyroid volume and its evolution during therapy, T3/T4 ratio, TSH to TRH responsiveness, serum thyroglobulin concentration, and thyroid stimulation activity (TSAb), have been proposed as predictors of remission or relapse of Graves' disease. However, only few studies demonstrated a relation between outcome and genetic markers like HLA class II region or CTLA-4 gene polymorphism.
Patients were investigated for a short-term (treatment duration before reaching euthyroid status; good < 90 days) and long-term responses (success – duration of remission >12 m; failure - persistent use (> 24m) of medication or relapse) as well as gene polymorphisms of HLA class II, CTLA-4, and VDR regions.
The GG genotype of CTLA-4 exon1 gene, a susceptible genotype of Graves' disease, showed an association with good response both during short-term (n=70, ptrend=0.043) and long-term (n=58, ptrend=0.042) follow-up. The duration of medication was also shorter in GG group than in AG group (15.0±8.0 m vs. 24.3±10.5 m; p=0.002). The AA genotype of vitamin D receptor (VDR) ApaI gene was also associated with a good short-term response (n=103, % of euthyroid, 94.7% vs. 70.2%, p=0.038), but not with long-term result (n=75). While the susceptible HLA haplotype HLA DRB1*0803-DQB1*0601 showed a poor short-term response (n=167; % of euthyroid, 64.8% vs. 83.2%, p=0.008), there was no difference in long-term result (n=119). The CTLA-4 promoter polymorphism yielded no association with the outcome.
In summary, the GG genotype of CTLA-4 exon1 gene and AA genotype of VDR gene were associated with a favorable response, while HLA DRB1*0803-DQB1*0601 haplotype was associated with a poor response to antithyroid drug treatment. Thus, it could be suggested that some genetic markers might help to predict outcomes of Graves' patients after antithyroid drug treatment.

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Program Number 74 Thyroid Diseases

The Human Leukocyte Antigen HLA-DRB1*0803-DQB1*0601 Haplotype Is Associated with Graves' Disease in Koreans

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The association of Graves' disease with HLA DR3 and DQA1*0501 in Caucasians has been described previously. Using a case-control study design, we have examined the role of HLA class II gene polymorphisms in the predisposition for Graves' disease in a group of Korean subjects. Oligotyping for HLA-DRB1 and DQB1 alleles was performed using the polymerase chain reaction (PCR) -single strand conformation polymorphism or PCR-restriction field length polymorphism technique in 198 Graves' disease and 200 normal controls. Clinical characteristics of Graves' patients were also analyzed.
We demonstrated that both HLA*DRB1 0803 (27.8% vs. 14.5%; RR = 2.27; Pc = 0.035) and DQB1*0601 (30.3% vs. 15.5%; RR = 2.37; Pc = 0.007) alleles conferred significant susceptibility in the DRB1*0803-DQB1*0601 haplotype. The DQB1*0601 allele was the primary susceptibility allele for Graves' disease, because the susceptibility provided by DRB1*0803 was most likely due to it being in linkage disequilibrium with DQB1*0601. The frequency of DRB1*0602 (5.1% vs. 0.0%; RR = 22.3; Pc = 0.025) was also increased significantly in the patients compared to the controls. The protective alleles for Graves' disease were DRB1*0101 (4.5% vs. 14.0%; RR = 0.29; Pc = 0.035), DQB1*0202 (4.0% vs. 12.5%; RR = 0.29; Pc = 0.033) and DQB1*0501 (7.6% vs. 18.5%; RR = 0.36; Pc = 0.018). DRB1*0101 was also in linkage disequlibrium with DQB1*0501. Some HLA class II alleles have shown significant associations with clinical characteristics of Graves¡¯ disease; DQB1*0601 with a positive TSH receptor antibody; DRB1*1302-DQB1*0609 with larger goiter (goiter size >80g); DRB1*1101 and DQB1*0609 with increased serum thyroid hormone levels (T3 and free T4) etc. These data suggest that HLA DRB1*0803-DQB1*0601 is more likely to be the primary susceptible locus of Graves' disease in Koreans. In addition, HLA class II gene may be associated with some clinical characteristics of Graves' disease.

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Program Number 75 Thyroid Diseases

Unsuspectedly High Frequency of Post-Head-Trauma Hypothyroidism

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Recent studies reported that hypopituitarism (Hypop) and its post-head-trauma (PHT) etiology are less rare than believed (Lancet, 2001; JCEM; 2000). Particularly, about 50% of patients with PHT-Hypop reported in the literature have TSH deficiency. To confirm that the thyrotropes are particularly fragile after head trauma (HT), we have carefully inquired for previous HT patients with low or borderline low serum TSH and FT4 in whom other causes of central hypothyroidism (CH) were excluded. Such patients would have otherwise been diagnosed as affected by idiopathic CH. Because HT can be minor and easily forgotten by patients, we also interrogated patients' relatives. We have thus collected a series of 40 patients with PHT-CH. They were 20 to 68-yr-old (47+/-13 yr, m+/-SD), but at trauma the 13 men were younger than the 27 women (19+/-19 vs 32+/-13 yr, $P<0.05$). The leading causes of HT were domestic and road accidents, and only one-fourth of patients had been hospitalized. Also, one-fourth was reminded of HT by relatives. The most frequent disturbances were fatigue (2/3), apathy (1/3), cold intolerance (1/5). Baseline TSH and TSH increment after iv TRH (delta-TSH) ranged <0.01 to 0.8 mU/L and 0.06 to 8.4 mU/L, respectively; in the subgroup with delta-TSH > 4.0 mU/L; about 3/4 had subnormal (<25%) post-TRH increment of serum T3, suggesting impaired TSH bioactivity. Based on pituitary dynamic tests, TSH deficiency was isolated in about 1/10; it was associated to deficiency of FSH/LH, ACTH or GH in about 2/3, 1/3 or 1/3, respectively. The leading neuroradiologic (CT/MRI) abnormalities were empty sella (about 1/2) or pituitary hypodensities (1/5). Conclusion: PHT-CH is indeed less rare than commonly believed. Physicians should be more inquisitive and alerted about PHT-Hypop, while patients (and their families) should be more informed about the endocrine complications of even minor HT.

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Program Number 76 Thyroid Diseases

Effects of Experimentally Induced Subclinical Hypothyroidism on Quality of Life and Mood

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Subclinical hypothyroidism (SCHypo) is common, but it is unclear whether it causes symptoms or affects quality of life. To address this issue, we recruited seven women (ages 21-75) with primary hypothyroidism receiving L-thyroxine (L-T4) who had normal TSH levels. In random order they received their usual dose of L-T4 or a lower dose, each given for 12 weeks in a double-blinded, placebo-controlled fashion. Dose adjustments were done at 6 and 18 weeks, with target TSH levels in the SCHypo arm of 10-39 mU/L. At the end of each arm, subjects completed the Billewicz Scale, Short Form 36 (SF-36, a validated measure of quality of life), and Profile of Mood States (POMS). Mean L-T4 dose decreased on the SCHypo arm from 1.53 to 0.73 ug/kg/day at 12 weeks (53% decrease). Mean free T4 levels decreased from 1.43 to 1.01 ng/dL on the SCHypo arm ($p = .001$), although all free T4 levels remained within the normal range. Mean TSH levels increased from 3.3 to 25.3 mU/L on the SCHypo arm ($p = .02$). All thyroid hormone levels remained normal on the euthyroid arm. Five subjects correctly guessed which arm was the SCHypo arm, and all 5 preferred the euthyroid arm ($p < .005$ by Chi-Square). Compared to the end of the euthyroid arm, there were no changes in Billewicz or SF-36 scores at the end of the SCHypo arm. However, mean POMS-F (fatigue) score was 41.6 at the end of the euthyroid arm and 46.4 at the end of the SCHypo arm ($p = .004$ by paired t-test). These results suggest that subjects with SCHypo have subtle decrements in quality of life. In addition, experimental induction of SCHypo is safe and feasible, and may provide a model for the study of other effects of SCHypo.

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Program Number 77 Thyroid Diseases

High Prevalence of Iodine Deficiency in Japanese-Brazilian Women of Childbearing Age

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Pregnancy has profound effects on thyroid physiology, particularly stimulating the maternal thyroid economy. An iodine-deficient status in the mother can cause goiter in the progeny and neuropsycho-intellectual impairment in the offspring. It is well known that iodine deficiency is not present in Japan, where the intake of iodine-rich seaweed is very high. In order to assess the influence of lifestyle changes in the dietary iodine content of a Japanese migrant population, we examined the urinary iodine excretion (IU) and thyroid function in 131 women of childbearing age (30 – 45 years old) from a Japanese descent population living in the city of Bauru, State of São Paulo, southeast of Brazil, an area considered to be moderate in iodine intake. The IU was analyzed in casual urine sample by a semi-automated method (normal: 10 to 29.9 micrograms/dL) and serum TSH concentration by an immunofluoroassay (Wallac-Delphia; sensitivity: 0.05 mU/L, normal range: 0.3–4.5 mU/L). The IU was low in 36/131 women (27.5%) and the deficiency was considered serious in 8/131 (6,1%; IU < 2.5 micrograms/dL), moderate in 9/131 (6.8%; IU between 2.5 and 4.9 micrograms/dL) and mild in 20/131 (15.3%; IU between 5 and 10 micrograms/dL). The prevalence of hypothyroidism (TSH > 5.0 mU/L) in this group was 6.1%, considered high for this range of age. These results show that an high proportion of women of Japanese descent ofchildbearing age in Bauru has iodine excretion levels in the range of iodine deficiency, in spite of the national program of dietary iodine supplementation.

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Program Number 78 Thyroid Diseases

The Possible Contribution of Anti-Gal to Graves' Disease

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Anti-Gal is a natural antibody found in all humans. Anti-Gal is specific for the alpha-galactosyl epitope. Etienne-Decerf et al., (Acta Endocrinol:15, 1987) found Graves' patients had elevated anti-Gal titers and Winand et al., (J Immunol:153, 1994) found anti-Gal increased cAMP synthesis in cultured thyrocytes from Graves' disease patients but not in normal controls. To study the contribution of anti-Gal to Graves' disease we developed an anti-Gal ELISA based on the specific binding of anti-Gal to laminin. Pooled normal serum was used as a standard. We found no significant correlations between anti-Gal (IgG, IgM, or IgG+IgM) and TRAb or freeT4 in untreated hyperthyroid Graves' patients (n=15) without opthalmopathy or Graves' disease patients without hyperthryoidism but with opthalmopathy. As expected, there was a significant regression between TRAb and free T4 in the hyperthyroid patients ($p<.01$). Interestingly, addition of total anti-Gal antibody to the regression improved the correlation ($p=0.15$), suggesting it may play a role in stimulating Graves' thyroid tissue. However, in contrast to previous studies we found hyperthyroid patients (n=20) had lower levels of both anti-Gal IgG (18.4+/- 4.0 vs 41.8+/-9.0) and IgG+IgM (30.6+/-4.8 vs 58.5+/-10.1) than normals (n=35, $p<0.05$ both comparisons). Additionally, the hyperthyroid patients tended to have lower anti-Gal titers than the opthalmopathy patients for anti-Gal IgG and for total anti-Gal ($p=0.1$ both comparisons). The ability of anti-Gal to improve the regression of TRAb on free T4 in hyperthyroid patients suggest that anti-Gal may make a contribution to the pathogenesis of Graves' disease. However, hyperthyroidism significantly lowers the level of anti-Gal. This latter effect may protect against more severe hyperthyroidism induced by both anti-Gal and TRAb. Lastly, the difference in anti-Gal levels between the Graves' disease patients with and without opthalmopathy suggests a possible role of anti-Gal in the pathogenesis of Graves' opthalmopathy.

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Program Number 79 Thyroid Diseases

Longitudinal Changes in Bone Mineral Metabolism and Hormonal Status in Young Patients with Graves' Disease

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Graves' disease (GD) may be associated with bone mass loss (BML). The effect of thyroid hormones (TG) on bone metabolism has been studied. We investigated the relationship between serum T3, T4, TSH, PTH (parathormone), Calcitonin (Ct), 1,25-vitamin D3 concentrations, serum calcium (Ca), phosphorus (P) and alkaline phosphatase (ALP) levels, 24-hour urinary calcium and phosphorus excretions and bone mineral density (BMD) by X-ray densitometry and ultrasound osteometry in 54 (24 males and 30 females, aged between 20 and 30 years) thyrotoxic patients. Data on 25 age-matched healthy controls were used for comparison. All patients were divided into two groups: 1) with moderate thyrotoxicosis (n=12); 2) with severe thyrotoxicosis (n=44). Both serum T3 and T4 were elevated ($p<0.001$), but TSH level was decreased in both groups. PTH was decreased by 82.4 % in first group and 91.5% in the second group. Ct was increased in the first group on 15% and 16% in the second group. 1.25-vitamin D3 levels in blood were not significantly different between two groups. Total serum Ca was increased in the second group. In the first group serum P and ionized Ca levels were increased by 67% and 17%, respectively; in the second group, by 53% and 23%, respectively. ALP was elevated (20%) in both groups. BMD data from the two groups were compared with those from healthy controls matched for age. The percentage of BML was dependently associated with the severity of thyrotoxicosis and means bone mass were decreased (10%, ultrasound osteometry and 38%, bone densitometry). Patients with GD had abnormalities in bone metabolism according to their thyroid status.

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Program Number 80 Thyroid Diseases

Frequency of the Late Diabetic Complications in Patients with Graves' Disease

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Background and aim. Toxic effects of thyroid hormones on tissue characterize Graves' Disease (GD). Diabetes Mellitus (DM) frequencies are as much as 3 times higher among of patients with GD. Toxic effect of hyperglycemia on DM leads to development of late complications (LDC) such as diabetic retinopathy (DR), nephropathy (DN) and foot vessels angyopathy. We study the frequency of the LDC in patients with GD and DM compared with DM only. Materials and Methods. Observed 80 patients with GD and DM (62 IDDM and 18 NIDDM), and 60s with DM only (32 IDDM and 28 NIDDM). Results. DR frequency at GD was 52.3% and at DM only registered in 74.3%. DN frequency at GD detected in 30.5% and at DM group in 36%. Renal blood flow was higher on 27.8% ($P<0.05$) at GD with DM than only DM. The leg vessels angyopathy were detected in 87.9% at GD group and in 85.47% at DM only, whereas diabetic foot was detected in 5.66% case in GD group and 15% with DM only. In GD group with DM 77.5% of patients has insulin injection (48.19 U/day) and 53.3% patients with DM only were insulin dependent (41.88 U/day). Data analysis according to duration of diabetes show that in the first 10 years the frequency of LDC was higher in the group with DM only, and after 10 years of disease duration, LDC frequency was higher in GD combined with DM group. Conclusions. Patients with GD in combination with DM became insulin dependence more often than those with only DM. Diabetic complications frequency in patients with GD was lower in the first 10 years and significantly increases after 10 years of DM.

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Program Number 81 Thyroid Diseases

Propylthiouracil-induced P-ANCA Positivity in Setting of Acute Renal Failure

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A 20-year-old woman was started on Tapazole in January 1993 for Graves' disease after presenting with weight loss and proptosis. Three years later, the patient developed urticaria associated with Tapazole and started PTU 300 mg orally per day. The patient was frequently noncompliant with PTU therapy. In March 1999, the patient developed an acute flu-like syndrome associated with left flank pain, hemoptysis, and gross hematuria. The patient was admitted to a hospital and was found to be in acute renal failure with a creatinine of 1.6. The patient was also found to have anemia and leukopenia at that time. The patient's PTU was discontinued while she remained in the hospital, and the hemoptysis resolved completely. Bronchoalveolar lavage was performed and was consistent with alveolar hemorrhage. The patient ultimately underwent a renal biopsy, which revealed acute tubular necrosis and mesangial proliferative immune complex-mediated glomerulonephritis, consistent with IgA nephropathy. The acute renal failure resolved. At that time, the patient's P-ANCA was elevated to greater than 1:640 FIU (fluorescence intensity units) with a significantly elevated proteinase 3 antibody of 80 EIA units. Myeloperoxidase antibody was also elevated greater than 200 u/mL. Glomerular basement membrane antibody was negative. Nine months later, the myeloperoxidase antibody was rechecked and had fallen to 16.9 u/mL. Proteinase 3 antibody fell to 42 EIA units. The patient's hyperthyroidism was treated with 20 millicuries of I131 in July 1999, and the patient became hypothyroid in November 1999. She remains on L-thyroxine replacement therapy with normal renal function and no hemoptysis. Review of the literature is significant for case reports of PTU-induced myeloperoxidase ANCA antibody positivity. Recently, a new case of ovelapping ANCA positivity and IgA glomerulonephritis in a patient treated with PTU was reported. This is, to our knowledge, the second reported case of such an overlap syndrome.

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Program Number 82 Thyroid Diseases

Treatment of Multinodular Goiter with Radioactive Iodine and Previous Administration of Recombinant TSH (rTSH)

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Radioactive iodine (131I) therapy has been used in the treatment of toxic and non-toxic multinodular goiter (MNG). However, patients with MNG frequently present with low 131I uptake, resulting in a poor treatment response. We describe a 79 years-old female patient with a large MNG with cough and compressive symptoms. Ultrasound (US) revealed a goiter with a volume of approximately 83 ml, predominantly at the left side, with hypoechoic and hyperchoic nodules (the largest one with 6 cm diameter), extending to intrathoracic region. Computed tomography (CT) showed important trachea deviation. In the thyroid scintillography, large cold areas with heterogeneous uptake were seen, with a 24h uptake of 9.5%. Cytologic diagnosis was colloid goiter. Thyroid function tests revealed a suppressed TSH value and mildly elevated free T4 levels. Due to the age of the patient, 131I therapy after rTSH was considered. Intramuscular injections of 0.1 mg rTSH (Thyrogen, Genzyme) were given on 2 consecutive days, resulting in a rise of 131I uptake to 59% and a homogeneous thyroid image, with a subsequent administration of 30 mCi of 131I. US and CT performed 6 months after treatment showed regression of the goiter, and the patient was clinically euthyroid, reporting a gradual improvement of her compressive symptoms. This case shows that rTSH might be a good tool to increase thyroid 131I uptake and to improve the therapeutic outcome of 131I therapy in patients with MNG.

	TSH mU/L	T4L (ng/dl)	US (ml)	131I 24 h uptake
Pre rhTSH	0.01	1.6	83	9.5 %
Pos TSH	29	3.1	---	59 %
6 months FU	4.3	0.9	52	---

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Program Number 83 Thyroid Diseases

Lipids and Subclinical Hypothyroidism in Older Patients with Metabolic Disorders and Arterial Hypertension

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OBJECTIVES: To analyze the frequency of subclinical hypothyroidism (SCH) and lipid concentration in patients with diabetes mellitus type 2, dislipidemia, obesity and arterial hypertension in patients over 60 years of age. METHOD: A transverse study in 88 patients. The entry criterias were:men over 60 year of age without thyroid disorders neither levotiroxine treatment in the last year, with diagnosis of diabetes mellitus and/or dislipidemia and/or obesity (BMI > 27 Kg/m2) and/or arterial hypertension. The diagnosis of SCH was done TSH > 5.5 and < 10 uUI/ml, with T4L normally. We evaluated the serum cholesterol, high-density lipoprotein(HDL) cholesterol, low-density lipoprotein(LDL) cholesterol and triglycerides concentration between patients with SCH and without SCH. Data were analyzed using chi-square and T test. RESULT: We studied 88 patients, 60 (68.2%) females and 28 (31.8%) males, with mean age of 68.6 years old. It was diagnosed 7 (8%) patients with HSC, 2 (2.3%) with subclinical hyperthyroidism and 79 (89.9%) euthyroids. Total cholesterol was higher in patients with SCH than patients without SCH (mean 294.6 vs 225.1 mg/dl)(p=0.003). There were not differences on LDLc, HDLc or triglycerides. We did not find differences between the presence of SCH and the type or number of metabolic disorders. CONCLUSION: The frequency of SCH is 8% in patients over 60 year of age with metabolic disorders. The serum cholesterol concentration is higher in patients with SCH as it is described by other authors.

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Program Number 84 Thyroid Diseases

Exopthalmos in Absence of Graves' Disease in Euthyroid Patients

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Thyroid associated opthalmopathy (TAO) occurs with Graves' disease in 90% of patients and the remaining 10% are euthyroid or have Hashimoto's thyroiditis. We describe here two cases of exopthalmos in euthyroid patients. A 79-year-old male, hypothyroid for 6 years and on 125 mcg of thyroxine had proptosis of right eye which got worse in November 2001. No history of hyperthyroidism or radioactive iodine therapy. He had severe proptosis of the right eye with chemosis and limitation of elevation, depression and abduction of right eye. Left eye was normal. Visual acuity was 6/12 on right and 6/6 on left. Optic discs normal. Pupils equal and reacting. Free T3 4.2pmol/l and TSH 2.1 uU/l. Thyroid peroxidase antibody 580 pmol/l.TSH receptor antibody negative. MRI scan of orbit showed right eye proptosis with gross enlargement of retro orbital muscles. Bones looked normal. No major intracranial abnormality was visualised. He was commenced on prednisolone 60 mg a day and had radiotherapy to the right orbit. Chemosis disappeared and ocular movements have normalised. A 58-year-old female with chronic obstructive airways disease due to smoking presented with right eye exopthalmos in March 2001. She had no hypo- or hyper- thyroid symptoms. She had right eye proptosis with periorbital oedema and chemosis; no restriction of ocular movements. Free T3 4.8 pmol/l and TSH 0.7uU/l. Thyroid antibodies negative. Ultrasound scan of the neck showed multinodular goitre. MRI scan of the orbit revealed extensive arterio venous malformation involving right middle cerebral artery, internal carotid artery and right cavernous sinus. Dilated veins entering the right orbit via the orbital apex and extend to the skin surface. Optic nerve atrophied. Right globe proptosed. She was referred to the neurosurgeons. Surgical intervention was not possible due the anaesthetic risk from her advanced obstructive airways disease. We have discussed the pathogenesis of thyroid associated opthalmopathy and various treatment modalities.

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Program Number 85 Thyroid Hormone Action

Raloxifene Prevents Osteoporosis in Postmenopausal Women under Suppressive Treatment with L-Thyroxine

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Background Postmenopausal patients under suppressive doses of LT4 are exposed to accelerated bone loss and increased risk of osteoporosis. Raloxifene (Rlx) is a selective estrogen receptor modulator (SERM) that prevents osteoporosis. However, the effects of Rlx and LT4 on bone mass have not been compared directly in postmenopausal women. **Methods** We therefore randomized 31 postmenopausal patients taking suppressive doses of LT4 to treatment with Rlx 60mg (Rlx/LT4, GrI, n=16) or placebo (LT4/P, GrII, n=15) for 1 year. Patients had measurements of bone mineral density (BMD) with DEXA, of the spine and femoral neck every 6 months. Biochemical markers of bone turnover were collected at randomization and at 12 months. Serum bone specific alkaline phosphatase (BSAP) was measured as a marker of bone formation and urinary deoxypyridinoline was determined as a marker of bone resorption. **Results** BMD increased 0.85% at the spine (T-score: -1.92 vs -1.83) and 1.3% at the femoral neck after 12 months of treatment (both $p<0.05$ vs baseline). There was no significant bone loss at any site in the Gr II. However, 95% CI exhibited a rate of bone loss >0.8% per year for the spine and >0.25% at the femoral neck. A greater decrease in both BSAP (-15%) and deoxypyridinoline (-18%) were found in GrI as compared with GrII at 12 months ($p<0.001$). **Discussion** Rlx (60mg) may prevent postmenopausal bone loss in patients under suppressive regimen with LT4. Furthermore, Rlx was found to significantly increase BMD and suppress biochemical markers as compared with LT4/P at 12 months. Thus, Rlx may be considered as a valuable agent for prevention of osteoporosis in patients under suppressive doses of LT4 who cannot take estrogens.

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Program Number 86 Thyroid Hormone Action

Does Low-T3 Syndrome Predict a Bad Prognosis in Patients with Dilated Cardiomyopathy?

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A low-T3 syndrome is a frequent finding in patients (pts) with dilated cardiomyopathy (DC). The aim of the study was to assess the role of thyroid metabolism in the prognosis of pts with DC. One hundred sixty-seven consecutive inpts (age 67±12 years, mean±SD) with post-ischemic (n=90) or hydiopathic (n=77) DC underwent thyroid function profile evaluation immediately after admission to the Cardiac Unit. Pts were divided in two subgroups: Group I, 57 pts (71±11 years) with low T3, i.e., with free T3 (fT3) < 2.0 pg/ml (fT3 normal range 2.1-4.2 pg/ml); Group II, 110 pts (66±12 years, p<.01) with normal fT3 i.e., >= 2.0 pg/ml. The event considered was overall death. Mean left ventricular ejection fraction (LVEF) was 30±8%. Free T3 was 1.46±0.42 pg/ml in the Group I and 2.63±0.53 pg/ml in the Group II. No correlation was found between fT3 and LVEF (r = 0.21, p=ns). TSH and fT4 were in normal range but TSH differed between the two groups (TSH: 2.97±4.23 microIU/ml in Group I vs 1.91±2.10 microIU/ml, p<.03; fT4: 13.9±4.3 pg/ml in Group I vs 14.3±4.4 pg/ml, p=ns). During the 31-days follow-up, there were 12 cumulative deaths in Group I and one death in Group II with a survival of 76.1% vs 98.8% (p<0.0001). In Group I, fT3 was lower in pts who died vs those alive (1.08±0.47pg/ml vs 1.56±0.34 pg/ml, p<.0001). At the univariate analysis, fT3 was the most important predictor of cumulative death (chi^2 27.5, p<.00001), followed by age (chi^2 9.3, p<.002) and obesity (chi^2 4.4, p<.03). At stepwise analysis fT3 was confirmed as the strongest prognostic predictor of death (Hazard Ratio 9.2, p<.00001) followed by age (Hazard Ratio 1.05, p<.04). In conclusion, in pts with DC, a low-T3 state is a strong predictor of early bad prognosis; administration of substitutive doses of synthetic T3 could improve the prognosis.

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Program Number 87 Thyroid and Development

Pre-natal Screening for Maternal Hypothyroxinemia to Reduce Source of Neurodevelopmental or IQ Deficits

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Early maternal hypothyroxinemia appears to be a significant risk factor for neurodevelopmental or IQ deficit in the child for which screening may be desired (Pop 1999; Morreale de Escobar, 2000; Dunn, 2002). Haddow demonstrated similarly with untreated hypothyroidism (Haddow, 1999). Human first-trimester fetal tissues are normally exposed to free thyroxine levels similar to (1/3 of) maternal serum levels (Calvo, 2002). Thyroxine is the required substrate for intracellular generation of T3 for binding to the nuclear hormone receptor in the developing brain. Since the fetal thyroid does not produce thyroxine until mid-gestation, maternal thyroxine is the only source of thyroxine to fetus in early pregnancy. This analysis uses data from the US National Health and Nutrition Examination Survey (NHANES III; 1988-1994) to estimate the US frequency of maternal hypothyroxinemia. The data showed that 0.7 % of the sampled (age 15-44 y/o) women had thyroxine levels below the normal range (4.5-13.2 ug/dL). Adjustment for age-specific probability of pregnancy (yr 2000) and for sampling weighting factor indicates a predicted maternal hypothyroxinemia prevalence of 0.4% or 1/240 live births. With 4 million US live births per year, it is predicted that 16,000 pregnancies may need assessment for treatment of maternal hypothyroxinemia. The estimated prevalence rate of maternal hypothyroxinemia is 10-20 times greater than that of congenital hypothyroidism (for which universal screening exists in the US). It is proposed that prospective feasibility and efficacy studies of screening and treating pregnancies with hypothyroxinemia be conducted.

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Program Number 89 Thyroid Hormone Action

T3 Receptor (TR) Activity Is Modulated by TRΔβ3 in a Cell-, Response Element-, and TR Isoform-specific Manner

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Alternative splicing of the rat TRβ gene produces TRs β1-3 and also TRΔβ3, which lacks the DNA binding domain but binds T3. TRβ1 is ubiquitously expressed; TRβ2 is predominantly in pituitary and hypothalamus, whilst TRβ3 and TRΔβ3 are widely expressed in T3-regulated tissue-specific ratios. We investigated the function of TRβ3 and TRΔβ3 by transient transfection and gel shift assay. TRβ3 was a more potent mediator of T3 induction of reporter genes containing malic enzyme (ME), α myosin heavy chain (MHC) or palindromic (PAL) TREs than TRβ1 in both Cos7 and osteoblastic Ros17⁄2.8 cells. TRΔβ3 did not influence reporter expression in either cell line. In equimolar co-transfections, TRΔβ3 antagonized T3-induced activation mediated by both TRβ1 and TRβ3 on all three TREs in Cos7 cells, whereas in Ros17⁄2.8 cells antagonism occured only for TRβ1 induction of MHC. However, low concentrations of TRΔβ3 relative to TRβ3 increased induction of the PAL TRE 2.5 ±0.9-fold and the MHC TRE 2.2 ±0.1-fold compared to activation in the absence of TRΔβ3. A similar potentiation of TRβ3 was also seen on the PAL element in Ros17⁄2.8 cells. Preliminary analysis of TRΔβ3 interaction with TRα1 in Cos7 cells showed antagonism on ME and MHC elements but potentiation on the PAL element. TRβ3 bound to ME, MHC and PAL TREs as a heterodimer with RXR in gel shift assays, but TRΔβ3⁄RXR heterodimers failed to bind these elements. TR⁄RXR binding to DNA was competed by TRΔβ3, suggesting that antagonism of TR action by increasing concentrations of TRΔβ3 may involve competition for RXR. Thus, TRΔβ3 is a modulator of T3 action, which acts as a dominant negative antagonist or a potentiator of TR activity depending on the TR isoforms present, their relative concentrations, the TRE sequence and the cell type.

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Program Number 90 Thyroid Hormone Action

Testicular Enlargement in TRα (P398H) Mutant Mice: Histology and Gene Expression Analysis

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Thyroid hormone is essential for normal testicular development and fertility in rodents. Neonatal hypothyroidism leads to a prolongation of the period of Sertoli cell proliferation, resulting in an increase in the number of Sertoli and germ cells in testis as well as an increase in testis size. TRα 1, but not other TR isoforms, is expressed at high levels in both proliferating Sertoli and germ cells. A recent published study of a TRα (PV) dominant negative mutation in mice reported reduced fertility. In this study, a dominant-negative "knock-in" mutation (P398H) was introduced into the TRα gene in mouse embryonic stem cells and TRα (P398H) mutant mice were created. We observed age-related testicular enlargement and reduced fertility in TRα (P398H) mutant mice but not in wild type (wt) mice. The testes of heterozygous mice for the P398H mutation at 6 months were enlarged 1.3-fold by weight compared to wt mice and further increased up to 2-fold in weight at 1 year. We performed DNA microarray and histological analysis to determine the gene expression profile and mophology of the testes. Histologic data indicated more active spermotagenesis in mutant mice compared to wt mice. DNA microarray revealed that the expression level of mRNA from 441 genes was altered in mutant mice compared to wt mice. Differential expression of known genes was confirmed by real-time PCR. Identification of differentially regulated genes in testes from TRα (P398H) mice may identify important T3 targets modulating testicular function and fertility.

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Program Number 91 Thyroid Hormone Action

The Truncated Thyroid Hormone Receptor α (TRα) Gene Products TRΔα1 and TRΔα2 Bind T4 and rT3 but Not T3

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Thyroid hormone (TH), specifically T4 and rT3, regulates actin polymerization and actin-based endocytosis in astrocytes by a non-genomic mechanism. Here we identify T4/rT3 binding proteins in astrocyte lysates that are candidate mediator(s) of these non-genomic actions of TH. Confluent cultures of rat astrocytes were incubated overnight in serum-free media and nuclei-free extracts prepared. Extracts were incubated with [125I]-TH and increasing concentrations of competitor for 30 min at 37°C, and bound ligand was separated from free ligand by Sephadex G25 chromatography. Both rT3 and T4 specifically displaced [125I]-T4 and [125I]-rT3 from astrocyte extracts (Kd's ~ 0.5-1.0 nM). T3 failed to displace either radioligand and there was no significant binding of [125I]-T3 to lysate proteins. Since the T4 analog, N-acetyl-T4-O-methyl, selectively regulates actin polymerization in astrocytes, we used the alkylating affinity label, BrAc[125]T4-OMe, to identify the T4/rT3 binding protein(s) in astrocyte lysates. Incubation of astrocyte extracts with BrAc[125]T4-OMe covalently labeled two proteins of ~26 kDa and ~16kDa, corresponding to TRΔα2 and TRΔα1, respectively. These two gene products correspond to the C-terminus of the ligand binding domain of the TRα gene and originate from an internal promoter located in intron 7. Antisera directed against the C-terminus of TRα1 and of TRα2 specifically immunoprecipitated BrAc[125]T4-OMe labeled ~16 kDa and ~26 kDa proteins, respectively, from astrocyte extracts. Finally, bacterially expressed TRΔα1 and TRΔα2 showed competitive displacement of bound [125I]-rT3 or [125I]-T4 with Kd's ranging from ~0.3 to 1 nM; neither truncated TRα gene product bound [125I]-T3. Both bacterially expressed proteins were specifically labeled by BrAc[125I]T4-OMe. These data show that TRΔα1 and TRΔα2 are present in the cytosol of rat astrocytes and specifically bind T4 and rT3 with properties that make them potential candidate mediators of the non-genomic actions of TH in astrocytes.

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The Truncated Thyroid Hormone Receptor α Gene Product, TRΔα1, Mediates T4-regulated Actin Polymerization in Astrocytes

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Thyroid hormone (TH), specifically T4 and rT3, regulates actin polymerization in astrocytes by a non-genomic mechanism. TRΔα1 is a ~16 kDa protein corresponding to the C-terminus of the TRα gene derived from a promoter located in intron 7. We found that TRΔα1 was a T4/rT3 binding protein suggesting that it was a potential mediator of non-genomic actions of TH. We used an in vitro actin polymerization assay to examine the role of TRΔα1 in TH-dependent actin polymerization. Confluent cultures of rat astrocytes were incubated overnight in serum-free media and nuclei-free extracts prepared. Lysate protein (100 mcg) was incubated ± 10 nM T4, T3 or no hormone for 30 min, mixed with pyrene-labeled (0.04 mg/ml) and unlabeled (0.4 mg/ml) G-actin, and equilibrium levels of F-actin (as measured by an increase in pyrene fluorescence) were measured after 24 h. As observed in intact cells, addition of T4, but not T3, to the nuclei-free astrocyte lysates doubled the quantity of F-actin fluorescence as compared to control, hormone-free lysates. Pre-incubation of cell lysates with antisera directed against the C-terminus of TRα1 completely blocked the T4-dependent increase in F-actin, while pre-incubation with anti-TRα2 IgG had no effect. To establish the role of TRΔα1 in TH-dependent F-actin organization in living cells, we grew astrocytes obtained from wild-type and TRα1 knockout mice (TRα1-/-) lacking TRΔα1 ±TH. Cells were fixed and stained with Alexafluor 448 phalloidin that specifically binds to F-actin. As shown in rat astrocytes, an extensive array of microfilaments was observed in T4-treated wild-type mouse astrocytes and these arrays were markedly reduced in the absence of TH. Importantly, the microfilament arrays of T4-treated TRα1-/- astrocytes were markedly reduced and identical to those observed in TH-free wild-type astrocytes. These data indicate that TRΔα1 is a mediator of T4-dependent actin polymerization in astrocytes.

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Hypothyroidism Induces Fos Expression in the Dorsal Motor Nucleus of the Vagus (DMV), Nucleus Tractus Solitarii (NTS), and Area Postrema (AP)

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Background: Extensive cardiovascular and gastrointestinal disorders, such as sinus bradycardia, hypertension, and altered gastric secretion and motility, are the main clinical symptoms of hypothyroidism that indicate vagal dysfunction. We reported that thyroid hormone feedback regulates TRH gene expression in brainstem TRH synthesizing neurons. The TRH-containing projections arising from these neurons innervate the DMV, which contains vagal motoneurons controlling the stomach and heart. NTS and AP serve as critical interface between peripheral visceral afferent and central autonomic efferent. Aim: To examine neuronal activation in the DMV/NTS/AP in different thyroid states using Fos expression as a marker. Methods: Adult male rats were treated differently with drinking water and a daily ip injection for 1, 2, 3, or 4 weeks: 1) Euthyroid: tap water and vehicle; 2) Hypothyroid: 0.1% propylthiouracil (PTU) and vehicle; 3) Hypothyroid+thyroxin (T4) replacement: 0.1%PTU and T4 (2 μg/100 g); 4) Hyperthyroid: tap water and T4 (10 μg/100 g). Cell counting of Fos positive neurons was expressed as mean±SE of 18 sections/rat and 4-6 rats/group from Bregma -14.08 mm to 13.30 mm. Results: The numbers of Fos positive neurons in the DMV, NTS and AP were low in euthyroid rats, but increased remarkably at 1 week after the induction of hypothyroidism. From the 1st week to the 4th week, Fos positive neurons were significantly higher compared with euthyroid controls. The Fos induction was prevented by simultaneous T4 replacement. There were significant negative correlations between T4 levels and the number of Fos positive neurons in the DMV ($r=-0.6388$, $P<0.008$), NTS ($r=-0.6741$, $P<0.003$) and AP ($r=-0.5622$, $P<0.004$). Double staining showed that most of Fos in the DMV of hypothyroid rats were in choline acetyltransferase neurons. Conclusion: These results indicate that thyroid hormone influences the DMV/NTS/AP neuronal activity. This action may contribute to the vagal-related visceral disorders observed in hypothyroidism. (Supported by DK 50255.)

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Program Number 94 Thyroid Hormone Action

Adenovirus 5-E1A-dependent Gene Activation of the Thyroid Hormone Receptor Is Regulated by the Cellular Context of Co-regulator and Adaptor Proteins

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The adenovirus type 5 early region 1A (Ad5-E1A) oncoprotein alters a wide variety of cellular processes involving transcription and chromatin remodeling in eukaryotes. In mammalian cells, Ad5-E1A functions primarily as a potent thyroid hormone (TH)-dependent activator of the thyroid hormone receptor (TR). However, we have observed that when Ad5-E1A is co-expressed in the cellular context of the yeast, *Saccharomyces cerevisiae*, it functions as a TR-specific constitutive coactivator which is down-regulated by TH. While we also find that Ad5-E1A binds directly to TR in a two-hybrid analysis, it is down-regulated by TH in yeast but maintained by TH in HeLa cells. Although the constitutive activation of full-length Ad5-E1A is confined to the N-terminal 1-82 amino acids, we observe that residues 4-29 are essential for Ad5-E1A binding to TR and its in vivo coactivator effects. To determine whether specific mammalian nuclear receptor co-regulatory and yeast chromatin remodeling adaptor proteins can modulate the effects of Ad5-E1A on TH-dependent gene activation by TR, studies were performed using a yeast Ad5-E1A/TR/TRE in vivo co-expression assay system. We observed that the N-CoR co-repressor could down regulate the observed constitutive transcriptional activation of TR by Ad5-E1A, while the presence of GRIP-1 coactivator reconstituted the Ad5-E1A induced pattern of enhanced TH dependent gene activation by TR observed in mammalian cells. Using yeast genetics, we also show that the substitution for wild-type of yeast strains with deletions of either a SWI2, SWI3, or SNF6 adaptor protein abrogated both TH/GRIP1- and Ad5-E1A dependent constitutive co-activator function, whereas yeast strains with a deletion of a GCN5 or ADA2 adaptor protein disrupted only TH/GRIP1-dependent gene activation but not Ad5-E1A-induced constitutive activation of TR. Taken together, these studies performed in a yeast eukaryotic in vivo co-expression system have demonstrated that constitutive and TH-induced Ad5-E1A-dependent gene activation of TR is regulated by the cellular context of N-CoR and GRIP1 co-regulators as well as SWI/SNF chromatin remodeling adaptor proteins.

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Program Number 95 Thyroid Hormone Action

Increased Mitochondrial Oxygen Consumption in Skeletal Muscle of Cold-acclimated Hypothyroid Rats

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The suppression of the thyroid gland function in cold-acclimated normal rats did not abolish the enhanced mitochondrial oxygen consumption (VO2) and UCP1 synthesis in brown adipose tissue (Amer J Physiol Endocrinol Metab, in press). The cold-acclimated hypothyroid rats were normothermic, thus suggesting that other thermogenic tissues were not affected by thyroidal suppression. We have now studied normal Wistar rats of 130 g BW, kept at 4°C for 2 months and thereafter given a single 131Iodine injection plus methimazole treatment. After 4 months in the cold, all hypothyroid rats were normothermic (37.5 to 38.1°C). Mitochondria from gastrocnemius and biceps femoral muscles were obtained to determine VO2. Results: Muscle VO2 in normal rats at room temperature (RT, 24°C, n=8) was 142±12 ng atoms oxygen/min/mg protein (units) rising to 192±13 units (+35%) in the cold (n=15, $P<0.001$). Hypothyroid muscle VO2 at RT (n=5) was 56±12 units and in the cold (n=18) 136±17 units (+143%, $P<0.001$). In the cold-exposed rats, hypothyroid muscle VO2 was 30% lower than normal muscle VO2 ($P<0.001$) albeit 2.6 times higher than hypothyroid muscle VO2 in the warm. T4 replacement increased VO2 in the cold to 153±9 units (+12.5%, n=8, $P<0.02$). Reserpine treatment of normal and hypothyroid rats reduced muscle VO2 to about one half of that in untreated controls ($P<0.001$). After acute (24-h) cold stress (data not shown), hypothyroid muscle VO2 failed to increase. Conclusion: Muscle requires T3 to initiate the thermogenic process. After cold-acclimation, suppression of T3 supply reduced but did not abolish muscle respiration. The data agree with recent reports suggesting that UCP3 is not a major determinant of muscle thermogenesis.

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Program Number 96 Thyroid Hormone Action

Regulation of Transferrin Gene Expression by Thyroid Hormone and Its Receptor

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Thyroid hormones (THs) regulate growth, development, differentiation, and metabolic processes by interacting with TH receptors (TRs) that bind to specific DNA sequences in the regulatory regions of target genes. Although much progress has been made in our understanding of the transcriptional regulation of TR target genes, little is known of the role of TRs in plasma proteins regulation. To investigate the role of TRs in plasma proteins expression, we examined several plasma proteins including transferrin, antitrypsin, albumin, and C3 expression in the human hepatocellular carcinoma cell lines. Our results indicate only transferrin is regulated by TH/TR. Transferrin is an iron binding protein expressed in all mammals, and mainly synthesized in the liver. Human hepatoma HepG2 cells, in which endogenous thyroid hormone receptor subtype alpha1 (TRalpha1) is expressed at a low level, were stably transfected either with expression plasmids encoding wild-type TRalpha1 or with the empty vector (yielding HepG2-Wt, and HepG2-Neo cells, respectively). The expressed levels of TRalpha1 protein in HepG2-Wt sub-lines were about 3- to 8-fold that of the control cell line. The growth rate of HepG2-Wt sub-lines was decreased by TH about 30-40%. Flow cytometric analysis indicated the growth inhibitory effect was mainly due to increase in the G1/G0 phase of the cell cycle. Immunoblot and Northern blot analysis revealed that exposure of HepG2-Wt sub-lines and HepG2-Neo cells to T3 induced time-dependent and dose-dependent increases in the abundance of transferrin mRNA and protein, with the extent of these effects correlating with the level of expression of TRalpha1 at several HepG2-Wt sub-lines. Cyclohexamide treatment did not eliminate transferrin induction by TH. Thus, our results implicate the induction of transferrin by TH is directed and mediated by the response element in the promoter region.

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Program Number 97 Thyroid Hormone Action

Effect of Thyroid Hormone on Sarcoplasmic Reticulum Ca2+-ATPase in Human Vascular Smooth Muscle Cells

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Human vascular smooth muscle cells (hVSMCs) express type 2 iodothyronine deiodinase (D2) and thyroid hormone receptors (TRs). The presence of D2 and TRs suggests that hVSMCs are physiological targets of the action of thyroid hormone. However, the target genes for T3 action in the hVSMCs remain unknown. Sarcoplasmic reticulum Ca2+-ATPase (SERCA2) expression is regulated by T3 in the cardiac myocytes. The SERCA2 gene encodes both SERCA2a (cardiac muscle type) and SERCA2b (smooth muscle type). In the present study, we investigated whether SERCA2a and 2b are expressed in hVSMCs, and whether T3 regulates SERCA2 expression, if any, in the cells. METHODS: Human aortic smooth muscle cells (HASMCs) were obtained from Cascade Biologics. Cells were grown in a medium (HuMedia-SG2, Kurabo, Japan), containing 5% FBS, bFGF, EGF, and insulin. Some cells were incubated with a medium (HuMedia-SD2), containing 1% FBS and heparin for induction of cell differentiation. For experiments, cells were incubated with thyroid hormone stripped (THS)-HuMedia-SG2 [SG2 (-)] or THS-HuMedia-SD2 [SD (-)] for 48 hours. Then, cells were further incubated with each medium in the presence or absence of 10-6 M T3 for 24 hours. SERCA2a and 2b mRNA levels were measured by RT-PCR and Northern analysis. RESULTS: In HASMCs, both SERCA2a and 2b mRNA were expressed, but the SERCA2b mRNA level was much higher than SERCA2a. SERCA2b mRNA level was higher in the cells cultured with SG2 (-) compared with SD (-). Neither SERCA2b mRNA level in the cells incubated with SG2 (-) nor SD (-) was altered by T3 treatment. SUMMARY: The present results indicate that SERCA2 gene is expressed in HASMCs but that the expression is not regulated by T3 in HASMCs. We suggest that the cell type-specific regulatory mechanism is involved in the induction of SERCA2 gene by T3.

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Program Number 98 Thyroid Hormone Action

Effect of Thyroid Hormone on Vascular Smooth Muscle Cell Growth

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Hypothyroidism is a risk factor for atherosclerosis, and thyroid hormone replacement for hypothyroidism protects arteries from development of atherosclerosis. Abnormal proliferation of vascular smooth muscle cells (VSMCs) is a key event in the formation of atherosclerosis. The present study was designed to determine whether thyroid hormone affects the growth of VSMCs. METHODS: Human aortic smooth muscle cells (HASMCs) were obtained from Cascade Biologics. Cells were grown in a medium (HuMedia-SG2, Kurabo, Japan), containing 5% FBS, bFGF, EGF, and insulin. For experiments, cells were pre-incubated with a medium containing 1% FBS for 48 hours, and the medium was replaced with HuMedia-SG2 containing [3H]thymidine in the presence or absence of thyroid hormone or iodide. After 24 hr incubation with the medium, [3H]thymidine uptake into the cells was determined. RESULTS: The addition of up to 10-5 M thyroid hormone or iodide did not alter the cell morphology. Thus, the highest concentration of thyroid hormone or iodide was set at 10-5 M. After 24- hour incubation with HuMedia-SG2 plus 10-5 M T4, T3, or rT3, [3H]thymidine uptake into the cells decreased by approximately 15% compared with HuMedia-SG2 alone. Furthermore, iodide exerts the same effects; [3H]thymidine uptake also decreased by approximately 30%, when cells were incubated with HuMedia-SG2 plus 10-5 M iodide. SUMMARY: T4, T3, and rT3 exert a growth-inhibitory effect in HASMCs at high concentrations. We suggest that iodine has a key role for the growth-inhibitory effect of thyroid hormone in HASMCs.

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Program Number 99 Autoimmunity

Randomized Trial of Intravenous versus Oral Steroid Therapy in Graves' Ophthalmopathy

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There are few effective treatments for severe Graves' ophthalmopathy (GO), which causes substantial morbidity. Because of the central role of congestive inflammation in GO, we performed a randomized, single blind trial of steroid monotherapy in GO subjects. Seventy euthyroid patients with untreated inflammatory GO of recent onset were randomly assigned to receive once weekly intravenous (i.v.) injections of methylprednisolone (0.5 g, then 0.25 g, 6 weeks each) or oral prednisolone for 12 weeks starting with 0.1 g/day, then tapering the dose by 0.01 g/week (cumulative doses of 4.5 and 4.0 g, respectively). At 3 months, the primary end point was a composite of improvements in measures of proptosis, lid fissure width, rate of diplopia in normgaze, intraocular pressure in upgaze, eye muscle diameter, and patient's assessment of quality of life. Treatment with i.v. steroids resulted in significant and sustained improvement. At 3 months, 26 of 35 patients (74%) in the i.v. group had a treatment response, as compared with 18 (51%) of those in the oral group ($p<0.01$). Improvements over base-line values for various measures of both disease severity, including visual acuity, diplopia rate, chemosis, as well as quality of life were significantly greater in the i.v. group. Longitudinal analysis showed that the treatment response was rapid and did not diminish over time. TSH-R antibody titers markedly decreased during i.v. steroid therapy. Additional forms of treatment, e.g., decompression surgery, were required less frequently in the i.v. group. Intravenous steroids were fairly tolerated, with significant differences in rates of adverse events between the two groups. A significant decrease of bone mineral density was noted in the oral group only. In conclusion, i.v. steroid pulse therapy was more effective than oral steroid treatment resulting in rapid, significant, and sustained improvement in patients with severe GO.

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Program Number 100 Thyroid and Development

Visual Processing Deficits in Infancy following Maternal Hypothyroidism and Congenital Hypothyroidism

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Thyroid hormone (TH) is essential for the developing visual system and structures belonging to primary visual pathway have unique ontogenies of TH requirements. We reported previously that 3-month old infants lacking TH in early pregnancy due to maternal hypothyroidism or later pregnancy due to congenital hypothyroidism (CH) showed contrast sensitivity deficits. However, it was not clear whether these deficits persist. Presently, we evaluated infants at 3, 4.5 and 6 months of age for visual acuity and contrast sensitivity using a sweep visual evoked potential (VEP) technique. The sample included 16 offspring of hypothyroid women, 10 children with early-treated congenital hypothyroidism (CH), and 31 normal controls of non-hypothyroid mothers. In infants fitted with electrodes over the occipital cortex, we observed rapidly changing sinusoidal gratings (bars) of varying contrast or thickness (spatial frequency). Three contrast-sweep conditions and 2 spatial frequency sweep conditions at 30% and 80% contrast were recorded. Evoked potentials synchronized to computer-generated stimuli were extrapolated to determine 3 contrast and 2 spatial frequency thresholds. These were fitted to an exponential "contrast sensitivity" function to yield parameters of peak sensitivity (contrast), spatial frequency (acuity), and goodness of fit. Compared to controls, offspring of hypothyroid mothers showed significantly reduced peak sensitivity at 3, 4.5, and 6 months ($p<.01$) while CH differed only at 3 months suggesting catch-up. Both showed reduced goodness of fit at 3 months ($p<.01$). In the maternal hypothyroid group, correlations between mothers' TSH levels and infant visual processing indices indicated that adequate TH levels are necessary during the first 2 trimesters of pregnancy for normal contrast sensitivity development. Findings on contrast sensitivity support a view that the developing visual system in the human is vulnerable to a lack of TH while the kind and persistence of deficit will reflect timing and type of TH deficiency.

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Program Number 101 Thyroid Diseases

Management Practices of Thyroid Specialists in the Diagnosis and Treatment of Subclinical Hyperthyroidism

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Subclinical hyperthyroidism is a commonly encountered condition for which prospectively derived evidenced-based management guidelines do not exist. A case-based mail survey was conducted to solicit opinions from American Thyroid Association (ATA) members about the management of these patients. A survey was mailed to all clinical physician members of the ATA. 62% completed the survey. Four hypothetical clinical case vignettes of subclinical hyperthyroidism were presented and respondents were asked whether they would order the following tests: 1)thyroid stimulating immunoglobulins (TSI) 2)antithyroid antibodies (TPO or TG) 3)radioactive iodine uptake (RAIU) 4)thyroid scan 5)thyroid ultrasound or offer any form of treatment. Cases 1 and 2 were young asymptomatic women with either a mildly suppressed or undetectable TSH. Cases 3 and 4 were older osteopenic women with either a mildly suppressed or undetectable TSH. Respondents were more likely to order a TSI ($p<0.001$), RAIU ($p<0.001$), and scan ($p=.001$) in the younger cases with an undetectable TSH. Similarly, respondents were significantly more likely to order a RAIU ($p<0.001$) and scan ($p=.003$) in older patients with an undetectable TSH. Clinicians were more likely to offer treatment to younger patients with an undetectable TSH (36%) than to those with a mildly suppressed TSH (13%) irrespective of underlying thyroid diagnosis ($p<0.001$). Additionally, treatment was more likely to be offered to the older osteopenic woman with an undetectable TSH (66%) than to the woman with a slightly low TSH (34%). In conclusion, clinician members of the ATA were more likely to request additional testing for patients with an undetectable TSH compared to a mildly suppressed TSH and were more likely to recommend some form of treatment to these individuals. In patients with advanced subclinical hyperthyroidism, as indicated by undetectable TSH, thyroid specialists were more likely to consider treatment of the older osteopenic patient than the younger asymptomatic patient.

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Program Number 102 Thyroid Diseases

Atrial Fibrillation Predicts Mortality in Hyperthyroidism

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Cardiovascular symptoms and signs are common in thyrotoxicosis and studies have highlighted increased vascular mortality (NEJM 1998,338,712-8). To identify risk factors for poor vascular outcome, we documented cardiovascular status in a consecutive series of 405 hyperthyroid subjects at hospital presentation compared with 405 age- and sex-matched community-based euthyroid controls. All completed a structured cardiovascular history and examination, resting 12-lead ECG and 24-hour Holter recording. All hyperthyroid subjects were re-investigated after treatment with thionamides and/or 131-I. The median age was 50.0 years, 317F, 88M. Median systolic lying and standing BP were higher in patients than controls ($P<0.0001$) as was median resting pulse ($P<0.001$). Persistent AF was evident on ECG and Holter monitoring in 26 patients and 4 controls (7.5 vs 1.2% $P<0.0001$); Holter monitoring detected paroxysmal AF in a further 6 (1.7%) patients and 1 (0.3%) control. Atrial and ventricular salvoes were both more common in patients (26% vs 18% and 3% vs 0.3%, $P<0.05$). Reinvestigation of 193 subjects when biochemically euthyroid revealed that differences in rhythm between patients and matched controls on ECG or monitoring were no longer evident. During a median follow-up of 13 months, 7 patients (no controls) have died. Multiple regression analysis revealed that independent risk factors for the presence of persistent AF at presentation were age ($P<0.0001$) and past history of ischemic heart disease or heart failure ($P<0.002$) but not biochemical severity of hyperthyroidism. The presence of AF on Holter monitoring was predicted by history of hypertension ($P<0.0001$) while atrial and ventricular salvoes/ectopics were predicted by age ($P<0.0001$). Mortality was predicted by the presence of AF on presenting ECG ($P<0.05$). We suggest that the marked prevalence of abnormalities of cardiac rhythm in hyperthyroid subjects at presentation accounts for previous findings of increased vascular mortality; better therapeutic targeting of those with AF may reduce this mortality.

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Program Number 103 Thyroid Diseases

Mutations in KCNE3 and KCNE4 Potassium Channel Genes Are Associated with Susceptibility to Thyrotoxic Hypokalemic Periodic Paralysis

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Hypokalemic Periodic Paralyses (HypoKPP) comprise diverse diseases characterized by low serum potassium and acute and reversible attacks of severe muscle weakness. The most prevalent causes of HypoKPP are thyrotoxic hypokalemic periodic paralysis (THypoKPP), secondary to thyrotoxicosis, and familial hypokalemic periodic paralysis (FHypoKPP), an autosomal dominant disease. Symptoms of paralysis are almost identical in both diseases, distinguished by thyrotoxicosis present in THypoKPP. Genetic defects in ionic channel genes have been identified as causes of FHypoKPP, as mutations in calcium (CACN1AS), sodium (SCN4A) and potassium (KCNE3) genes, but none in THypoKPP. Since both diseases are similar, we tested the hypothesis that THypoKPP could carry the same mutations described in FHypoKPP, being the paralysis a genetically conditioned complication of thyrotoxicosis. In 15 patients with THypoKPP, using target-exon PCR, CSGE screening, and direct sequencing of aberrant shifts, we excluded known mutations in CACN1AS and SCN4A genes. In contrast, we were able to identify, initially, a R83H mutation in the KCNE3 gene in one sporadic case of THypoKPP, a 44-year-old man who had been asymptomatic until developing thyrotoxicosis caused by Graves' disease; we confirmed the disease-causing mutation in 2 of 3 descendants. R83H was recently found in two FHypoKPP unrelated families, in which the R83H-KCNE3 mutant decreased outward potassium flux, resulting in a more positive resting membrane potential. Since we found a mutation in the third member of the KCNE gene family, we thereafter screened the remaining potassium-channel genes (KCNE1-4) and discovered a new defect, a M53V mutation in the KCNE4 gene in one kindred, whose father reported paralysis during hyperthyroidism 30 years ago. Therefore, in this report we identified two genetic defects in THypoKPP, adding this disorder in the group of channelopaties, and revealing its genetic heterogeneity.

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Program Number 104 Thyroid Diseases

An Examination of the Relationship between Coronary Artery Calcium Scores and Serum TSH in Patients Undergoing Non-contrast Electron Beam Computed Tomograpy (EBCT) at Walter Reed Army Medical Center (WRAMC)

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Objective: Overt hypothyroidism is an established risk factor for coronary artery disease (CAD). This risk is largely conferred by the associated atherogenic dyslipidemia found in overtly hypothyroid patients. Though varying degrees of dyslipidemia have also been described in subjects with subclinical hypothyroidism (SCH), defined as the finding of elevated serum TSH concentrations with normal free thyroid hormone levels, the association between SCH and CAD remains uncertain. Non-contrast electron beam computed tomography (EBCT) is a noninvasive screening tool to assess degree of coronary artery calcification. Studies have shown that patients with high EBCT scores are at increased risk for coronary events. We sought to examine the relationship between serum TSH concentration and EBCT score in a large group of patients receiving screening EBCTs at Walter Reed Army Medical Center (WRAMC). Methods: A database of 10,000 subjects who had undergone EBCT since its inception at WRAMC was reviewed. EBCTs were obtained in asymptomatic subjects through both physician and self-referral. EBCT data for each subject were expressed in terms of total calcium score and calcium score for each major coronary artery. For each subject, thyroid function test data were gathered through review of a computerized laboratory database dating to 1990. Results: Of the approximately 2,000 patients who underwent EBCT whose data have been analyzed to date, 600 had available TSH values. EBCT values greater than 300 were found in 49 patients. The mean peak TSH value in these patients was 2.38 ± 1.59 mU/L (0.04-8.04). Linear regression analysis showed a strong correlation between EBCT score and highest documented value of TSH ($p = 0.005$). Conclusion: In the subset of patients at higher risk for the development of complications of CAD (EBCT score >300), TSH was found to correlate linearly with total EBCT calcium score. The mechanism and clincial implications of this association remain unknown.

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Program Number 105 Cell Biology

Transforming Growth Factor-β1 (TGF-β1) Up-regulates Pendrin (PDS) Gene Expression: It Acts by Modulating a Thyroid Transcription Factor-1 (TTF-1) Promoter Element That Also Controls Constitutive PDS Expression in the Thyroid

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TGF-β1 regulates the growth, differentiation, and immune responsiveness of many different cell types. In thyrocytes it decreases growth and function, including concentrative iodide uptake and sodium iodide symporter (NIS) gene expression. The PDS gene is suggested to be an apical iodide porter capable of transferring iodide concentrated in the thyrocyte to the follicular lumen. In this report we show that TGF-β1 increases PDS, whereas it decreases NIS gene expression; we additionally describe a transcriptional mechanism to explain the TGF-β1 action as well as constitutive thyroid expression of the PDS gene. In Northern analyses, TGF-β1 increased Pendrin but decreased NIS mRNA levels in a time and concentration-dependent manner. Approximately 2 kb of 5' flanking sequence was cloned from a rat FRTL-5 cell genomic library. The transcriptional start site of the Pendrin gene is at -492 bp, if A in the ATG initiation codon is +1; an intronic sequence lies between the two sites. The 2 kb 5'-flanking sequence attached to a luciferase reporter gene expressed both significant basal activity and TGF-β1 responsiveness in FRTL-5 thyrocytes, but not in CHO-K1 or COS-7 cells. Deletion chimeras revealed that the TGF-β1 responsive element was at -1650 to -1618 bp and that the same region was important for constitutive expression of the PDS gene in FRTL-5 thyrocytes. This region contains a putative TTF-1 consensus sequence with which FRTL-5 cell nuclear extracts and recombinant TTF-1 form a specific DNA-protein complex that is supershifted by anti-TTF-1. Overexpression of TTF-1 cDNA in CHO-K1 cells caused significant PDS-reporter chimera expression. Additionally, mutations of the TTF-1 core (CTTG to CTaG) decreased TGF-β1-responsiveness. We conclude that a TTF-1 element between -1650 to -1618 bp of the rat PDS promoter, which we have cloned and characterized, confers constitutive PDS gene expression in thyrocytes and mediates its TGF-β1 responsiveness by an unknown mechanism.

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Program Number 106 Thyroid Hormone Metabolism

Identification of MCT8 as a Major Thyroid Hormone Transporter

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Transport of thyroid hormone across the cell membrane is required for thyroid hormone action and metabolism. Iodothyronine uptake in different tissues is mediated by a T type amino acid transporter (TAT). Recently, rat and human TAT1 were cloned and shown to transport the aromatic amino acids Phe, Tyr and Trp but not iodothyronines. TAT1 belongs to the monocarboxylate transporter (MCT) family, and is far more homologous to MCT8 than to other members. As the function of MCT8 is unknown, we cloned rat MCT8, tagged it with FLAG and tested it for iodothyronine transport in Xenopus oocytes. The FLAG tag confirmed expression of MCT8 at the plasma membrane of cRNA-injected oocytes.
For transport studies, oocytes were injected with 4.6 ng rat MCT8 cRNA, and after 3 days they were incubated for 2-60 min at 25 °C with 10 nM 125I-iodothyronines. MCT8 cRNA induced a dramatic, ~10-fold increase in uptake of T4 and T3 that was only linear for 2 min and independent of sodium. MCT8 was much more active than all other iodothyronine transporters tested so far. However, MCT8 did not transport Phe, Tyr or Trp. Similar uptake rates were observed with T4, T3, rT3 and 3,3'-T2. T4 transport was strongly inhibited by 10 microM Triac (95%), BrAcT3 (91%), D-T4 (91%), D-T3 (91%), L-T4 (70%), L-T3 (70%), MIT (65%) and DIT (36%). Iodothyronine transport was also strongly inhibited by 100 microM BSP (93%) but less by 10 microM Tyr (36%) and Trp (22%) and not by taurocholate. T3 uptake was less affected by inhibitors than T4 uptake. Km values for T4, T3 and rT3 were 2-5 microM. In conclusion, we have identified MCT8 as a very active thyroid hormone transporter. Its physiological relevance remains to be determined.

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Program Number 107 Cancer

Mice with a Mutation in the Thyroid Hormone Receptor β Gene Spontaneously Develop Thyroid Carcinoma: a Mouse Model of Thyroid Carcinogenesis

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The molecular genetic basis of thyroid carcinogenesis is not well understood. Most of the existing models of thyroid cancer only rarely show metastatic spread, and this has limited progress in the understanding of the molecular events in thyroid cancer invasion and metastasis. We have recently generated a mutant mouse by introducing a dominant negative mutant thyroid hormone nuclear receptor gene, TRβPV, into the TRβ gene locus. In this TRβPV mouse, the regulation of the thyroid-pituitary axis is disrupted, leading to a mouse with high levels of circulating thyroid stimulating hormone and extensive hyperplasia of follicular epithelium within the thyroid. The weights of thyroid increased with age, with a12-,18- and 36-fold increase at the ages of 3-4, 5-7 and >12 months, respectively, as compared with wild-type mice. Importantly, as $TR\beta^{PV/PV}$ mice, but not $TR\beta^{PV/+}$ mice, aged, metastatic thyroid carcinoma developed. Histological evaluation of thyroids of 5-14 month-old mice showed capsular invasion (91%), vascular invasion (74%), anaplasia (35%) and metastasis to the lung and heart (30%). These findings suggest that thyroid carcinogenesis is a multi-step process with sequential capsular invasion, vascular invasion, anaplasia and eventually metastasis. cDNA microarray analyses of RNAs prepared from the thyroids of 5-month old $TR\beta^{PV/PV}$ and wild-type mice indicate that the expression of TRβPV gene led to an up-regulation of 200 genes and down-regulation of 95 genes. One gene, cyclin D1, was activated ~8-fold, which was confirmed by Northern blot analysis. These results are consistent with the findings in human thyroid carcinoma that show a high frequency of over-expression of cyclin D1. These mice provide a unique model for clinically important parameters in human thyroid cancer, namely, invasion and metastasis. Importantly, this model provides an unusual opportunity to study the alterations in gene regulation that occur during progression and metastasis in a predictable fashion.

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Program Number 108 Cancer

The PAX8/PPAR-γ Putative Follicular Thyroid Carcinoma Oncogene Down-regulates Expression of the TRAIL-related Death Receptor 5

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We have previously confirmed frequent expression in follicular thyroid carcinoma (FTC), of a fusion gene between the thyroid specific transcription factor PAX8 and the peroxisome proliferator receptor gamma (PPAR-γ). Further, we have demonstrated that this fusion gene (PAX8/PPAR-γ) decreases apoptosis after transfection into thyroid cell lines, through unknown mechanisms. Wild-type PPAR-γ (wt-PPAR-γ) is a transcription factor, affecting expression of a broad range of genes. Activation of wt-PPAR-γ by ligand binding induces apoptosis in cell lines, including breast, lung and thyroid. We hypothesize that PAX8/PPAR-γ inhibits wt-PPAR-γ altering expression of apoptosis-related genes, some of which may be important in the development of FTC. An immortalized normal thyroid cell line (NT cells) was transfected in triplicate with the PAX8/PPAR-γ fusion gene, engineered into the pCDNA3.1 expression vector, or with vector alone. mRNA was isolated at 24, 48 and 72 hours post-transfection, using TRIzol reagent. Following reverse transcription and 32P labelling, cDNA was hybridized to nylon membrane arrays, spotted with 1176 cancer-related genes (Clontech). Changes in gene expression were confirmed using Northern blots. Analysis of the expression arrays demonstrated significant (>2-fold) down regulation of the Death Receptor 5 gene (DR5), within 24 hours of PAX8/PPAR-γ transfection, at a time when apoptosis is known to be inhibited. Northern blots confirm significant (3- to 5-fold) down-regulation of DR5 at 24 and 48 hours after transfection. DR5 is a member of the TRAIL (TNF-related apoptosis-inducing ligand) receptor system, and TRAIL binding to DR5 induces apoptosis. PPAR-γ activation enhances TRAIL-induced apoptosis in several cell models, though the mechanism remains unknown. Our data suggest that PAX8/PPAR-γ may inhibit wt-PPAR-γ activity, reducing expression of DR5. Subsequent impaired DR5 signalling in response to TRAIL may partly explain the lower rates of apoptosis seen following PAX8/PPAR-γ expression. TRAIL signaling may be important in the development of FTC.

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Program Number 109 Cell Biology

Administration of Recombinant Adenoviruses Expressing Antiangiogenic Factors Blocks Goitrogenesis in Mice

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In vivo models of goiter have shown that endothelial cell proliferation precedes that of follicular cells, consistent with the hypothesis that vascularisation is a prerequisite for thyroid growth. Several angiogenic growth factors are produced by thyroid follicular cells and we have investigated their role in goitrogenesis using recombinant adenoviruses (RAd) to block their actions. RAds expressing a dominant negative form of fibroblast growth factor receptor 1 (DN-FGFR1) and a secreted, truncated form of human Tie2, the receptor for the angiopoietins, were prepared and a RAd expressing a soluble form of VEGFR1 (Flt) was kindly given to us (Dr Mulligan, Harvard). Using these viruses in vitro and in vivo we have defined the role of these angiogenic receptors in thyroid follicular cells and their role in goitrogenesis. RAds were administered to mice at the time of dietary change to low iodine chow, methimazole (0.15%) and $NaClO_4$ in the drinking water. Mice (4 per treatment) were followed for 14 days and no adverse effects on weight and general well being were seen. Efficacy of treatment was assayed by measurements of TSH, T3 and T4. RAd-DN-FGFR1 inhibited human and FRTL5 thyroid cell growth in vitro and significantly prevented goiter formation (by 55%), assessed by thyroid lobe weight. RAd-Tie2(s) prevented human and FRTL5 thyroid follicular cell adhesion in vitro but had no significant effects on goiter weight. RAd-Flt(s) had no observed effect on thyroid follicular cells in vitro, and in vivo the weight of goiter formed was less although this did not reach statistical significance. The effects of combinations of RAd-DN-FGFR1, RAd-Tie(s) and RAd-Flt(s) were not additive. The combination of the 3 RAds however prevented goiter formation completely. We conclude that angiogenic factors orchestrate goitrogenesis cooperatively and that prevention of angiogenesis may impact clinically in hyperplastic or malignant cell growth.

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Program Number 110 Thyroid Hormone Action

The Human Type 2 Iodothyronine Selenodeiodinase (D2) Is Ubiquitinated via Interaction with the Mammalian Ubiquitin Conjugases MmUBC7 and MmUBC6

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The critical homeostatic role of D2 in thyroid hormone action is contingent on its low Km(T4) and short half-life (~45 min). An endoplasmic reticulum (ER) resident selenoprotein, D2 is deactivated via selective ubiquitination and proteasomal degradation, a process accelerated by interaction with its substrates (T4 or rT3). Heterologous expression of D2 in *S. cerevisiae* indicated that the ER-associated ubiquitin ligases (E2s) Ubc6p and Ubc7p are involved in D2 ubiquitination. To investigate the role of these E2s in D2 ubiquitination in mammalian cells, murine MmUBC6 and MmUBC7 along with inactive mutants in which the active site Cys residue was converted to Ser (SerUBC6 and SerUBC7) were coexpressed with hD2 in HEK-293 cells. While wild type E2s had no effect on hD2 activity alone or in combination, coexpression with both mutants together increased D2 activity 2 to 3 fold and inhibited the substrate-induced loss of D2 activity. D1 and D3 activities were not affected. To ascertain whether hD2 interacts directly with these E2s, GST fusion constructs of the selenodeiodinases (D1, D2, D3) produced in bacteria were used in GST-pulldown assays against *in vitro* translated, 35-S methionine-labeled E2s and E2 mutants. Both MmUBC7 and SerUBC7, but not MmUBC6 or SerUBC6, interacted with GST-D2 but not GST-D1 or D3, consistent with our earlier observation that D1 and D3 are not ubiquitinated. Studies with truncated GST-D2 proteins revealed that MmUBC7 interacts with the carboxy terminal region of D2. In agreement with this result, hD2 activity in HEK-293 cells was increased ~2 fold when wild type hD2 was coexpressed with an inactive carboxy-terminal fragment of D2, but not when coexpressed with D1 or D3. These results confirm that MmUBC7 and probably MmUBC6 are rate-limiting in the basal and substrate-accelerated ubiquitination of D2. The site of MmUBC7-D2 interaction maps to the carboxy terminal region of D2.

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Program Number 111 Thyroid Diseases

The Impact of Treatment of Overt and Subclinical Hyperthyroidism

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Introduction: Skeletal muscle weakness is a common finding among overtly hyperthyroid (OH) patients but there are no reports on muscle strength in patients with subclinical hyperthyroidism (SCH). Methods: We measured 16 lower extremity muscle strength (MS) variables using a Cybex II dynamometer, thigh muscle cross sectional (TCS) area was assessed by CT and total muscle mass (TMM) determined by DPX in subjects with OH (n=27), SCH (n=22) defined as TSH<0.1, normal fT4, T3, and euthyroid controls, EC (n=42). Subjects were studied both prior to treatment of hyperthyroidism and 6-12 months following restoration of an euthyroid state. Results: OH Group–16/16 MS variables improved significantly (P<0.001) and both TCS and TMM also showed significant increases (p<0.001). SCH Group–6/16 MS variables improved significantly (p<0.05), 4/16 showed borderline improvement (p=0.05), TCS area also increased (P<0.003) while small increases in total TMM did not reach significance (p>0.1). EC Group–No significant changes were noted in 14/16 MS variables, TCS or TMM. Conclusion: Increases in muscle strength and thigh muscle cross-sectional area occur following treatment of both OH and SCH while significant increases in total TMM occurs only in OH subjects. These findings may influence treatment decisions regarding SCH particularly among the elderly.

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Program Number 112 Thyroid Diseases

Thyroid Disorders and Smoke Exposure: Complex Associations in NHANES III

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OBJECTIVE: To assess relationships between active/passive smoking exposures and thyroid outcomes. METHODS: Data from 16,046 adult participants in the Third National Health and Nutrition Examination Survey (NHANES III) were analyzed. Smoke exposures were defined as active (serum cotinine > 0.15ng/mL) and second-hand (cotinine detectable but < 0.15ng/mL). Outcomes were serum TSH <0.1mU/L or >4.5mU/L, presence of anti-microsomal or anti-thyroglobulin antibodies >0.5, and goiter. Relationships between smoke exposure and outcomes were assessed with STATA 7.0, using weighted logistic regression models, and adjusted for age, gender, race/ethnicity, and urinary iodine. RESULTS: After adjustment, active smoking was associated with 40% decreased odds of having TSH <0.1mU/L and 42% decreased odds of TSH >4.5 mU/L versus normal TSH level (ORs 0.60 [CI 0.40,0.92] and 0.58 [CI 0.45,0.74], respectively). Smoking was associated with 31% decreased adjusted odds of having thyroid autoantibodies (OR 0.69 [CI 0.59,0.80]). There was no significant association between goiter and smoke exposure (OR 1.28 [CI 0.85,1.91]). Analyses of ever-smokers (active or self-reported) showed a significant relationship only for thyroid autoantibody status (OR 0.84 [CI 0.73,0.95]), suggesting reversibility of the smoke effect or increased prevalence of outcomes in ex-smokers. Unadjusted analysis showed second-hand smoke exposure was associated with decreased odds of TSH >4.5 mU/L (OR 0.67 [CI 0.50, 0.90]); however, multivariate analyses adjusting for participant characteristics failed to demonstrate significant relationships between second-hand smoke and any outcomes. CONCLUSION: Active tobacco smoke exposure is associated with apparent decreased risk of laboratory evidence of hyperthyroidism, hypothyroidism, and thyroid autoantibodies. The basis for these relationships is enigmatic but might be attributable to diminished cytokine or immune modulator production in smokers.

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Program Number 113 Thyroid Hormone Metabolism

Overexpression of the Type 2 Deiodinase in Large or Widely Metastatic Follicular Thyroid Carcinoma Causes Increased Efficiency of Peripheral Thyroxine-to-Triiodothyronine Conversion

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Thyroid function is normally undisturbed in patients with thyroid carcinoma. The rare exceptions include hyperthyroidism due to functional metastases from well-differentiated thyroid carcinoma and release of preformed thyroid hormone (malignant pseudothyroiditis). We have recently identified 3 patients with large or widely metastatic follicular thyroid carcinomas who had a persistently increased serum ratio of T3 to T4 in the absence of autonomous production of thyroid hormone by the tumor. With an intact hypothalamic-pituitary-thyroid axis, these patients' serum TSH values were in the normal range. However, when given excess levothyroxine, TSH was suppressed with high or high normal T3 and low or low normal T4 values. A potential explanation would be tumor-mediated T4 to T3 conversion, with serum T3 replacing T4 as the predominant regulator of TSH secretion. We were able to investigate this possibility by assaying type 1 and type 2 iodothyronine selenodeiodinase (D1 and D2) activity in 6 samples of a 965g mediastinal tumor resected from one of these patients. The Km(T4) and Km(rT3) for D1 and the Km(T4) for D2 in the tumor homogenates were typical for these enzymes. The Vmax for D2 was about 10 fold higher than in normal human thyroid tissue using similar conditions. In agreement with this high activity, 73±3% (mean ±standard error) of T4 to T3 conversion at approximately physiologic free T4 concentration (2 nM) was catalyzed by tumor D2. This contrasts with values of 22-35% in homogenates of normal human thyroid, in which D1 catalyzes the bulk of T4 to T3 conversion. Resection of this tumor, leaving the thyroid intact, normalized the serum T3/T4 ratio. The clinical data, supported by direct measurements of deiodination in tumor tissue, illustrate that increased T4 to T3 conversion in follicular thyroid carcinomas, probably by D2, will cause a significant alteration in serum T4 and T3 concentrations.

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Program Number 114 Thyroid Hormone Metabolism

Polymorphisms in Thyroid Hormone-related Genes Are Associated with Serum Thyroid Parameters in Normal Subjects

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Introduction: Single nucleotide polymorphisms (SNPs) in genes involved in thyroid hormone metabolism may affect thyroid hormone bioactivity. We investigated the occurrence and possible effects of SNPs in the deiodinases (D1-D3), the TSH receptor (TSHR), and the T3 receptor beta (TRβ) genes. **Methods:** SNPs were identified in Public and Celera databases or by sequencing of genomic DNA from 15 randomly selected individuals (30 alleles). Genotypes for the identified SNPs were determined in 156 healthy blood donors and related to serum T4, FT4, T3, rT3, and TSH levels. **Results:** Six SNPs of interest were identified, 4 of which have not yet been published. Three are located in the 3'-UTR: D1a-C/T (distribution: 65 CC, 76 CT, 15 TT), D1b-A/G (124-32-0) and D3-T/G (112-42-1). Two are missense SNPs: D2-A/G (58-75-23) and TSHR-C/G (127-29-0). One is a silent SNP: TRβ-T/C (145-10-0). D1a-T was associated with higher rT3 levels in a dose-dependent manner (CC : CT : TT = 0.29±0.01 vs 0.32±0.01 vs 0.34±0.02 nmol/l (mean±se), $P<0.01$ tested for linear regression), with higher rT3/T4 ($P<0.01$), and with lower T3/rT3 ($P<0.01$). D1b-A/G showed opposite relations: rT3/T4 was highest ($P<0.05$) and T3/rT3 lowest ($P=0.07$) in D1b-AA. There was no significant effect on rT3. TSHR-C/G showed a significant relation with TSH levels. TSH was highest in TSHR-CC (CC : CG = 1.38±0.07 vs 1.06±0.14 mU/l, $P<0.05$). TSH/FT4, TSH/T3, and TSH/T4 were higher in TSHR-CC ($P=0.05$, $P=0.05$, $P=0.07$, respectively). No associations were found for the other SNPs. **Conclusion:** We analyzed 6 SNPs in 5 thyroid hormone-related genes and found significant associations of 3 SNPs in 2 genes (D1, TSHR) with serum thyroid parameters in a normal population. The genotype-dependent effects we found could be clinically relevant. Association with thyroid disease-related endpoints can now be investigated in population studies.

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Program Number 115 Thyroid Nodules and Goiter

Comparative Effects of L-Thyroxine (L-T4) and 3,5,3´Triiodothyroacetic Acid (TRIAC) on Euthyroid Goiter and Peripheral Parameters

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Euthyroid goiter is usually treated with TSH-inhibitory doses of L-T4. Since TRIAC also decreases TSH levels, the following study was perfomed: 33 euthyroid goitrous female patients without evidence of cancer or chronic thyroiditis were randomized to TRIAC (23.4 ug/kg) (n=18) or L-T4 (2 ug/kg) (n=15) treatment during 12 months. Goiter volume, bone lumbar and femoral densitometry; serum osteocalcin (OC); deoxypyridinoline (d-pyr); TSH; free T4; Total, HDL and LDL cholesterol and triglycerides were measured before and at the end of the study period. Non-parametric and Student´s t test analysis were performed.
Average ± SEM age was 50 ± 2 (TRIAC) and 48 ± 3 (L-T4), BMI was 28.8 ± 7.3 and 24.1 ± 6.1, respectively. TSH values (uU/ml) in the TRIAC group were: 1.87 ± 0.33 (basal) and 0.14 ± 0.04 (after), while the L-T4 group had 2.05 ± 0.51 (basal) and 0.11 ± 0.03 (after). Free T4 values (ng/dl) of the TRIAC group decreased from 1.31 ± 0.07 to 0.73 ± 0.09, while for L-T4 they increased from 1.39 ± 0.06 to 1.92 ± 0.19 ($p< 0.0001$ vs TRIAC). Thyroid volume decreased 51.18 ± 6.05 % in the TRIAC patients and 33.55 ± 5.67% in the L-T4 group ($p< 0.04$). Lumbar t score decrease was 0.44 ± 1.3 (TRIAC) and 0.90 ± 1.51 (L-T4) , while femoral decrease was 1.30 ± 1.67 (TRIAC) vs. 5.11 ± 2.21 (L-T4) (both were n.s.). Percent increase of serum d-pyr was 68.4 ± 18.2 (TRIAC) vs 63.8 ± 26.9 (L-T4) (n.s.), while OC increased 84.7 ± 72.5 % (TRIAC) and 46.5 ± 34.4 (L-T4) (n.s.). No differences in the changes of lipid profile and glycemia were observed between both groups. The present results show that TRIAC is more effective than L-T4 in the reduction of goiter size, with comparable effects on peripheral parameters.

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Program Number 116 Cancer

The Clinical Utility of Thyroglobulin Messenger RNA Quantification in the Monitoring of Patients with Differentiated Thyroid Carcinoma

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Thyroglobulin messenger RNA (Tg mRNA) testing has been suggested as an alternative for overcoming the actual limitations of the protein measurement. However, the few previous studies reported discordant conclusions about its utility in the follow-up of patients with differentiated thyroid carcinoma (DTC). The aim of this study was to compare the diagnostic performance of Tg mRNA and Tg protein measurement in the detection of residual or recurrent thyroidal cells in DTC. Peripheral blood Tg mRNA was quantified by RT-PCR on Light Cycler in 63 patients with DTC. Simultaneously, TSH, Tg and anti-Tg autoantibodies (anti-TgAb) were measured in serum. All patients had been treated by total thyroidectomy and radioiodine ablation. The last ^{131}I whole body scan (^{131}I WBS) was chosen as a reference for the classification of patients into two groups: those with residual or recurrent thyroidal cells (n = 23) and patients without any evidence of disease (n = 40). Tg mRNA was positive in 14/23 patients with positive ^{131}I WBS and in 8/40 of patients with negative ^{131}I WBS. Diagnostic sensitivity and diagnostic specificity were 61% and 80%, respectively, versus a sensitivity of 78% and a specificity of 65% for Tg protein. Tg mRNA sensitivity was particularly low (50%) in patients with elevated TSH after thyroid hormones withdrawal (n = 10). No Tg mRNA was detected in patients with serum Tg >1000 μg/L (n = 2). In patients with anti-TgAb, Tg mRNA was positive in 3/14 versus 4/14 for Tg protein (2/4 were negative for Tg mRNA). In conclusion, our results do not support the use of Tg mRNA testing as an alternative to Tg protein measurement. At best, it could be used jointly with Tg protein to enhance its sensitivity or as a marker of the mobility of thyroidal cells in the body.

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Program Number 118 Cancer

Differentiated Thyroid Cancer (DTC) Patients with Detectable Preoperative Serum TgAb Have Higher Risk of Recurrent/Persistent Disease (R/P)

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We previously reported that 2-year postoperative serum Tg levels (+T4Rx) have prognostic value for TgAb-negative DTC patients, whereas serial serum TgAb levels have prognostic value for DTC patients with interfering TgAb (~20%) that preclude serum Tg monitoring. This study evaluates the prognostic significance of serum TgAb detected at thyroidectomy (Tx). PATIENTS: 287 DTC patients had serial TgAb(RIA), physical examination, and anatomic or isotopic imaging until R/P was detected, or for >5 years when judged disease-free (DF) at end of follow-up (FU). 46 patients with TgAb detected at Tx. (Ab+) had median FU=5.5 (range 3.2-8.2) years. 241 patients with no TgAb detected at any time (Abneg) had median FU=7.6 (1.9-17.7) years. ANALYSES: Preoperative clinical characteristics and %R/P during FU were compared for the Ab+ vs. Abneg groups. In addition, serum TgAb concentrations of Ab+ patients with R/P detected were compared with Ab+ patients classified as DF. RESULTS: 1) Preoperatively, there was no difference in tumor diameter (median 2.5 vs. 2.0cm, Ab+ vs. Abneg, respectively), TNM score or %LN metastases (47.4 vs. 29.8%, respectively). 2) Some 28.3% Ab+ patients had R/P detected at median 3.9(0.8-5.5) FU years compared with only 15.8% for Abneg at median 4.2 (0.2-13.3) years, $p< 0.04$. 3) Analyses of Ab+ patients who developed R/P vs. Ab+ patients judged DF showed comparable tumor diameter but a trend for both more LN metastases (69 vs. 39 %, $p<0.07$, R/P vs. DF, respectively) and higher preoperative serum TgAb (median 18.3 vs. 7.3 U/mL, $p< 0.09$, respectively). 4) Serum TgAb was detectable at R/P in 92% of patients compared with 67% for DF patients at end of FU (12.1, 0.9-246 vs. 1.2, 0.9-53 U/mL, R/P vs. DF, respectively). CONCLUSIONS: R/P risk is increased for DTC patients with TgAb detected at initial surgery. Pre-operative TgAb status may be a prognostic risk factor that should be considered when planning post-operative management of DTC patients.

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Program Number 119 Cancer

The National Cancer Institute's *Making Choices. Screening for Thyroid Cancer: From Concept to Product*

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The National Cancer Institute has developed a public information campaign addressing the effects of iodine-131 (I-131) exposure from the Nevada nuclear weapons tests during the Cold War. A key component of NCI's campaign is a screening decision aid designed to aid people in making informed choices about screening options for thyroid conditions, particularly cancer. Thyroid cancer screening and treatment decisions are not clear-cut. The benefits of early detection are uncertain. The difficulty inherent in determining an individual's level of I-131 exposure, and in assessing subsequent cancer risk, further complicates decision-making. A clear need exists for an educational tool that health care providers and consumers can use to make sound decisions. NCI's thyroid screening decision aid builds on risk communication models, coupled with decision-support methodologies. The result is a tool that consumers can use to 1) increase knowledge about screening benefits and risks; 2) obtain accurate perception of estimated risk; and 3) help them make specific, deliberate choices among screening options for thyroid conditions. NCI will disseminate this tool in conjunction with other consumer education materials dedicated to I-131 exposure from above-ground nuclear testing in Nevada. This presentation will highlight 1) the process NCI employed in developing this tool, including extensive work with advocates and health professionals; 2) findings from focus group research; and 3) a Web-based preview of the tool.

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Health Profiles and Quality of Life of 518 Survivors of Thyroid Cancer

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Differentiated thyroid cancer is generally a readily treatable disease that carries the anticipation of prolonged survival and cure for most patients. It is common practice that thyroid cancer survivors remain under medical surveillance for many years both to monitor for the possibility of late recurrences and for adjustment of thyroid replacement over time. Available literature describes the long-term outcome of thyroid cancer survivors with respect to thyroid cancer but not their overall medical and social well-being. In conjunction with the M. D. Anderson Cancer Center Life After Cancer Care Program we developed a survey to learn about special health needs, if any, of cancer survivors. 518 thyroid cancer survivors responded to a survey regarding medical and social impacts of their cancer experience. All thyroid cancer survivors had surgery and 417 (80.5%) also had some radiation. Two thirds (64.5%) reported that cancer created health effects varying by gender and passage of time; neurological, musculoskeletal and psychological problems appeared most prominent. They reported more memory loss and psychological problems than other cancer survivors and more migraine headaches than both other cancer survivors and the general population. Regarding family and work they integrated well in society, overall. However, unsolicited comments by 24.5% of responders disclosed symptoms reminiscent of thyroid hormone imbalance. Thyroid cancer survivors generally report good health long-term but describe distinct, lasting medical problems including symptoms of thyroid dysregulation. However, age and the passage of time appear to affect the survivors' perceived impact of their cancer experience on their overall health. The extent and manner in which cancer therapy contributes to the health profile of the group merits further inquiry.

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Predictive Value of Serum Thyroglobulin after Surgery for Well-differentiated Papillary-Follicular Thyroid Carcinoma

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A retrospective chart review was performed on 213 consecutive patients with well differentiated papillary-follicular thyroid carcinoma treated between 1983 and 1998 to evaluate risk factors for predicting disease recurrence. The stimulated serum thyroglobulin (Tg) value obtained after thyroid hormone (L-T3) withdrawal 2-3 months post total thyroidectomy and taken just prior to radioiodine ablation therapy was compared to the clinical/pathological variables (age, gender, tumor pathology, size and stage I-IV) and evidence for later residual or recurrent disease (based upon annual examination, serum Tg, neck/thyroid ultrasound, CT scan, fine needle aspiration cytology, repeat surgical pathology and post-radioiodine therapy whole body scan when indicated). Patients with a positive serum anti-Tg antibody were excluded from the study. Among the 213 patients, 141 (66%) were stage I, 50 (23%) stage II, 18 (8%) stage III and 4 (2%) stage IV. The median TSH level for the whole group of patients was 38 mIU/L and the median time to recurrence or follow-up was 40 months (range = 1-182 months). Twenty-five patients (12%) had a documented recurrence of which 5 were local, 14 regional and 6 distant. The post-surgical Tg was significantly associated with an advanced disease stage at presentation (p=0.005, Kruskall Wallis) but not with other clinical/pathological variables. Patients with a Tg >20 pmol/L, had a significantly increased risk of disease recurrence on both univariate (n=213, p=0.0001, log rank test) and multivariate (n=213, p=0.0001, Cox's regression model) analyses. In the Cox proportional hazards model, advanced tumor stage (p=0.001, relative risk 3.4 (2.4-4.9) and a Tg level >20 mnol/l (p=0.001, relative risk 5.1 (2.0-13.1) were significant predictors of recurrence. Conclusion: Patients with advanced tumor stage at diagnosis and a hypothyroid stimulated Tg >20 pmol/L 2-3 months after total thyroidectomy are at increased risk for recurrence and should be considered for more intensive follow-up and/or additional treatment.

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New Insight into rhTSH-stimulated Serum Tg Testing as Revealed by a Recently Developed Second Generation Tg Assay

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BACKGROUND: A conventional rhTSH-Tg stimulation test is a pivotal diagnostic procedure for staging, monitoring and assessing the need for additional treatment for occult differentiated thyroid cancer (DTC) in patients with undetectable basal serum Tg (BTg) during L-T4 Rx. The diagnostic utility of the rhTSH-stimulated serum Tg (STg) value is highly dependent on the sensitivity and specificity of the Tg assay. Current 1st generation (1stG) Tg assays have wide between-method biases and sub-optimal sensitivity, as reflected by a <10-fold difference between assay functional sensitivities (~0.3-1.0 ng/ml) and lower normal reference limits (~0.5-3.0 ng/ml). STUDY DESIGN: A 2nd generation (2ndG) Tg assay with enhanced sensitivity (sensitivity = 0.09 ng/ml; lower normal reference limit = 3.0 ng/ml) was developed. This 2ndG Tg assay was used to remeasure serum Tg in 22 pairs of rhTSH-Tg test sera previously obtained from TgAb-negative DTC patients who had undetectable BTg (<1.0 ng/ml) measured by 1stG assay. RESULTS: Comparisons of rhTSH test results measured by 1stG vs. 2ndG Tg assays showed: (1) 12/22 responded using the 1stG assay while 14/22 responded using the 2ndG assay. (2) 4/5 tests with undetectable 2ndG BTg (<0.09 ng/ml) had no STg response. (3) In responders, 2ndG BTg values averaged 0.39±0.07(se) (range<0.09-1.15 ng/ml) compared with 0.11±0.02, (<0.09-0.22 ng/ml) for non-responders, $p< 0.024$. (4) Responder fold-response (STg/BTg) averaged 14±5, range 3-80. There was no correlation in responders between either BTg and STg values, or BTg and fold-response. CONCLUSIONS: (1) Failure to detect a Tg response with rhTSH-Tg testing is most likely a function of inadequate Tg assay sensitivity and warrants the development of 3rd generation Tg assays characterized by sensitivities that are 100-fold below the current lower normal reference limit. (2) More accurate and sensitive Tg measurements likely would improve the diagnostic value of BTg measurement and reduce the need for rhTSH-Tg testing for monitoring DTC patients.

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131-I Ablation Success after Iodine Depletion in Thyroid Cancer Patients

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Clinicians prepare post-thyroidectomy, thyroid cancer patients with low iodine diets prior to radioactive iodine ablation hoping to improve the chance of successful ablation through total body iodine depletion. However, no direct link between total body iodine depletion and success of ablation has been established. We retrospectively reviewed the outcomes of fifty-nine, thyroprivic, thyroid cancer patients receiving radioactive iodine ablation after near total thyroidectomy. We instructed all patients to follow a low iodine diet (<50 μg iodine/day) prior to the ablation and determined the degree of iodine depletion using the urinary iodine to urinary creatinine ratio, UI/UCr. Six to thirty months after ablation we evaluated thyroglobulin levels and thyroid scan results with the patients either thyroxine deprived or rhTSH stimulated. The fifty-nine patients had a mean UI/UCr of 78.7+/-97.8(SD) μg/g. The mean radioactive iodine dose administered was 110.5+/-30.3 mCi. On thyroid scan, fifty of fifty-nine patients (84.7%) had no visible activity and we considered this group successfully ablated. These patients had a mean UI/UCr of 77.1+/-101.8 μg/g. Seven of fifty-nine (11.9%) had thyroid bed activity only. Their mean UI/UCr was 91.9+/-83.5 μg/g. Two of fifty-nine (3.4%) had disease outside the thyroid bed with a mean UI/UCr of 73.5+/-60.1 μg/g. Among forty-one patients with negative thyroglobulin antibody titers, thirty-one (75.6%) had undetectable thyroglobulin levels. These individuals had a mean UI/UCr of 72.9+/-100.5 μg/g. Ten of the forty-one (24.4%) had thyroglobulin levels ≥2 ng/ml with mean UI/UCr of 73.2+/-58.1 μg/g. We found no correlation between the degree of iodine depletion from a low iodine diet and thyroid scan result ($p=0.6375$), nor thyroglobulin levels ($p=0.5847$). Varying degrees of total body iodine depletion prior to radioactive iodine ablation within this range had no measurable effect on the success of ablation as assessed by these outcomes.

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Program Number 124 Cancer

Thyroglobulin Responses following Recombinant Human TSH Stimulation in Thyroid Cancer: Influence of Site of Metastases

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Thyroid hormone withdrawal (THW) is a standard approach for stimulating residual thyroid cancer to produce thyroglobulin (Tg). However, THW results in variable degrees of hypothyroidism and TSH elevations. Administration of recombinant human TSH (rhTSH) provides a more reproducible elevation of serum TSH. Using such a standardized approach we compared Tg production by mets in different tissues. We hypothesized that the location of mets would significantly influence the degree of change in serum Tg following rhTSH. We analyzed the results of all thyroid cancer patients (pts) undergoing routine evaluations during a 1-year interval. Pts with Tg assay interference were excluded. Stimulated Tg levels were obtained 72 hours following the second of two 0.9 mg IM doses of rhTSH. Pts were categorized as: no evidence of disease (NED; n=136), thyroid bed only (TB; n=60), cervical lymph nodes only (Cv; n=41); lung mets (n=55); and, bone mets (n=30). The median baseline Tg for the entire group was 1 ug/L and it rose to 2.6 ug/L at 72h. The median baseline Tg values (in ug/L) were: 0.4 (NED), 0.55 (TB), 1.0 (Cv), 46 (lung), and 700 (bone). The median incremental changes in Tg (ug/L) were: 0 (NED), 0.5 (TB), 2 (Cv), 37 (lung), and 415 (bone) ($p = <0.001$ ANOVA). The median fold increase in Tg for the entire group was 2.0. The median fold increases based on site of metastasis were: 1 (NED), 1.6 (TB), 3.3 (Cv), 3.2 (lung), and 2.3 (bone)($p = 0.64$ ANOVA). We conclude that the site of mets affects the amount of Tg produced, but not the responsiveness to rhTSH.

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Program Number 125 Cancer

Detecting Residual Differentiated Thyroid Carcinoma with Serum Thyroglobulin

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We tested the hypothesis that the serum thyroglobulin (Tg) level following recombinant human TSH (rhTSH) stimulation (stim) is a more sensitive test for residual differentiated thyroid cancer (DTC) than Tg drawn when TSH is suppressed (supp). We retrospectively reviewed the relationship between the supp or stim Tg and the presence of residual thyroid carcinoma in a cohort of thyroid cancer patients (pts) undergoing routine surveillance. 364 pts were evaluated over a 2-year period. Exclusion criteria: non-DTC cancer, Tg assay interference and, thyroid bed uptake only. Using these criteria, 291 pts were eligible for analysis. The functional sensitivity of our Tg assay is 0.6 ug/L. The stim Tg was drawn 72 hours following the second of two 0.9 mg IM injections of rhTSH. Residual DTC was found in 18% of those with a supp Tg <0.6 ug/L. The likelihood of residual DTC began to rise sharply with a supp Tg >2 ug/L. 88% of pts with a supp Tg between 3 and 8 ug/L had residual DTC. All pts with a supp Tg >7 ug/L had residual disease. Only 6% of those with a stim Tg <0.6 ug/L had residual DTC. All pts with a stim Tg above 10 had residual DTC. The positive likelihood ratio increased sharply >2 ug/L for the supp Tg, and gradually >6 ug/L for the stim Tg. At any given Tg value, the supp Tg was more sensitive than the stim Tg. However, ROC curve analysis demonstrated that the stim Tg was significantly more accurate than the supp Tg, especially for Tg <2 ug/L. Based on this cohort, we suggest that a stim Tg should be considered for pts with an undetectable supp Tg. If the stim Tg is undetectable, the presence of residual DTC is less than 6%.

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Program Number 126 Cancer

Age-dependent Expression of NIS Protein: A Clue for the High Sensitivity of Childhood Thyroid Gland to the Carcinogenic Effects of Radioiodine

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The high sensitivity of the thyroid gland to the carcinogenic effects of radiation during childhood contrasts with the absence of demonstrable carcinogenic effects of radiation exposure in adults. In a series of 26 thyroid glands removed for RET mutation in MEN 2A family members, we studied by immunohistochemistry on formalin-fixed paraffin embedded samples, the expression of NIS and of other functional proteins (pendrin, TPO, thyroglobulin) and of proteins associated with the cell cycle (ki67, cyclin A). Age at surgery ranged from 3 to 39 years (median: 14 years). NIS expression was restricted to the baso-lateral membrane and pendrin to the apical membrane of thyrocytes. The percentage of cells stained for NIS ranged from 20% to 80%, and pendrin staining was less heterogeneous, with 50%-80% of thyrocytes being positive. A stronger NIS staining was associated with a younger age ($p<0.05$), whereas the expression of other functional proteins did not vary with the age at surgery. In contrast, the expression of ki67 and of cyclin A did not vary with age at surgery, and thus differences in growth rate cannot explain differences in sensitivity to radiation exposure. In conclusion, NIS expression was much higher in younger subjects and this may explain higher exposure and thus higher risk to develop a thyroid tumor following exposure to radioiodine

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Program Number 127 Cancer

The Axilla as a Rare Site of Metastatic Thyroid Cancer with Ominous Implications

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Background: Thyroid carcinomas generally metastasize to regional lymph nodes and distant sites such as the lungs, liver and bones. Axillary metastases are an unusual and rare occurrence in thyroid cancer patients. Methods: The Endocrine Surgical Oncology database at the University of California San Francisco was searched to identify patients with thyroid cancer and axillary metastases. The records of these patients were retrospectively reviewed. Results: Of 895 patients treated surgically for thyroid cancer between 1985 and 2002, three patients with axillary metastases were identified. This subset consisted of two males and one female aged 65, 59, and 45 years, respectively, at presentation with axillary disease. The first patient had undergone two previous surgeries for thyroid cancer at age 24 and 63 years. The second presented with a hypercoagulablity syndrome causing strokes, and the third with a pericardial effusion. All patients had disseminated disease with multiple systemic metastases along with axillary metastases. All patients underwent total thyroidectomy with modified radical neck dissections and one patient underwent an axillary dissection as well. Final pathology revealed poorly differentiated papillary carcinoma in two patients and poorly differentiated medullary thyroid cancer in one patient. The patients with papillary tumors died within 1 and 10 months of diagnosis of axillary metastases, whereas the patient with medullary cancer and a hypercoagulable syndrome is alive 6 months after diagnosis. Conclusions: Thyroid cancers may occasionally be associated with axillary metastases in the context of disseminated disease, and must be considered in the differential diagnosis of an axillary mass. Furthermore, the axillae should be examined in thyroid cancer patients with extensive cervical nodal metastases. Axillary metastases appear to be associated with poorly differentiated cancers and a poor prognosis.

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Program Number 128 Cancer

Comparison of Five Prognostic Scoring Systems for Differentiated Thyroid Cancer (DTC) in a Series of 1053 Patients

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Prognostic scoring systems (PSS) based on multiple regression analysis of prognostic factors have been proposed as a tool to distinguish DTC patients in low- or high-risk category, to be followed and treated with less or more aggressive protocols, respectively. In our series of 1053 patients with DTC followed in our Institution since 1969 (minimum and maximum follow-up =11 and 30 years), 87 (8.2%) patients died of thyroid cancer. In this series we have performed a multifactorial analysis (Pisa), based on age at diagnosis and tumor extension and we compared its clinical impact with 5 PSS reported by other Institutions. The PSS compared are: the EORTC scoring system, taking into account age, sex, histology, extra-thyroidal invasion and distant metastases; the TNM staging system, considering age, extent of primary tumor, lymph node status, and distant metastases; the Clinical Class system (University of Chicago), considering only the extent of primary and metastatic tumor tissue; the MACIS system (Mayo Clinic), considering distant metastases, age, completeness of surgery, invasion of extra-thyroidal tissues and size of the primary tumor; the Ohio University scoring system, considering tumor size, lymph node status, multifocality, local tumor invasion and distant metastases. The results of our analysis are reported in the table:

% 10-YEAR SURVIVAL IN EACH PROGNOSTIC GROUP					
PSS	I	II	III	IV	V
Pisa	100	85.5	44.4		
Clinical	100	96.5	76.8	52.0	
Class					
MACIS	99.1	94.2	80.8	25.0	
EORTC	99.7	96.3	76.5	47.2	15.4
OHIO	100	98.4	82.5	48.5	
TNM	99.2	93.1	82.9	35.5	

This analysis shows that all the PSS examined have similar prognostic values. The practical implication is that also changing the significant factors from center to center, any PSS can be applied to a given population of DTC patients without altering the prognostic information, provided that the series is large enough.

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Program Number 129 Cancer

Fatal Outcome of a Young Woman with Papillary Thyroid Carcinoma and Graves' Disease: Possible Implication of the Crosstalk (Cross-Signalling) Mechanism

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Crosstalk is used to call the interactions between signal transduction pathways. Such interactions have numerous examples. However, this is the first time this mechanism may explain the aggressive behavior of a papillary carcinoma in a young woman with Graves' disease. A 29-year-old patient was referred to our hospital due to generalized convulsions. She had Graves' disease treated with methimazole. Her MRI showed 4 metastatic lesions in the brain. She had a goiter with a "cold" nodule and a palpable ipsilateral lymph node. The FNA biopsies have disclosed papillary carcinoma. Under MMI treatment she had T3 = 138 ng/dl, FT4 □.5 ng/dl, TSH □.05 mUI/L and TRAb = 47.8 %. The CT scan also showed lung metastatic lesions. She underwent total thyroidectomy plus modified neck dissection and received an accumulated dose of 700 mCi of 131– I during the following two years. She died for the brain metastases. Studies were performed in DNA extracted from paraffin-embedded tissues from the thyroid tumor, the metastatic lymph node and the non – tumoral thyroid. Two different mutations in the carcinomatous tissue, both in the thyroid and the lymph node were found. No mutations were observed in the non-tumoral thyroid. The mutations were:1) the normal GCC sequence at codon 623 of the TSHR gen was replaced by TCC, changing ala by ser. 2) the wild type CAA at codon 61 of N-ras mutated to CAT, replacing gln by his. Since this activating mutation of the TSHR was unable to reach the end point of the PKA cascade in tumoral tissue, it is concluded that the N-ras mutation was probably involved in this particular cross-signalling mechanism.

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Program Number 130 Cancer

Outcome Analysis for Neck Recurrences following Surgical and I-131 Treatment (RAI) of Differentiated Thyroid Cancers

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137 patients who had surgery for differentiated thyroid cancer were followed for 1-45 years (mean 12.3, SEM 0.7) to look for neck recurrences using mainly sonography, with palpation, and scintiscanning. 96 patients (70%) had total, near total or subtotal thyroidectomies (total surgery) and 41 (30%) had partial thyroidectomies (partial surgery). The 2 groups showed similar gender, age and pathology distributions. They were different for post surgery RAI therapy: 34.2% partial group, 56.8% total group (fisher's p=0.02). The frequencies of recurrence were not significantly different (19.5% partial and 30.2% total). However, mean time to recurrence was (mean±SEM) 72.0±15.3 months in the total group and 157.0±34.2 months in the partial group (t test, p=0.03). The difference was also evident in the group without RAI therapy (mean time to recurrence: mean±SEM, partial group 326±52.7 months, total group 91.7±28.1 months, p=0.005). Kaplan Meyer curves for this group were also significantly different (p=0.03). All 29 recurrences in the total group occurred by 207 months after surgery, whereas 2/8 recurrences in the partial group were significantly later (at 233 and 418 months). When stratified for RAI a non-significant trend was noted with greater recurrence in the total group compared to partial in the absence of RAI (recurrence with RAI: 42.9% partial, 39% total, recurrence without RAI: 7.4% partial, 19.5% total). We conclude that perhaps related to high risk factors that led to therapeutic decisions: 1) In the absence of RAI, total surgery is followed by significantly earlier and possibly greater frequency of recurrence compared to partial surgery. 2) RAI is associated with increased frequency and decreased time to recurrence after partial thyroidectomy. 3) There is need to follow patients with partial surgery lifelong because recurrences may occur as late as 35 years whereas recurrences from total surgery appeared before 17.25 years in our cohort.

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Program Number 131 Cancer

Lack of Mutation in Exon 10 of p53 Gene in Thyroid Tumors

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The nuclear protein p53 has an important role in the negative control of cellular proliferation, and in masterminding signaling cascades important in DNA repair and/or apoptosis. Mutations of p53 have been reported with high frequency in many cancer types and are highly prevalent in poorly differentiated and undifferentiated thyroid carcinomas, but they are not found in benign tumors and are infrequent in well-differentiated cancer. Most mutations have been described in exons 5-8 of the gene. Recently, an inherited mutation in exon 10, codon 337, of p53 was described in Brazilian children and adults with sporadic adrenocortical tumors. The high prevalence of this point mutation in these patients and their relatives led us to examine the same region of exon 10 in 74 patients from the same region of the country but presenting thyroid tumors: 5 follicular carcinomas, 22 papillary carcinomas, 11 follicular adenomas, 1 medullary carcinoma and 35 benign goiters. DNA was extracted from a central part of all tumors. In addition, we obtained DNA from contralateral normal thyroid tissue samples and/or blood from 38 of these patients. The products of PCR for exon 10 of p53 were examined by single strand conformation polymorphism (SSCP) analysis using a sample harboring the Arg337His mutant as a control. The automatic sequencing of exon 10 from 5 samples suspected of presenting aberrant migrating bands and 12 additional PCR products from tumor samples with normal SSCP patterns revealed wild type sequences. We conclude that exon 10 of p53 gene does not present mutations in thyroid tumors, suggesting that this mutation is specific to adrenocortical cancers.

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Program Number 132 Cancer

Prognostic Factors and Therapy in Huerthle Cell Thyroid Carcinoma: Analysis of the Disease-free Interval

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Background: Huerthle cell thyroid carcinoma (HCTC) is a rare tumor with relatively favorable prognosis for which surgery is the mainstay of therapy. On the other hand, it is generally believed RAI therapy is unlikely to be beneficial in HCTC. The aim of this study was to find out if the extent of thyroidectomy and ablation of thyroid remnant with RAI prolongs the disease-free interval of the patients with HCTC.
Materials and methods: A total of 1435 patients with thyroid carcinoma were seen at the Institute of Oncology Ljubljana from 1972-2000. In 48 patients (3.3%) histopathology confirmed HCTC. Since distant metastases were present in 10 patients, this retrospective study of disease-free interval was carried out in remaining 38 patients (31 females, 7 males; median age 62 years). The data on patients' gender, age, extent of disease, morphological characteristics of tumor, mode of therapy, recurrence, disease-free interval and survival rate were collected. The influence of prognostic factors and therapy on the disease-free interval was analyzed using univariate log-rank test.
Results: During the follow-up period of 0.66-28 years (median 5.8 years), the recurrence was diagnosed in 12 patients, whereas 6 patients died of HCTC. Favorable prognostic factors related to disease-free interval were: primary tumor size T3 or less, microscopically radical excision and total or near-total thyroidectomy, while unfavorable prognostic factors were: primary tumor size T4, non-radical excision and subtotal thyroidectomy. We observed that the uptake of RAI was present in 10 of our patients with the recurrent and/or metastatic HCTC. However, there was no statistically significant difference of disease-free interval according to RAI ablation.
Conclusion: Total or near-total thyroidectomy prolongs the disease-free interval of the patients with HCTC in comparison to less extensive surgical procedure. We could not demonstrate that RAI ablation of thyroid remnant prolongs the disease-free interval.

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Program Number 133 Cancer

Therapy with rhTSH and Radioactive Iodine for Metastatic Huerthle Cell Thyroid Carcinoma

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Background: It is generally believed that Huerthle cell thyroid carcinoma (HCTC) does not accumulate radioactive iodine (RAI). However, the uptake of RAI was confirmed in 10/16 of our patients with the recurrent and/or metastatic HCTC. The aim of this study was to find out if RAI accumulates in metastatic HCTC after application of rhTSH.
Materials and methods: This study deals with two patients (2 females, age 73 and 72 years) with metastatic HCTC who already had 5 and 7 therapies with RAI, but in whom 1) another withdrawal of thyroid hormones was contraindicated, and 2) tumor progressed during previous endogenous elevation of TSH concentration. Both patients were on low iodine diet for two weeks before the beginning of the treatment. Two intramuscular injections of 0.9 mg rhTSH (Thyrogen, Genzyme corporation, MA, USA) on two consecutive days were administered and on the third day, 4 mCi (148 MBq) of RAI was ingested, followed by a diagnostic whole body RAI scintigraphy. If RAI uptake was confirmed, the therapy with 150 mCi (5.6 GBq) of RAI was performed after another two intramuscular injections of 0.9 mg rhTSH on two consecutive days.
Results: In one patient the uptake of RAI was not found. The uptake of RAI in the other patient was 0.1%. She was treated by rhTSH and RAI therapy and had no serious side effects. A post-therapy scintigraphy showed good uptake of RAI in the metastatic lesion. Three months after the therapy with rhTSH and RAI, serum thyroglobulin level decreased.
Conclusions: RAI may accumulate in metastatic HCTC after application of rhTSH.

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Program Number 135 Cancer

Cytology Can Predict Histology of Follicular Thyroid Neoplasms

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Preoperative differential diagnosis for benign and malignant follicular thyroid neoplasms is difficult. To determine if preoperative cytologic diagnosis of follicular neoplasm predicts the postoperative histologic diagnosis, we compared the diagnoses in 94 cases of follicular neoplasm detected by FNB that subsequently underwent thyroidectomy, from 1991 through 2000. The cytologic diagnoses were: follicular adenoma (21 cases), cellular adenoma (22 cases), follicular carcinoma (13 cases), and follicular variant of papillary carcinoma (8 cases). Thirty cases were designated as indeterminate follicular neoplasm because they did not fulfill the cytologic criteria for further differentiation.
Of the 94 cases of follicular neoplasm, 21 were cancer by cytology (22%). Of these 21 cases, 18 were cancer by histology (86%). Of the 73 cases benign by cytology, 13 were cancer by histology (18%). Thirty-one cases of cancer were detected by histology (33%), and 17 of these (55%) were cancer by cytology.
Of the 21 cases of follicular adenoma, 16 were adenoma by histology (76%), and 3 were cancer by histology (14%). Cellular adenoma (22 cases by cytology), 15 were adenoma by histology (68%), and 1 was cancer by histology (4%). Follicular cancer (13 cases by cytology), by histologic diagnosis, 10 were cancer (77%): 8 were follicular cancer (62%), 1 follicular variant of papillary cancer and 1 was papillary cancer. For the cytologic diagnosis of follicular variant of papillary cancer (8 cases), 7 were follicular variant by histology (88%), and 1 was follicular cancer. Of the 30 cases of indeterminate cytologic diagnosis, 9 were cancer by histology (30%). These data show: 1) the cytologic diagnosis of follicular thyroid cancer or follicular variant of papillary thyroid cancer predicts a concordant histologic diagnosis; 2) the cytologic diagnosis of follicular adenoma or cellular adenoma risks a histologic diagnosis of follicular thyroid cancer of 14% and 4%, respectively.

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Ablation and Remission Rate of Differentiated Thyroid Cancer at King Faisal Specialist Hospital & Reseach Centre (KFSH&RC) and Factors Affecting Outcome

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Thyroid cancer is the second cancer among Saudi population. Differentiated Thyroid Cancer (DTC) represents 90% of all thyroid malignancies. Several studies have found age, gender, tumor size, histological type, grade of the tumor, and distant metastasis are significant prognostic factors. At KFSH&RC, Riyadh, Saudi Arabia, the therapeutic strategies of DTC management for a tumor size 1.5cm or more are including total or near-total thyroidectomy followed by ablation or thyroid remnant using I131 and long-life thyroxine suppressive therapy. We have conducted a retrospective study to assess ablation (negative follow-up I123 scan) and remission (negative follow-up I123 scan and thyroglobulin <0.4 ng/ml) rates and factors affecting outcome.
There were 100 randomly selected patients. Female to male ratio was 3.6:1. The commonest presentation was goiter 92%. Pathological examination: 89% papillary thyroid cancer (PTC), 37% soft tissue invasion, and 33.3% cervical lymph node metastases (CLN). All patients received I131 Rx once (mean dose is 137 mCi, mode 150 mCi and average 29-204 mCi), and 25% received twice (mean dose is 137 mCi, mode 200 mCi and average 100-212). The ablation rate is 92.8% and remission is 70%. The prognostic factors for remission that reached statistical significance are age (mean 33-year vs 43 year, P-value 0.0005), thyroglobulin level post surgery (mean is 24ng/ml vs 320ng/ml, P-value 0.0005), and CLN metastases (53% vs 25% with P-value 0.003). Tumor size, perithyroidal extension, and soft tissue invasion were about to reach statistical significance (P-value 0.07 and 0.09, respectively). Gender, presentation, multifocality, scans uptake, and I131 ablative dose did not reach statistical significance. Remission rate of DTC at KFSH&RC is 70% (CI: 59.9-77.8) which is lower than reported from other centers. The most significant prognostic factors that influenced remission in our study are age, thyroglobulin level post surgery, and CLN metastases.

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Program Number 137 Cancer

Giant Follicular Thyroid Carcinoma Arising in Human Cranium

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A case is described of a giant follicular thyroid carcinoma arising in human cranium with no evidence of a primary tumor in the thyroid gland. The cranium has not previously been identified as a site known to harbor ectopic thyroid tissue and certainly not as the origin of metastatic thyroid cancer. A previously healthy 41-year-old female presented to her primary care physician complaining of severe headaches in November of 1992. A mass was palpated in the scalp and the patient was refered for excisional biopsy. Pathology revealed hyperplastic thyroid tissue that stained positively for thyroglobulin (TG). Subsequent thyroidectomy and regional node sampling failed to reveal thyroid carcinoma. Total body iodine scanning revealed a very large destructive lesion in the skull and a small focus in the upper left thoracic area, both suggestive of metastatic thyroid cancer. After thyroidectomy, the patient received three high doses of I131 (178, 178 and 209 millicuries) over the next 6 months. Although the thoracic lesion resolved and the patient developed premature menopause, very little progress was made on the cranial lesion and thyroglobulin remained elevated at around 20. After Endocrine consultation, the patient was referred to neurosurgery for complete excision of the skull lesion. Craniotomy was performed and pathology confirmed the mass to be a very large, well-differentiated, follicular thyroid carcinoma. Post-operative TG levels became undetectable and subsequent thyroid scans have been negative. Eight years later, the patient is doing well and remains free of disease.

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Program Number 138 Cancer

A Combination of a Thiazolidenedione and a Retinoid Synergistically Inhibits Thyroid Cancer Cell Proliferation

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Nuclear hormone receptors are variably expressed in normal tissues and different types of cancer. Our laboratory has shown that the RXRγ isoform is undetectable in normal thyroid and variably expressed in malignant tumors, and this receptor predicts response to retinoid treatment in cell lines. Recently PPARγ expression was identified in some thyroid carcinoma cell lines, and this expression appeared to predict inhibition of cell growth by treatment with a thiazolidenedione (TZD). PPAR and RXR can influence gene expression as a heterodimer, which can occur through liganded PPAR, liganded RXR or both liganded receptors. We measured receptor expression in three thyroid carcinoma cell lines (MRO, WRO, and DRO). All three cell lines expressed PPARγ mRNA and protein. RXRγ mRNA and protein were detectable in DRO cells only. We therefore predicted that the DRO cells would respond to TZD or retinoid therapy, and that a combination of the two ligands may augment this response. DRO cells were treated with increasing concentrations (0.1, 1, 10 μM) of pioglitazone (PIO) or LG346 (RXR-selective retinoid) separately or in combination (0.05, 0.5, 5 μM each) for 9 days. Proliferation was measured by a nonradioactive cell proliferation assay. Each ligand suppressed cell proliferation when compared with vehicle. PIO was more effective at inhibiting cell proliferation than LG346 at similar concentrations. At the lowest concentration (0.1 μM), each ligand inhibited cell proliferation compared with vehicle, but cells continue to grow over 9 days. The combination of PIO and LG346 (50 nM each) completely inhibited cell growth at 3, 6, and 9 days of treatment. Higher concentrations of PIO (1 μM) and LG346 (10 μM) were required for complete inhibition of cell growth. In summary, combination therapy with PIO and LG346 appears to act synergistically to inhibit cell growth in a thyroid cancer cell line expressing PPARγ and RXRγ.

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PTC1 Decreases NIS Expression and Confers TSH-Independent Growth by Two Distinct Phosphotyrosine Signaling Pathways

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Ret/PTC1, a thyroid-specific oncogene, has been reported to decrease NIS expression in thyroid cells in vitro and in vivo, as well as to induce TSH-independent growth in vitro. However, the signaling pathways underlying these effects have not been elucidated. The OBJECTIVE of this study was to investigate the roles of the signaling pathways mediated by pY404 and pY451 of PTC1 on NIS expression and TSH-independent growth. To abolish the signaling pathways mediated by pY404 or pY451, we have generated retroviruses carrying PTC1 mutants in which tyrosine 404 or 451 was substituted with phenylalanine (Y404F and Y451F). Stable PC Cl 3 rat thyroid cell lines expressing these mutants were established via retroviral transduction. Our data showed that PTC1-Y451F expression decreases radioiodide uptake activity in thyroid cells to a similar extent as wild type PTC1 (PTC1-wt). Therefore, signaling pathways mediated by pY451 are not critical for down-regulation of NIS-mediated radioiodide uptake in thyroid cells. However, cells expressing PTC1-Y404F failed to decrease radioiodide uptake activity, indicating that signaling pathways mediated by pY404 are essential for NIS-mediated radioiodide uptake. Immunocytochemistry and Western blot analysis are being performed to demonstrate that the decreased radioiodide uptake in cells expressing PTC1-wt or PTC1-Y451F is mainly due to reduced NIS expression. While the growth rate of cells expressing PTC1-wt did not change significantly in the absence of TSH, cells expressing PTC1-Y451F demonstrated decreased growth in the absence of TSH. This finding indicates that signaling pathways mediated by pY451, while not important for mediating decreased radioiodide uptake, are important in the regulation of hormone-independent thyroid cell growth. Thus, decreased NIS expression and TSH-independent growth appear to be mediated by two distinct PTC1 phosphotyrosine signaling pathways.

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Program Number 140 Cancer

Estradiol Promotes the Expression of Metallothionein II and Provides Resistance to Apoptosis in Thyroid Tumor Cells

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Although there are a number of predisposing factors to the development of thyroid carcinoma, the underlying pathogenesis remains unknown. A striking feature of this cancer is its predilection for females of reproductive age relative to males. The present study investigated the expression of metallothionein II (MT II) in human thyroid follicular carcinoma cells (WRO cells) treated with 17b-estradiol and whether the treatment would alter the response of the cells to apoptotic stimulation. WRO cells were treated with 0-1000 nM of 17b-estradiol. After 24 hours, RNA was isolated and analyzed for the expression of MT II RNA. To test the response of the cells to the apoptotic stimulator staurosporine (STS) WRO cells were treated with 17b-estradiol and then incubated with STS for 24 hours. Apoptosis was determined by staining cells with fluorescein diacetate (FDA) and propidium iodide (PI), which was quantitated by flow cytometry. The results showed that 17b-estradiol increased the expression of MT II RNA in a dose-dependent manner. The increased expression became detectable with concerntrations of 17b-estradiol between 10-100 nM. The appearance of the increased expression of MT II RNA was recorded at 8 hours. Apoptosis of WRO cells induced by 1 mM of STS was significantly lower in the cells treated with 17b-estradiol than those treated with the vehicle control. The time course study revealed that the increase of MT II preceded the occurrence of apoptosis. MT II is known to be required for cell survival in several types of cells. Therefore, our study suggests that 17b-estradiol protects human thyroid follicular carcinoma cells from apoptosis via a pathway related to MT II. These findings may provide some clues to explain the relative high incidence of thyroid carcinoma in females

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Program Number 141 Cancer

Loss of Heterozygosity (LOH) on Chromosome 7q21 Is an Early Event in the Development of Thyroid Follicular Carcinoma

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Since loss of heterozygosity (LOH) affecting chromosome region 7q21 was observed only in malignant tissues and in few adenomas, it was suggested that it could represent a marker of thyroid tumor transformation. To test this hypothesis we analyzed LOH pattern of three microsatellite markers, located in different loci of this region, namely D7S-660 (7q21.1), D7S-492 (7q21.2) and D7S-657 (7q21.3), by PCR amplification, electrophoresis on 12% polyacrylamyde gel, and visualization after silver staining. 121 thyroid glands, containing 260 different histological patterns were evaluated, including 126 goiters (GO), 27 follicular hyperplasias (FH), 47 follicular adenomas (FA), 12 oncocytic hyperplasias (OH), 14 oncocytic adenomas (OA), 18 papillary thyroid carcinomas (PTC), 11 follicular thyroid carcinomas (FTC), and 5 anaplastic thyroid carcinomas (ATC). Laser capture microdissection was used to isolate pure populations of follicular cells from each lesion. 7.9% of GO, 11.1% of FH, 27.6% of FA, 0% of OH, 0% of OA, 16.7% of PTC, 9% of FTC, and 20% of ATC showed LOH on D7S-660. 16.6% of GO, 29.6% of FH, 29.8% of FA, 0% of OH, 0% of OA, 22.2% of PTC, 81.8% of FTC, and 20% of ATC presented LOH on D7S-492. 7.9% of GO, 22.2% of FH, 23.4% of FA, 16.6% of OH, 0% of OA, 11.1% of PTC, 45.4% of FTC, and 20% of ATC had LOH on D7S-657. In conclusion, our study demonstrates that LOH on 7q21 is a very early event in thyroid tumorigenesis, since it can be detected in benign lesions, and its frequency constantly increases as the cells progress toward malignancy. It is correlated with the development of an FTC, while it appears to be less relevant in the formation of PTC. The chromosome region included between the loci 7q21.2 and 7q21.3 may contain one or more genes involved in the pathogenesis of FTC.

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Program Number 142 Cancer

TSH and Cyclic AMP Enhance RET/PTC-3-mediated Akt Activation

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Akt is an oncogenic serine threonine kinase, downstream of PI3 kinase, that is regulated in vitro by serum and growth factors. In FRTL-5 cells, Akt activation is associated with regulation of cell cycle proteins, growth, and apoptosis. We have previously demonstrated that Akt is constitutively activated by stable expression of RET/PTC3 in thyroid cells, and that Akt is frequently overexpressed and overactivated in thyroid cancers. The aim of this study was to evaluate if TSH could enhance the chronic activation of Akt induced by stable overexpression of RET/PTC3 in FRTL-5 thyroid cells. RET/PTC3 transfected (PTC3) cells and control transfectants were grown in 6H medium containing TSH, insulin, hydrocortisone, transferrin, glycyl-L-histidyl-L-lysine acetate, and somatostatin, with 5% calf serum. After reaching 70-80% confluence, cells were kept in a 3H medium (minus somatostatin, TSH and insulin) without serum for 5 days. The cells were stimulated with TSH in increasing concentrations (1, 10 and 100 mU/L) for 15 min and proteins were isolated. Activation of Akt was determined by Western blotting using 20 mcg of protein lysate and a specific antibody for activated (phosphorylated) Akt (Ser-473). In PTC3 cells, Akt activation was present basally, and was maximally stimulated by 10 mU/L TSH, while basal Akt activity was lower and TSH was unable to stimulate Akt activity in control transfectants. Similar results were obtained using cholera toxin (100 ng/ml), forskolin (50 mcM) and 8 Br-cAMP (2 mM) after 15 minutes of incubation. These data suggest that RET/PTC3 overexpression activates Akt, and that this activation can be further enhanced by TSH stimulation and cyclic AMP accumulation. This pathway may account, in part, for the growth-stimulatory and anti-apoptotic effects of TSH in RET/PTC3-expressing thyroid cancers.

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Cyclin D1 Overexpression in Thyroid Tumors after the Chernobyl Accident and Its Relations with Aberrant Beta-catenin and Pin1 Expression

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Cyclin D1 is a target molecule transcriptionally activated by aberrant beta-catenin expression in Wnt-signaling. Regardless of a high prevalence of cyclin D1 overexpression in thyroid cancers, beta-catenin mutations were restricted to familial or undifferentiated thyroid carcinomas. While, recent reports suggest prorlyl isomerase Pin1 promotes cyclin D1 overexpression through accumulation of beta-catenin or activated Ras. This study aimed to clarify a role of Wnt-signaling during thyroid tumorigenesis in the radio-contaminated area after the Chernobyl accident. We examined 12 cases of follicular adenomas and 20 cases of papillary carcinomas from the contaminated area. In immunohistochemistry for beta-catenin and cyclin D1, all carcinomas displayed a strong cytosolic beta-catenin and/or a marked decreased in membrane beta-catenin immunoreactivity and cyclin D1 overexpression. An analysis for beta-catenin gene exon 3 with PCR-SSCP could not demonstrate any mutations in carcinomas. Up-regulation of cyclin D1 transcripts were observed in 40.0% of carcinomas and 36.4% of adenomas by quantitative real-time PCR. While, only a case of 9 Japanese papillary carcinomas showed overexpression of cyclin D1 transcripts. Pin1 overexpression was revealed in carcinomas by immunohistochemistry, and its expression was confirmed by RT-PCR at the transcriptional level. This study demonstrated a higher prevalence of cyclin D1 overexpression during thyroid carcinogenesis in the radio-contaminated area after the Chernobyl accident. Up-regulation of cyclin D1 transcription could be an early event in these tumorigenesis. Overexpression of Pin1 may play a role on cyclin D1 overexpression in thyroid cancers.

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Oligodeoxyribonucleotide Phosphorothioates (ODNs) Complementary to p53 Nucleotide Sequences Inhibit Proliferation, VEGF Secretion and Induce Chemosensitivity in the Follicular Thyroid Cancer Cell Line FTC 133

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Background: Genetic mutations leading to inactivation of wild type p53 tumor suppressor gene are common in anaplastic thyroid cancer and associated with poor prognosis in differentiated thyroid cancer (DTC). Tumor cells deficient in p53 display a diminished rate of apoptosis and decreased chemo- and radiosensitivity. Mutated p53 has also been suggested as a positive regulator of angiogenesis. Aim: To evaluate the potential of oligodeoxyribonucleotide phosphorothioates (ODNs) complementary to p53 nucleotide sequences to inhibit proliferation, induce apoptosis and alter angiogenetic potential in an in vitro model of DTC using the FTC 133 cell line, known to have a p53 point mutation. Main methods: Transient transfection with ODNs (20 bp, ODNs recognizing complementary portions of exon 10 (A-ODN) and 4 (C-ODN), control ODN (HIV-ODN), was carried out using the lipofectin protocol. Positive transfections were documented by immunostaining (IHC) transfected cells and by p53-Western blots from cell lysates. Proliferation was evaluated by MTT-colorimetric cell quantiation, apoptosis by flow-cytometry (FACS) following incubation of cells with propidium iodine (10 µg/ml) and Annexin (5 µg/ml). Vascular endothelial growth factor (VEGF) secretion into culture medium was quantitated by EIA. Main results: Transfection of FTC133 cells with A-ODN decreased proliferation by up to 50% at 48 hours, without inducing apoptosis as determined by FACS. IHC and EIA confirmed a significant reduction of VEGF secretion of FTC cells to as low as 26% of control samples, lasting for up to 5 days post transfection. Transfection with A-ODN rendered FTC133 cells sensitive to 100 µg/ml VP16 (Etoposide) treatment, inducing apoptosis in 29% of cells, as compared to 17% following transfection with HIV-ODN and 8.6% of untransfected cells treated with VP16. Conclusions: These results suggest that ODNs complementary to p53 nucleotide sequences transiently inhibit proliferation, impair secretion of VEGF and may increase chemosensitivity in the follicular thyroid carcinoma cell line FTC 133.

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Expression of Human Epididymalprotein 1 (HE-1) in Papillary Carcinoma

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Papillary carcinoma exhibits characteristic papillary projectile proliferation as the name indicates. Also, portions of benign goiters may form papillae. It has been totally unknown how papillary proliferation takes place in some cancer tissues. In this study, we describe unique localization of human epididymal protein 1 (HE-1) in the papillary projections including papillary carcinoma. The HE-1 is the major secretory protein from the epididymis and has multiple functions. Recently, Niemann-Pick type 2C disease was linked to the mutation of this gene. When we analyzed gene expression in papillary carcinoma and normal tissues by two-dimensional cDNA electrophoresis (unbiased screening), up-regulation of HE-1 gene was found in papillary carcinoma tissues. This was confirmed by northern blot in 3 papillary carcinoma tissues and 4 normal tissues. Immunohistochemical stain of HE-1 protein showed an intense stain in all papillary carcinomas tissues so far examined (n=10) and NPA papillary carcinoma cell line. Follicular carcinomas (n=5), medullary carcinomas (n=5), anaplastic carcinomas (n=3), and normal tissues (n=4) showed negative or weak staining. In benign goiters, the HE-1 protein was detected only in the portion of papillary projection. We also examined the presence of HE-1 protein in human tissues displaying papillae, such as kidney, breast, brain, and small intestine. Indeed, all papillae-forming tissues showed a strong stain for HE-1 protein. This protein was uniquely localized in the cytoplasm of papillae in all tissues. In conclusion, HE-1 protein may be involved in the formation of papillae including papillary carcinoma of the thyroid gland.

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Program Number 146 Cancer

A Soluble TGF-beta Inhibitor Lowers Tumor Interstitial Fluid Pressure in Experimental Human Anaplastic Thyroid Carcinoma

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Solid tumors are characterized by a high interstitial fluid pressure (TIFP), which results in a low uptake of anticancer drugs in the tumor tissue. The aim of the present investigation was to study the role of members of the transforming growth factor (TGF)-beta family on the high TIFP, in an in vivo model of human anaplastic thyroid carcinoma (ATC), using a soluble inhibitor of TGF-beta1 and -beta3. Treatment of KAT-4 ATC tumors grown in athymic mice with 1 or 10 mg/kg of the TGF-beta inhibitor for 10 days resulted in a 48% reduction in TIFP compared to untreated control tumors. The mice that received the inhibitor had initially a higher tumor growth rate. However, at day 10 the apoptotic index, as well as the protein level of the cell cycle inhibitor p27Kip1, were higher in tumors from treated mice compared to the control mice. This was followed by a decreased tumor growth rate between days 15 to 29 in the treated mice. Since the KAT-4 cells in vitro did not respond to TGF-beta1 stimulation, measured as phosphorylation of Smad2 protein and growth inhibition, the effects observed in the tumors by the inhibitor are believed to be caused by the effects on the tumor stroma. Taken together, the present data indicate that members of the TGF-beta family are involved in the generation of the high TIFP observed in the ATC model, as well as in regulation of tumor growth, by changing the properties of the tumor stroma. These results identify TGF-beta1 and -beta3 as potential targets for novel anticancer treatment directed to the tumor stroma.

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Program Number 147 Cancer

P16 Dominates over P21 for the Cell Cycle Arrest Induced by Decorin in Thyroid Carcinomas

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Decorin-mediated tumor invasive growth repression has been reported in a variety of tumor cell lines. The signalling pathway involved begins with auto-phosphorylation of tyrosine kinase receptors, finally causing activation and up-regulation of P21 (a potent cell cycle regulatory protein), repression of the phosphorylation activity of CDK2 and arrest of the cell cycle in the G1-phase. Here we show that in contrast to reports in other types of tumors, decorin–induced dose dependent growth repression in thyroid carcinoma cells (previously reported by us) is associated with up-regulation and activation of P16 rather than P21 to induce a G1-phase arrest.
The differential expression profiles of P21 and P16 were studied in HuSD-24 (normal thyroid) cells and FTC-236 (a follicular thyroid carcinoma cell line), using a standard LightCycler RT-PCR protocol. The regulatory effect of decorin on P21 and P16 was also examined in FTC-236 with or without pcDNA3.1 harbouring the decorin cDNA gene for transgenic expression. Kinase activity of CDK2, CDK4 and CDK6 was measured in the presence of 2 mCi [33P]ATP using 1 mg pRb as a substrate.
Expression levels of P21 and P16 in FTC-236 were both up-regulated (+64.8% and +239% respectively) compared to HuSD-24 cells. Induction of decorin expression in FTC-236 cells caused growth repression, down-regulation of P21 (to 17% of that of HuSD-24 cells) and marked up-regulation of P16 (to 900% of that of HuSD-24 cells). These findings were verified by the observations that the down-regulation of P21 was associated with increased phosphorylation activity of CDK2 while the up-regulation of P16 induced repression of CDK4 and CDK6 activities.
Thus decorin-induced growth repression in thyroid carcinoma cells has a unique signalling pathway, in which P16 rather than P21 is the important cell cycle regulatory protein causing cell cycle arrest.

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Decorin Down-regulation in Thyroid Carcinomas Is Associated with Unusual Regulation of Its Binding Proteins (EGFR and ErbB2)

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The extracellular matrix protein decorin is an influential tumor growth suppressor in many tumor cell lines, including breast and squamous cell carcinomas. The signalling pathway which induces cell-cycle repression begins with decorin binding to tyrosine kinase receptors such as EGFR and ErbB2, finally causing up-regulation of cell-cycle regulating proteins which then induce G1-phase arrest. The regulatory status of decorin, EGFR and ErbB2 has been partially investigated in some tumor cell lines but not in thyroid carcinoma, one of the most frequent endocrine tumors.
The differential expression profiles of decorin, EGFR and ErbB2 were studied in HuSD-24 (normal thyroid) cells and FTC-236 (a follicular thyroid carcinoma cell line). A standard LightCycler RT-PCR protocol was used in the quantification process, and the expression profile of beta-actin was used to normalise differences between the cell types used in this experiment. The regulatory effect of decorin on both receptors investigated was examined using FTC-236 with or without pcDNA3.1 harbouring the decorin cDNA gene.
Decorin expression was down-regulated in FTC-236 cells to 1.1% of the level in the normal thyroid cells. Both receptors that bind decorin, EGFR and ErbB2, showed different regulatory patterns. EGFR expression was up-regulated in FTC-236 (+1606% compared to HuSD-24 cells), while ErbB2 showed a concomitant down-regulation (-76%). Decorin caused a non-significant reversal of these regulation trends in both receptors. This contrasts directly with findings in mammary carcinomas where ErbB2 is over-expressed, and following induction of decorin expression is initially further stimulated and then significantly down-regulated.
The down-regulation of decorin in FTC-236 cells reflects a possible role in the invasive growth of these cells. The regulatory status of EGFR and ErbB2 receptors seems to be independent of decorin expression in contrast to other tumor cells, reflecting abnormal regulatory features of both receptors in thyroid carcinoma cells.

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Program Number 149 Cancer

2-Methoxyestradiol (2-ME) Induces Apoptosis in Anaplastic Thyroid Carcinoma Cells

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Anaplastic thyroid carcinoma is a rapidly growing and infiltrating tumor. It is one of the most malignant tumor in humans, and today there is no effective treatment. The aim of the present study was to investigate the effect of an endogenous estrogen metabolite, 2-methoxyestradiol (2-ME), on the growth pattern of human anaplastic thyroid carcinoma cells. Addition of 2-ME led to a decrease in cell growth in 5 out of 6 human anaplastic thyroid carcinoma cell lines. Cell cycle analysis (FACS) of cells treated with 20 μM of 2-ME and stained with propidium iodide showed an increased amount of cells in the G2/M-phase, indicating a 2-ME induced G2/M-arrest. This was followed by an increased sub-G1 fraction, indicating nuclear fragmentation. To analyse if this fraction represented cells undergoing apoptosis, TUNEL assay (TdT-mediated dUTP nick end labelling) was used. An increased fraction of positive cells was observed after 24 hours of 2-ME treatment. This was also shown by DNA fragmentation using DNA-laddering technique. However, the sensitivity to 2-ME varied between cell lines. Apoptosis was not observed in the non-responding carcinoma cell line (KAT-4). 2-ME caused an activation of caspase-3 as detected by using Western blot analysis. Furthermore, the activity of p38 MAPK was induced in both HTh 7 and KAT-4 cells, after 2-ME treatment. However, 2-ME caused an activation of SAPK/JNK MAPK only in the responding HTh 7 cells. Addition of inhibitors of SAPK/JNK MAPK and p38 MAPK attenuated the 2-ME effect. Taken together, our data show that 2-ME induces cell death via apoptosis in a majority of human anaplastic thyroid carcinoma cell lines, maybe involving an activation of p38 MAPK- and SAPK/JNK MAPK-pathways. It is possible that the stimulatory effect of 2-ME on apoptosis might, in the future, be useful *in vivo* in the treatment of anaplastic thyroid carcinomas.

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IL-18 Expression in Human Thyroid Carcinomas

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IL-18 was originally cloned by the action of interferon-gamma inducing factor. It has been reported that IL-18 up-regulates Fas ligand expression in NK cells and in myelomonocytic cells. Recently, Fas ligand was found in papillary thyroid cancer cells, suggesting that it helps them escape immune surveillance by eliminating infiltrating lymphocytes. In view of the potential importance of IL-18 in immune responses, we have analyzed IL-18 expression in human thyroid cancer cells by RT-PCR and immunohistochemistry. When tissue samples from thyroid cancer patients were immunostained with anti-IL-18 antibody, dominant IL-18 expression was observed in tumor sites. IL-18 expression was detected in 7 of 10 (70%) papillary thyroid carcinomas. Similarly, 4 of 6 (67%) follicular thyroid carcinomas were positive. Notably, none of 6 cases of anaplastic thyroid carcinomas were positive. Consistent with previous reports, Fas ligand was also detected in the IL-18 positive lesions in papillary thyroid carcinomas. Finally, we investigated whether TSH and MMI regulate IL-18 mRNA expression in human thyroid cancer cells in vitro. Human thyroid cancer cell line 8505C constitutively expressed IL-18 mRNA. In contrast to FRTL-5 cells, TSH and MMI did not affect the IL-18 mRNA level. These findings suggest that separate signal transduction mechanisms might be involved in the regulation of IL-18 mRNA expression in malignant and non-malignant thyroid cells. RT-PCR also revealed that 8505C cells expressed IL-18 receptor. These results suggest that IL-18 produced by human thyroid cancer cells can regulate the physiological functions of tumor cells and act as an autocrine factor to regulate Fas ligand expression. In conclusion, the present data indicate the constitutive and frequent expression in human thyroid cancer cells, suggesting that IL-18 up-regulation is an immunological feature of differentiated thyroid cancer, and that IL-18 may play a role in promoting the local immune response.

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Program Number 151 Cancer

Enhanced Expression of Nicotinamide N-methyltransferase in Human Papillary Thyroid Carcinoma Cell Lines

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To better understand the molecular pathogenesis of thyroid cancer, we used DNA microarray to study the expression profiles of 10 different human thyroid carcinoma cell lines. These included papillary lines BHP 2-7, BHP 7-13, BHP 10-3, BHP 18-21, NPA 87 and TPC1, anaplastic lines ARO 81-1 and DRO 90-1, follicular line WRO 82-1 and medullary line HRO 85-1. As compared with a profile generated from a primary normal thyroid cell culture, about 2-6% of the 12,626 transcripts in the cancer cells showed altered expression with at least a 2-fold change. Among the genes with increased expression in the cancer cell lines, a gene coding for nicotinamide N-methyltransferase (NNMT) was identified for being highly expressed only in the papillary cell lines. NNMT catalyzes N-methylation of nicotinamide and other structurally related compounds, and is highly expressed in the human liver. Using semiquantitative RT-PCR, NNMT message was detected in higher levels in all the papillary cell lines, much lower levels in the primary normal cells and DRO 90-1, but not detected in ARO 81-1, HRO 85-1 and WRO 82-1. The level of NNMT in BHP 2-7 was about 30 times higher than that in the primary normal cells after normalization to hGAPDH. Total RNAs isolated from six representative cell lines were further analyzed for NNMT message by Northern blotting using a single-stranded NNMT cDNA probe. Consistent with the microarray and RT-PCR data, NNMT expression was high in the papillary cell lines BHP 2-7 and NPA 87, low in DRO 90-1 (about 6% of BHP 2-7), and not detectable in ARO 81-1, HRO 85-1, and WRO 82-1. These results suggest that NNMT could be a potential biomarker for papillary thyroid carcinoma.

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Program Number 152 Cancer

Expression of Wild Type PPAR Gamma in Medullary Thyroid Carcinoma

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Peroxisome proliferator-activated receptor gamma (PPARg) is a nuclear receptor involved in several cellular processes. Inactivating PPARg gene point mutations were found in sporadic colon cancer while the chimeric translocation PAX8-PPARg were reported in follicular thyroid carcinoma suggesting a role as tumor suppressor gene. PPARg expression has been reported in several human normal and tumoral tissues and its activation has been demonstrated to inhibit cell growth and to participate in apoptosis. The aim of this work was to study the expression of PPARg in medullary thyroid carcinoma (MTC) and in a human MTC cell line (TT). We analyzed 10 primary MTC and 15 lymph node metastases. After reverse transcription of RNA, cDNAs were amplified by PCR for calcitonin (CT) to confirm the parafollicular origin of the tissues. All samples were amplified for PPARg using primers localized on exon 5 and 6. PCR products were analyzed by agarose gel electrophoresis. All MTC cases, both primary and metastatic, and the TT cell line were positive for CT mRNA expression. PPARg was expressed in the TT cell line at a very low degree and different degrees of expression were found in 8/10 (80%) primary MTC and 14/15 (93%) lymph nodes metastases showed PPARg expression. These results suggest that PPARg mRNA expression is present in the majority of MTC and their lymph nodes metastases. In papillary thyroid cancer, overexpression of wild-type PPARg by its thiazoledinedione ligands is associated with an increased rate of cell deaths through apoptotic mechanism in vitro, and with a decrease in experimental tumor mass in vivo. The finding of PPARg expression in MTC and preliminary evidence of a similar in vitro effect of one thiazoledinedione ligand (i.e., ciglitazone) in TT cells, may suggest that the pharmacological modulation of PPARg expression may play a therapeutic role also in MTC patients.

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Differential Effects of Transforming Growth Factor-β1 on Telomerase Activty in Human Anaplastic Thyroid Carcinoma Cells

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An important step during tumorigenesis is the acquisition of cell immortality. Normal cells have a continuous loss of telomeric DNA, which eventually results in senescence. Cells may overcome telomeric loss and become immortal by activating the enzyme telomerase. Transforming growth factor-β (TGF-β) is a multifunctional cytokine that plays an important role in the regulation of growth of various cell types, and it has been previously shown that TGF-β can inhibit telomerase activity in human cancer cells. The aim of the present study was to investigate the effect of TGF-β on the telomerase activity in human anaplastic thyroid carcinoma (ATC) cells. Analysis of telomerase activity in two ATC cell lines (HTh 74 and C 643) showed a cell density-dependent regulation; the activity decreased when the cell density increased. Addition of TGF-β1(10 ng/ml) to HTh 74 cells resulted in a decreased telomerase activity, however, when added to the C 643 cells an increased activity was found. Western blot analysis showed a down-regulation of the hTERT protein level in TGF-β treated HTh 74 cells, compared to controls. No differences in hTERT protein levels were observed in TGF-β-stimulated C 643 cells. To investigate the effect of TGF-β on the hTERT promoter, cells were transfected with luciferase reporter constructs containing parts of the hTERT promoter. Addition of TGF-β decreased the luciferase reporter activity in the HTh 74 cell line, but no effect was seen on transcriptional activity of hTERT after TGF-β treatment in the C 643 cells. In summary, our data show that TGF-β can both decrease and increase the telomerase activity in ATC cell lines. Inhibition of telomerase activity by TGF-β is probably caused by a direct interaction with the hTERT promotor, resulting in decreased protein levels of hTERT. The mechanism for the activation of telomerase seen in C 643 cells is not known.

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Program Number 154 Cancer

Differential Expression of the Selenium Binding Protein-1 in Thyroid Cancer Cell Lines

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Low levels of dietary selenium are associated with increased risk of malignancy of several organs. Selenium binding protein-1 (SBP-1) is expressed at a higher level in well-differentiated compared to less-differentiated prostate cancer cell lines. Our preliminary cDNA microarray analysis showed reduced expression of SBP-1 in a thyroid papillary cancer cell line BHP 2-7 compared with normal human thyroid cells. To further investigate the expression of SBP-1 in thyroid cancer, we performed RT-PCR of thyroid cancer cell lines and normal human thyroid tissue. Papillary cancer cell lines, BHP 2-7, BHP 15-3, NPA, and a follicular cancer cell line WRO 82-1, did not express SBP-1, while modest expression was detected in anaplastic cancer cells ARO, medullary cancer cells HRO, and normal thyroid tissue. Northern blot analysis demonstrated a similar pattern of expression as determined by RT-PCR. To investigate whether the regulation of the proximal promoter is involved in the differential expression of SBP-1, we cloned the 5'-flanking sequence of the human SBP-1 gene between –1597 and –67 by genomic PCR, fused it to the upstream of the firefly luciferase gene, and transfected the construct into some thyroid cancer cell lines. No significant correlation, however, was observed between the mRNA expression level and the promoter activity. SBP-1 mRNA expression in BHP 2-7 cells was not enhanced by treatment with 5-azacytidine, a demethylating agent, or trichostatin A, a histone deacetylase inhibitor, suggesting that these epigenetic aberrations are not sufficient to restore the expression of SBP-1 in the BHP 2-7 cells. Activation of unknown cell-selective enhancer(s) may be required for the full expression of SBP-1. Differential expression of SBP-1 between differentiated thyroid cancer cell lines and other thyroid cancer cells demonstrates the potential of SBP-1 as a differential marker of thyroid cancer.

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Program Number 155 Cancer

Cathepsin D as a Prognostic Marker in Thyroid Carcinoma of Endemic Origin

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Background: Cathepsin D is a widely distributed acidic endopeptidase. It is an estrogen-regulated protein that is a prognostic factor in breast cancer. The aim of this study was to measure cathepsin D concentration in thyroid tissues and to correlate these concentrations with clinical & pathologic parameters and to see whether cathepsin D can serve as a prognostic marker.
Methods: Cathepsin D and thyroglobulin concentration were measured in the cytosol of normal thyroid tissues, benign nodules, thyroid carcinoma (different stages of papillary carcinoma) with an immunoradiometeric assay.
Results: The mean level of Cathepsin D expressed as picomoles per milligram was higher in 26 carcinomas, 24.1 + 15.3, than in 3 normal thyroid tissue 14.1 + 9.8 (p=.142) or in 6 benign tumors 18.4 + 16.8 (p=.274). Cathepsin D concentrations were higher in papillary Ca than follicular Ca (p=0.06). Cathepsin D concentration correlated negatively with urinary iodine excretion and positively with thyroglobulin level (Spearman rank correlation coefficient r=-338 (p=.03) and r=.957 (p=.0001), respectively. Cathepsin D concentration does not correlate with tumor staging (r=.403, p=.06).
Conclusions: Cathepsin D concentration is higher in thyroid carcinoma than in normal thyroid tissue. There is increased cathepsin D concentration in higher tumor stage though it was statistically not significant. Cathepsin D can be used as a potential prognostic marker in papillary thyroid carcinoma.

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Down-regulation of Thyroid Hormone Receptor Expressions by Thyroid Hormone in Human Neuroblastoma SH-SY5Y Cells and Medulloblastoma HTB-185 Cells

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The role of thyroid hormone (T3) in the regulation of growth and development of the central nervous system has been established. Thyroid hormone actions in the brain are exerted through their receptors and gene transcription mediated by these receptors depends on recruited co-activators. Recent works have suggested the repression of the growth of brain tumor in hypothyroid status, differential expression of coactivator SRC-1 in brain and the decreasing of the number of thyroid hormone receptors (TRs) by T3 in glioma C6 cells. To explore further whether T3 affects the expression of TRs and cofactors for TRs, we performed RT-PCR analysis in human neuroblastoma SH-SY5Y cells and glioblastoma HTB-185 cells. We demonstrated that TR alpha1 and beta1 expression in both SH-SY5Y and HTB-185 cells decreased after 24 hours of incubation with 100nM T3. In contrast, the expression of co-factors (SRC-1, Trip-1 and p120) for the TR in the same cell lines did not change during incubation with T3. Next to test the transactivation activity of endogenous TRs, we tried transient transfection analysis in the same cell lines using the two positive TREs (DR+4, pal) constructs in the pA3Luc vector. Incubation of T3 did not activate the luciferase activity in the absence of co-transfected TRs, while co-transfected TRs increased the transcriptional activity of luciferase in the presence of T3, indicating that endogenous TRs in SH-SY5Y and HTB-185 cells may have little function as transcriptional factors in the presence of T3. In conclusion, our data suggested that T3 might repress the gene transcription through TRs in brain tumors.

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Program Number 157 Cancer

Alterations of Mitochondrial DNA in Radiation-associated Human Thyroid Tumors

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To evaluate the suitability of mitochondrial DNA (mtDNA) as a bioindicator of radiation exposure, mutational status of mtDNA was examined in paired DNA samples extracted from tumor and corresponding non-tumor thyroid tissue obtained from adult patients possibly exposed to radioactive Chernobyl fallout. Three mitochondrial (mt) genes and two polymorphic D-loop regions were screened for point mutation by PCR-SSCP and sequencing. Heteroplasmic point mutation variants, both described previously and novel, occurred with overall frequency of 5-10%. No homoplasmic mutations were found in this series of samples in the selected regions. Most often observed were the transitions whereas a transversion was detected in only one case. No preferential association of the presence of point mutations with DNA origination from tumor was noted. Large-scale deletions of mtDNA were assessed using PCR employing pairs of primers annealing to distal regions of the intact mitochondrial genome (8–10 kb apart from each other) while short elongation time (1 min) of the reaction would allow only those templates with deleted interstitial fragments to be amplified. Most of specimens harbored multiple aberrant mtDNA species whether the case was presented by malignant or benign neoplasm. Sequencing analysis of truncated mtDNA fragments revealed patches of short regions of sequence homology or direct and/or inverted direct repeats 3-6 bases long exactly at or in close vicinity to breakpoint sites. The abundance of deleted mtDNA variants in control cases was lower compared to radiation associated tumors although no significant correlation with radioiodine contamination of the place of patient's residence was found. Model experiments with irradiated primary cultures of thyrocytes demonstrated a tendency to the increase in number of truncated mtDNA fragments at larger exposure dose. Thus, quantitative interpretation of the spectrum of random mtDNA deletions may be an informative approach for evaluation of radiation exposure.

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Program Number 158 Cancer

Implication of hSNK in Thyroid Cancerogenesis

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Background and specific aim: A recently cloned hSNK gene was identified by our group as one of radiation response genes whose expression is rapidly upregulated in thyroid cells after the stress. Here we address issues of hSNK protein function and biological relevance in thyroid cancerogenesis by investigating its properties with newly developed anti-hSNK monoclonal antibody. Methods: The antibody was raised in a mouse using GST-hSNK fusion protein as an eliciting antigen. Western blot, immunoprecipitation and immunofluorescence analysis of thyroid carcinoma cell lines and immunohistochemical staining of thyroid cancer specimens were carried out to assess intracellular distribution of hSNK and to reveal its possible molecular targets. Results: Cytoplasmic localization of the hSNK was observed in FRO and NPA cell lines treated with proteasome inhibitor. Without the inhibitor only weak nuclear staining occurred in NPA cells indicative of a very high hSNK turnover and rapid degradation in proteasomes. Immunoprecipitation of lysates of COS7 cells ectopically overexpressing hSNK demonstrated that hSNK can associate with CIB (calcium integrin binding protein). In thyroid cancer sections weak nuclear staining was observed in normal thyroid parenchyma whereas the tumor counterpart displayed strong cytoplasmic accumulation of the hSNK protein. Conclusion: In normal thyroid cells small amount of hSNK may localize to the nucleus at that main part of the protein is rapidly degraded in proteasomes. On the contrary, in thyroid cancer cells hSNK is specifically targeted to the cytoplasm suggestive of the impairment of normal regulatory pathways, such as its own alteration of hSNK or abnormalities of protein(s) interacting with it. Thus, hSNK may possibly be involved into thyroid tumorigenesis either directly due to self alteration, or indirectly by means of interfering with its downstream intracellular targets.

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Demonstration of Mutations in the Promoter of the Manganese Superoxide Dismutase Gene in Post-Chernobyl Papillary Thyroid Carcinomas from Belarus

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The manganese superoxide dismutase (MnSOD) enzyme is a major cellular defense against oxidative stress. MnSOD has putative tumor suppressor activity, and its expression is reduced in a number of human cancer cell types. Xu and colleagues identified three types of MnSOD promoter mutations in cancer cell lines resulting in altered AP-2 binding and decreased MnSOD gene expression. We hypothesized that MnSOD might act as a tumor suppressor in thyroid cells, and that MnSOD promoter mutations might be involved in the pathogenesis of radiation-induced thyroid cancers. As a preliminary test of this hypothesis, we extracted genomic tumor DNA from four post-Chernobyl papillary thyroid carcinomas arising in Belarus. The affected individuals were from 6 months to 3 years old at the moment of the accident, and ranged from 15 to 18 years at the time of surgery. The MnSOD promoter region was amplified by PCR and the PCR products were sequenced on an automated sequencer. Two of the four samples demonstrated MnSOD promoter mutations. In one case, mutations affecting all three promoter sites described by Xu and colleagues were present. In the second case, a mutation at only one of these sites (position –93) was present. These data demonstrate that MnSOD promoter mutations can exist in radiation-induced papillary thyroid carcinomas. Additional studies will be required to further define the role of MnSOD promoter mutations in the pathogenesis of these tumors.

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Molecular Analysis of Thyroid Nodules That Developed following External Beam Irradiation for Tinea Capitis

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During the mass immigration to Israel in the 1950s, at least 20,000 children received low-dose external beam irradiation as primary therapy for a benign fungal infection of the scalp (tinea capitis). Also, an unknown number of children were irradiated abroad before their arrival to Israel. A long-term follow-up study of 10,834 of these children (with matched controls) demonstrated an excess risk for the development of thyroid cancer (RR= 5.4, 95% CI=2.7-10.8). In order to improve our understanding of the molecular basis of radiation-induced thyroid cancer, we identified, and collected samples of the paraffin embedded thyroid surgical specimens (benign and malignant) from 50 irradiated children and from 23 non-irradiated controls. All paraffin-embedded tissue samples were histologically verified, and sectioned for molecular analysis. Total RNA was recovered using RNeasy Mini kit (Qiagen) with substantial modifications. Quality and quantity of RNA was determined using RT-PCR for beta-actin (154 base pair) and spectrophotometry. The quantity of RNA recovered from 3-4 paraffin-embedded tissue sections (20 microns in thickness) ranged from 0.1 to 1 mcg of total RNA. In the initial 43 samples analyzed, the 154 base pair beta-actin product was detected in 76% (33/43). However, the rate of detection of beta-actin was lower in the older samples than in the more recent surgical specimens: 75% (15/20) for sample from the 1970s, 88% (8/9) for samples from the 1980s, and 100% (12/12) for samples from the 1990s. Molecular analysis of this cohort will allow us to directly compare the rates of ret/PTC activation in thyroid nodules (both benign and malignant) in subjects exposed to external beam irradiation for tinea capitis with their age- and gender-matched non-irradiated controls.

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Program Number 161 Thyroid Nodules and Goiter

Recombinant Human TSH (hrTSH) Is Highly Effective in the Preparation of Multinodular Goiter for Radioiodine (RAI) Ablation

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Multinodular goiter (MNG) is a very common disorder in most countries with chronic iodine deficiency. Periodic surveys conducted in Brazil have indicated that possibly 6-8 million people have MNG. Some of these patients have other co-morbidities that would increase the risks associated with surgery. Most are over 60 years old and the MNG frequently had moved downward to the upper mediastinal space. Due to relatively high concentration of iodine in the salt in Brazil (40-100 mg/kg) or the use of iodine-rich medication (amiodarone), about half of these patients will present with clinical or subclinical hyperthyroidism, with a suppressed serum TSH level. This results in poor radioiodine uptake and less effective radiation delivered to the MNG. We have used hrTSH (0.45 mg IM) 24 hours before the thyroid ablation in group I (17 patients) (age 63±9 yrs, goiter 138±48 g). Another group II of 14 control patients (age 64±10 yrs, goiter size 126±38 g) were not pretreated with hrTSH. After hrTSH the 24h uptake in group I rose to 41-60% (mean 51±8%). This was reflected in a higher uptake of the RAI therapeutic dose and a significantly higher permanency of the radionuclide in the gland (10.1±1.2 days versus 5.1±1.9 days in the control group). After RAI there was a significantly higher serum concentration of total T3 and free T4 in group I as compared with group II. Serum Tg was also higher in the hrTSH-treated group. During the 12-month follow-up, group I had a significantly higher reduction of goiter size (68% vs 38%) but also a significantly higher proportion of hypothyroidism (65% vs 21%). We concluded that pretreatment of MNG in elderly patients with hrTSH is a safe procedure and highly effective in increasing the beneficial effects of RAI in reducing the MNG size and associated compressive symptoms, while correcting the subclinical hyperthyroidism.

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Three Brazilian Families with Congenital Goiter and Defective Thyroglobulin Synthesis Associated with a Novel Homozygous Mutation (A2234N) in the Thyroglobulin Gene

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Primary congenital hypothyroidism may be caused by developmental defects of the thyroid gland or inborn errors of metabolism in one of the multiple steps required for normal thyroid hormone biosynthesis. Among the latter, mutations in the thyroglobulin *(TG)* gene may result in abnormal TG secretion and/or function. In most instances, the mode of inheritance is autosomal recessive and the phenotype is characterized by goiter with compensated or overt hypothyroidism. In this study, we analyzed the coding region of the *TG* gene in the index patients from three unrelated Brazilian families with a putative TG defect. In Family 1, the propositus presented with euthyroid congenital goiter with a low serum TG level (TSH 4.0 mU/L; T4 7.8 ng/dL; TG 9.6 ng/mL). At age 16, he underwent thyroidectomy because of a large multinodular goiter. In Family 2, the two affected brothers presented with goiter and mild thyroid failure (TSH 10.0 and 4.0; T4 4.0 and 6.0 ng/dL; TG 9.5 and 29.2 ng/mL). In the inbred Family 3, the index patient had goiter and mild hypothyroidism (TSH 12.7 mU/L; T4 6.0 ng/dL; TG 3.0 ng/mL). Total RNA extracted from thyroid tissue of the propositi was reverse transcribed and the whole *TG* coding region of 8307 bp was amplified by PCR. In all cases, direct sequence analysis revealed the presence of the same homozygous nucleotide substitution 6701C>A in exon 38, resulting in substitution of alanine by asparagine at position 2234 (A2234N). Analysis of genomic DNA of the index patients and their relatives confirmed segregation of this alteration, which does not appear to be a polymorphic TG variant, with the abnormal phenotype. These findings suggest that the substitution A2234N may be associated with impaired secretion and/or function of TG. Further elucidation of the molecular consequences of this TG mutation will require in vitro analysis.

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Program Number 163 Thyroid Nodules and Goiter

Administration of a Single Dose of Recombinant Human Thyrotropin May Increase the Efficacy of Radioiodine Therapy for Multinodular Goiter

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Iodine-131 therapy has been reported to reduce the size of non-toxic multinodular goiters by approximately 40% at 1 year. Previous reports show that pre-treatment with recombinant human thyrotropin (RHT) may result in a much greater reduction in goiter size after radioiodine therapy.
PATIENTS AND METHODS. Four 47-73 year-old patients (1-4) had multinodular goiters of 45, 50, 100, 107 c.c. calculated size. Patient 2 was on hemodialysis.
On a low iodine diet, they received 0.45 mg RHT intramuscularly (patient 2 received 0.9 mg) at the time of iodine-123 tracer dose administration. Radioiodine uptake and I-131 administration were performed 24 hours later. Uptakes were 64, 19.8, 27.9, 25.7%, and I-131 therapy doses were 15, 60, 70, 80 millicuries, respectively.
RESULTS. The patients experienced only minor soreness in the neck area. In patient 2 total T-3 levels were: 489 ng/dL twenty four hours after RHT injection (while TSH was 331.9 mIU/L), 390 ng/dL one week later, and 140 ng/dL fifty five days later. In patient 3 total T-3 level was 766 ng/dl and TSH level was 38.92 mIU/L 24 hours after RHT. Neither had any symptom of thyrotoxicosis. Patient 4 had a TSH of 10.11 mIU/L 20 days after RHT. Visible goiters were deemed to be reduced in size by clinical evaluation at 6- and 12-week follow-ups. Patient 1 and 2 were found to have a normal TSH and started on thyroid hormone replacement. Imaging studies are planned at 6-month visits.
CONCLUSION. We found that pretreatment with a single dose of recombinant human thyrotropin in patients receiving radioiodine therapy for nontoxic, multinodular goiter was safe in the short term. Clinical trials are needed to evaluate its efficacy and long term safety.

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Program Number 164 Thyroid Nodules and Goiter

Diagnostic Approach to a Thyroid Nodule, Utilizing Decision-Tree Analysis

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Introduction: Thyroid nodules are frequently encountered in the clinical setting. However, there remains considerable diversity and controversy in the diagnostic approach to the patient with a thyroid nodule. The use of decision-tree analysis has been implemented in various clinical scenarios to allow a quantitative evaluation of the outcomes that result from a set of choices. Our study utilized decision-tree analysis to compare the cost-effectiveness of common diagnostic strategies with regards to a thyroid nodule. Methods: A systematic review of data on the diagnostic approach towards a thyroid nodule was performed on MEDLINE, OVID, COCHRANE, and ACP JOURNAL CLUB databases. Following synthesis of data, decision analysis was used to model four diagnostic strategies for thyroid nodules: Fine-needle aspiration biopsy (FNA), ultrasound (US), I-131 scintigraphy and ultrasound-guided FNA (USGFNA). The decision tree was parameterized using costs from our institution based on a sample of patients treated for thyroid nodules and probabilities from a review of the literature. Effectiveness was defined as cases correctly diagnosed, and we computed the incremental cost-effectiveness ratios (ICER) for each four diagnostic strategies. Results: As seen in the table below, the USGFNA strategy had the lowest expected cost and second highest effectiveness. The I-131 strategy had the highest expected effectiveness, largely because all I-131 scans were followed by FNAB. Overall, FNAB was cost-effective compared to the US strategy and the I-131 strategy. The USGFNA strategy may be considered cost-effective compared to FNAB if the decision maker is willing to pay $2,861 for each additional correctly diagnosed case.

Diagnostic Strategy	Expected Cost	Expected Effect	ICER
	$895	0.913	-

FNAB			
	$1,091	0.914	$195,900
I-131			
	$787	0.875	$2,861
USGFNA			
	$1,146	0.757	-$1,610
US			

Conclusion: Based on our decision-tree, FNAB is the most cost-effective initial diagnostic strategy for a thyroid nodule.

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Program Number 165 Thyroid Nodules and Goiter

Changes in Thyroid Function in Subjects Using Oral Iodized Oil for the Treatment and Prevention of Endemic Goiter in Vietnam

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Iodine deficiency is a worldwide problem, resulting in endemic cretinism and goiter. To evaluate the effects of iodine supplementation on the thyroid status of individuals living in Vietnam's iodine deficient Mekong River delta area, a trial involving the administration of a single 400 mg dose of iodized oil (IO) or a placebo dose of vegetable oil in volunteers with endemic goiter between 10 and 40 years of age was conducted. SUBJECTS: Eighty subjects received IO (G1) and 40 received placebo (G2). Urinary iodide and serum TSH, T4 and T3 levels were obtained on days 0 and 5, repeated at months 6 and 12. RESULTS: Urinary iodide levels of G1 and G2 were similar at baseline (2.7 mcg/dl vs 4.2 mcg/dl, respectively) but significantly higher at all time points in G1 (440 mcg/dl at day 5, 15 mcg/dl at month 6, and 11.9 mcg/dl at month 12) G2 demonstrated unchanged iodide values. Serum T4 levels at day 5 were lower in G1 compared to baseline (92.7 nmol/L vs 86.7 nmol/L), TSH levels rose (2.1 mU/L vs 3.1 mU/L), T4 values returned to baseline in G1 at 6 and 12 months, TSH levels were significantly less than baseline (1.1 mU/L and 1.4 mU/L at 6 and 12 months, respectively). Serum T4 and TSH levels were stable throughout the study in G2. T3 was unchanged in G1 and G2. Four subjects (14.3%) between the ages of 16 and 40 years of G1 developed iodine-induced thyrotoxicosis (IIT). CONCLUSIONS: IO can maintain adequate iodine exposure for up to 12 months. IO should be limited to individuals less than 16 years of age, to avoid IIT.

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Program Number 166 Thyroid Nodules and Goiter

Nodular Lesions Detected by Ultrasonography in the Thyroid Gland of Patients with Graves' Disease under Treatment with Antithyroid Drugs

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It is well known that some patients with acromegaly have adenomatous goiter. Long-term stimulation by IGF-1 is considered responsible for thyroid enlargement, presence of functioning lesions and the subsequent formation of multiple nodules. In order to ascertain whether long-term stimulation by TSH receptor antibody causes formation of nodules, ultrasonography was performed in patients with Graves' disease under treatment with antithyroid drugs, and the relation of the thyroid nodularity with age, duration of illness and TSH binding inhibitor immunoglobulin (TBII) activity was studied. One hundred forty-one treated patients as well as 115 untreated patients were included in this study. The sonographic findings were classified into 4 groups: group 1 without nodules (homogeneous echogenicity), group 2 with heterogeneous echogenicity or nodular lesions less than 0.5 cm in diameter, group 3 with one nodule greater than 0.5 cm, and group 4 with multiple nodules greater than 0.5 cm. There was no significant difference in age or TBII among these 4 groups (n=89, 8, 10, and 8 for groups 1, 2, 3, and 4, respectively) of the 115 untreated patients. On the other hand, in case of treated Graves' disease, the age (54.6 +/- 10.4 years) and the duration of illness (15.0 +/- 8.0 years) in group 4 patients (n=33) were significantly greater (both $P<0.01$) than those (46.6 +/- 12.2 and 10.8 +/- 5.6 years, respectively) in group 1 patients (n=83). The mean TBII activities in groups 2 (39.9 +/- 30.5 %; n=11), 3 (32.3 +/- 30.7 %, n=14) and 4 (39.0 +/- 30.3%) were significantly higher than in group 1 (17.3 +/- 15.8%)($P<0.001$, $P<0.01$, $P<0.001$, respectively). In conclusion, Graves' disease patients in whom TBII remains to be elevated in spite of long-term treatment with antithyroid drugs have a high frequency of having thyroid nodules.

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Case Report: Ectopic Intratracheal Thyroid Tissue Presenting as New-onset Asthma in a 19-Year-Old

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This 19-year old girl presented with increasing shortness of breath on exercise. She was treated with bronchodilators for asthma. Her symptoms progressively worsened. Four months later, she developed acute shortness of breath at rest. She was taken to the emergency room where she was treated with steroids for asthma. The following day she saw her pulmonologist. Flow-volume loops were done which revealed a fixed obstuction. A CT scan revealed a large subglottic mass. She underwent emergent tracheostomy, laryngoscopy and bronchoscopy. This revealed a near obstructing mass in the subglottis that was submucosal and broad based. The pathology revealed ectopic thyroid tissue in the submucosa. There was no evidence of malignancy. MRI of the neck revealed an intraluminal, mucosal, uniformly enhancing subglottic tracheal lesion measuring 1.3-cm in maximum diameter. There was no apparent extension beyond the tracheal wall or lymphadenopathy in the neck. The thyroid gland adjacent to this region was also unremarkable. The patient was clinically euthyroid and examination revealed a normal sized thyroid gland with no palpable nodules. Thyroid function tests were within normal limits.

Discussion: Ectopic intratracheal thyroid tissue is an uncommon cause of upper airway obstruction. Intratracheally located ectopic thyroid tissue accounts for 6-7% of all primary endotracheal tumors. It most commonly occurs in the third and fourth decades of life and favors women over men in the ratio of three to one. Dyspnea may vary cyclically with menses and with pregnancy secondary to hormonal stimulation.

Conclusion: Intratracheal thyroid, though rare, must be considered in cases of adult onset "asthma", refractory to treatment. Our patient has been referred for surgical excision of the lesion followed by thyroid hormone suppression therapy to prevent hypertrophy of the residual thyroid tissue.

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Program Number 168 Thyroid Nodules and Goiter

Cytological Studies of Fine-needle Aspiration Specimens and the Risk of Malignancy in Thyroid Nodule: Importance of Nuclear Atypia.

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Thyroid nodules are relatively common in the general population and ultrasound studies have shown that 30-50% of asymptomatic adults may have thyroid nodules. The primary goal in clinical practice has been to select for surgery only those patients whose thyroid nodules are at risk for malignancy. Fine needle aspiration cytology (FNAC) is the best test for preoperative detection of malignant nodules. The present study was undertaken to evaluate the cytological characteristics of benign and malignant nodules and compare them with histologic findings. Cytologic studies included nuclear enlargement, prominent nucleoli, abnormal chromatin, intranuclear inclusion and nuclear crease. We reviewed surgical reports of 97 patients referred to Hospital das Clínicas for evaluation of a thyroid nodule with corresponding FNAC (82 females and 15 males, ages between 12 and 75 years). Of these surgical cases, 43 were benign (24 adenomatous goiter and 19 follicular adenoma) and 54 were malignant (33 papillary carcinoma, 16 follicular carcinoma, 3 medullary carcinoma and 1 anaplastic carcinoma). Abnormal chromatin was found exclusively in neoplastic lesions: Follicular adenoma 26.3%, follicular carcinoma 37.5%, papillary carcinoma 42.4%, medullary carcinoma 42.4% and anaplastic carcinoma 100%. Nuclear enlargement was more frequent in neoplastic lesions (83.8%) but no difference in nuclear size was observed between follicular adenomas (78.9%) and follicular carcinomas (81,2%). Prominent nucleoli was more frequently seen in malignant neoplasias (61.8%) as compared with adenomatous goiter (20.8%) and follicular adenoma (42.1%). Intranuclear inclusion was observed only in malignant neoplasia (22/33 papillary carcinoma and 1/16 follicular carcinoma). Crease was seldon found in adenomatous goiter (4.2%) or follicular adenoma (15.8%) and follicular carcinoma (6.2%) but were present in papillary carcinoma (48.5%). We concluded that the presence of prominent nucleoli and abnormal chromatin should be considered to harbor risk factors for cancer and the presence of intranuclear inclusion and crease are characteristics of papillary carcinoma.

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Thyrotropin Alfa (Thyrogen) in the Treatment of Toxic Multi-nodular Goiter

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Determination of the appropriate therapy in the patient with a toxic multi-nodular goiter is challenging for both the endocrinologist and surgeon. In the past, the treatment of the patient with toxic multi-nodular goiter with low radioiodine uptake has been surgery. Achieving adequate uptake of radioiodine has been limited by the variable iodine uptake of tissues in the multi-nodular goiter. A recent addition to the practice of thyroidology had been thyrotropin alfa (Thyrogen) which is approved for imaging of thyroid malignancies. Goals of therapy in multi-nodular goiter include both elimination of hyperthyroidism and reduction of goiter size. Previous reports on thyroid size reduction have shown greatest cyto-reductive effect in the first six months post therapy. However, large doses of radioiodine may be required. With the ability of thyrotropin alfa to increase the uptake of radioiodine in a multi-nodular goiter, new modalities of therapy exist. Both published studies and anecdotal reports have described the use of Thyrogen in over 50 patients, although most of these patients were non-toxic. We recently had the occasion to treat two patients with thyrotropin alfa to increase radioiodine uptake. The first patient was a ventilator-dependent patient admitted with cardiac arrest from supraventricular tachycardia and thyrotoxicosis. His I-131 uptake was 8 percent. The second patient presented with a toxic sub-sternal multi-nodular goiter while being treated for a hypernephroma. He refused surgery. The initial patient was stabilized after I-131 therapy but subsequently required surgery for tracheal decompression. The second patient showed resolution of hyperthyroidism with 6-month follow-up for reduction in goiter size. Dosing issues and safety issues are discussed. Our data demonstrate that thyrotropin alfa (Thyrogen) can be utilized to treat toxic multi-nodular goiter. Usage may reduce the dosage requirements for radioiodine. Multi-center randomized controlled studies further evaluating Thyrogen in the multi-nodular patient are needed.

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Successful Thyrogen-assisted Treatment of Non-toxic Multinodular Goiter

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Present treatment options for non-toxic multinodular goiter consist of surgical excision and radioiodine therapy. Here we present a case of compressive non-toxic multinodular goiter, recurrent despite hemithyroidectomy, successfully treated with Thyrogen-assisted radioiodine therapy. A 59-year-old female with a history of non-toxic multinodular goiter was referred to our hospital in 2001 for evaluation of compressive symptoms. In 1961 the patient was initially treated with sub-total thyroidectomy for biopsy-proven compressive multinodular goiter. Over subsequent years the patient developed hypothyroidism successfully treated with levothyroxine, but otherwise remained healthy. Unfortunately, in June 2001 the patient suffered an acute respiratory arrest believed secondary to reactive airway disease superimposed upon a recurrent compressive multinodular goiter. CT imaging revealed a large 7.5 by 8.0 by 3.5 cm thyroid gland compressing the trachea into a crescent shape with a 5mm opening at its narrowest dimension. Of note, the patient's past medical history includes Bannayau-Riley-Ruvalcaba syndrome, a rare autosomal dominant mutation of chromosome 10 that is associated with goiter. As the patient refused surgical excision of her thyroid, we undertook Thyrogen-assisted radioiodine treatment. A diagnostic uptake and scan following administration of 0.03 mg rhTSH revealed a 24-hour uptake of 29%. Subsequently, the patient was treated with 69.1 uCi 131-I 24 hours post-administration of 0.03 mg rhTSH. The patient tolerated the treatment well with no compromise in her airway. Three months post-treatment the patient was doing well clinically and her follow up CT revealed a moderately smaller thyroid gland of 7.5 by 7.2 by 3.4 cm, with a 50% improvement in trachea area at the point of maximal compression. This represents the first United States report of a successful Thyrogen-assisted therapy of benign goiter. We believe Thyrogen-assisted radioiodine therapy may present a safe and effective treatment alternative for non-toxic multinodular goiter.

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Program Number 171 Thyroid Nodules and Goiter

Papillary Carcinoma of the Thyroid in a Patient with Congenital Generalized Lipodystrophy: A Case Report

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Congenital generalized lipodystrophy (CGL) is a rare autosomal recessive disorder with an estimated prevalence of less than 1 case in 12 million people. Incidence is equal between males and females. Patients have a nearly complete absence of adipose tissue from birth associated with severe insulin resistance, leptin deficiency, hyperglycemia, hypertriglyceridemia, fatty liver, organomegaly, and generalized muscular hypertrophy. We report a case of a 19-year-old Lebanese male who is a product of a consanguineous marriage. Physical examination was remarkable for a height of 5 feet 8 inches; weight of 195 pounds; body mass index of 30; generalized muscular hypertrophy; severe acanthosis nigricans in the neck, axillae, and trunk; a 2- x 2-centimeter firm, non-tender, movable left thyroid nodule; Grade 2/6 systolic murmur heard best at the left parasternal border; and hepatosplenomegaly with a liver span of 18 centimeters. Laboratory findings include a fasting insulin of 410 mU/mL (Normal: 5-15); HgbA1c of 7.8%-12.4% (Normal: <6%); serum leptin of 1 ng/mL (Normal: >3ng/mL);IGF-1 of 153 ng/mL (Normal: 182-780); serum triglycerides of 452 mg/dl (Normal: <200 mg/dL); TSH of 2.35 mIU/mL (Normal: 0.5-5.5) and free T4 of 1.2 ng/dL (Normal: 0.8-1.8). Dual energy X-ray absorptiometry showed a total body fat of 6% (Normal:15-20%). A 2-D echocardiogram showed mild to moderate concentric left ventricular hypertrophy with good ventricular function and renal sonogram revealed bilaterally enlarged kidneys. Fine-needle aspiration of the left thyroid nodule revealed few follicular cells with Hurthle cell changes and nuclear atypia (rare pseudonuclear inclusions and nuclear grooves) suspicious for papillary carcinoma. Surgical pathology findings after total thyroidectomy showed papillary carcinoma. To our knowledge, this is the first reported case of a patient with CGL and papillary carcinoma of the thyroid.

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Program Number 172 Thyroid Nodules and Goiter

High-intensity Focused Ultrasound—Potential for Thyroid Pathology: Feasibility Study in a Sheep Model

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Thyroid pathology leading to surgery is extremely common. Surgical complications as recurrent nerve and parathyroid injury are possible. HIFU was investigated as a possible minimally invasive therapeutic option for thyroid pathology. Aim of the study: To determine the HIFU ability of inducing a precise necrotic area in the thyroid gland while preserving the structures located close to the target. Material and methods: A HIFU device designed for human prostate cancer treatment was used. The targeting of the lesion was done with the embedded ultrasonic 7.5-MHz sector-scanned imaging. HIFUs are generated by a 3-MHz spherical piezocomposite transducer. The sheep model was used because its thyroid gland is easily accessible with US although its size is smaller than human's. Eight sheep were anaesthetised and exposed to HIFU. During follow-up, animals were examined for adverse effects collection, including skin examination for possible burns. At day 11 +/- 4, the animals were sacrificed. The anterior part of the neck was formalin fixed and examined on transversal sections perpendicular to the trachea. Results: Thirteen thyroid lobes from 8 sheep aged 7 years (1-9) with an average volume of 3.4 cm^3 were partially treated. Three lobes were left untreated and used as controls. An average of 24 ultrasound bursts (9-44) per lobe was applied, covering an average volume of 0.7 cm^3 (0.2-1.8). The centre of each lobe was targeted. At histology, the following lesions were observed: central necrotic area, on the periphery, haemorrhagic congestion, fibroblastic and inflammatory granulation tissue, and thyroid follicular cell regeneration. At day 15, there was a retraction of the necrotic area. Conclusion: Our preliminary study confirms the possibility to obtain a necrotic thyroid volume with HIFU, matching the pre-defined targeted volume in location and dimension.

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Program Number 173 Thyroid Nodules and Goiter

Two Novel TSH Receptor Gene Mutations in Autonomously Functioning Thyroid Nodules*

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Objective: To determine the relationship between TSH receptor gene mutations and autonomously functioning thyroid adenomas (AFTA). Methods: The thyroid samples from 14 diagnosed AFTAs were analyzed, with normal thyroid specimens adjacent to the tumors as controls. The 155 base pairs DNA fragments which encompassed the third cytoplasmic loop and the sixth transmembrane segments in the TSH receptor gene exon 10 were amplified by PCR and analyzed by the single-strand conformation polymorphism(SSCP). Direct sequencing of the PCR products was performed with ABI Prism Dye Terminator Cycle Sequencing Core Kit. Results: In SSCP analysis, 6 of 14 AFTA specimens displayed abnormal migration. In sequence analysis of 3 abnormally migrated samples, one base substitution at nucleotide 1957 (A to C) and two same insertion mutations of one adenosine nucleotide between nucleotide 1972 and 1973 were identified. No mutations were found in controls. Conclusion: This study confirmed the presence of TSH receptor gene mutations in AFTAs; two novel mutations, especially one-base insertion mutation, were first discovered.
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Program Number 175 Cancer

Up-regulation of CITED1 in Papillary Thyroid Carcinoma: Discovery via Gene Expression Profiling and Validation by Tissue Microarray-based Immunohistochemistry

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Background: Robust diagnostic markers of common thyroid lesions are needed. Comprehensive gene expression technologies, such as oligonucleotide arrays, promise to discover differentially expressed genes amongst follicular cell lesions that should prove to be clinically useful markers. These methods were used to identify up-regulated genes in papillary thyroid carcinoma (PTC). The expression of one such gene (CITED1) was validated at the protein level using a thyroid tissue microarray and immunohistochemistry. Methods: Gene expression profiles of seven cases of PTC and four normal thyroids were generated using oligonucleotide arrays that contain 12,200 probe sets which represent approximately 10,000 genes (Affymetrix). A thyroid tissue microarray with 105 thyroid tissues representing the common thyroid lesions and 15 non-thyroid tissues was constructed from paraffin-embedded tissues. Sections were immunohistochemically stained using a rabbit anti-CITED1 polyclonal antibody. Results: CITED1 mRNA was significantly increased in the PTC cohort compared to the normal thyroid cohort (fold change = 63.4, $P<2x10^{-7}$). Immunohistochemistry showed increased expression in 22/26 PTCs (84.6%), with similar rates of positivity observed across classical, follicular and tall cell PTCs. Among 79 other thyroid lesions, 4 (5.1%) were positive for CITED1 immunoreactivity, which included 1/8 (12.5%) poorly-differentiated carcinomas, 1/9 (11.1%) nodular hyperplasias, and 2/5 (40%) follicular carcinomas. The remaining lesions (normal thyroid (5), medullary carcinoma (11), oncocytic carcinoma (5), anaplastic carcinoma (4), follicular adenoma (5), and oncocytic adenoma (1)) were all negative. The sensitivity and specificity of CITED1 immunoreactivity in PTC was 84.6% and 94.9%, respectively. The positive predictive value was 84.6%. Other non-thyroid tissues were negative. Conclusion: CITED1 gene expression, as assessed by routine immunohistochemistry, may represent a clinically useful diagnostic marker of PTC. Furthermore, as a transcriptional activator, CITED1 protein likely plays a role in the pathobiology of PTC.

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Program Number 176 Cancer

Successful Ultrasound-guided Percutaneous Ethanol Ablation of Neck Nodal Metastases in 20 Patients with Postoperative TNM Stage I Papillary Thyroid Carcinoma Resistant to Conventional Therapy

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The most common site for tumor recurrence in TNM stage I papillary thyroid carcinoma (PTC) is in neck nodal metastases (NNM). Sonographically guided (SG) percutaneous ethanol injection (PEI) was recently reported (AJR 178: 699, 2002) to be a valuable treatment option for patients with limited NNM from PTC, unsuitable for further surgery or I-131 therapy. The aim of the present study was to evaluate the efficacy of PEI in treating recurrent NNM in 20 selected patients (median age 35 years) with stage I PTC. All had undergone complete primary resection, and 11 (55%) had 1 to 6 (mean 2) neck re-explorations. 18 (90%) had I-131 treatment with a mean dose of 169 mCi (range 28-359) ; 2 (10%) also received cervical irradiation. Biopsy-proven recurrent NNM were discovered on average at 46 postoperative months (range 7-196). 31 NNM (mean diameter 12 mm, range 4-32) were typically injected on 2 consecutive days with a mean dose of 0.7 cc (range 0.2-1.6 cc) of 95% ethanol. There were no significant complications. Patients have been followed for up to 9.0 years since PEI (mean 24 months). At latest follow-up, none had untreated disease and, to date, 24 further neck re-explorations have been avoided. Sixteen (80%) have had repeat sonograms from 3-106 months after PEI (mean 22 months). All 23 injected nodes significantly decreased in volume and 6 disappeared. Seven of the 16 re-evaluated patients (44%) required a second PEI within 9 months (median 5 months) because of persistent flow on color Doppler. Five patients (25%) developed 6 new NNM during follow-up that were amenable to PEI. Only one patient (5%) required further surgery for NNM not amenable to SGPEI. PEI was successful in controlling all metastatic adenopathy in 15 (94%) of 16 re-evaluated patients. Conclusion: SGPEI appears efficacious in eliminating recurrent NNM in stage I PTC.

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Program Number 177 Cancer

Empiric Radioactive Iodine (RAI) Dosing Regimens Frequently Exceed Maximum Tolerated Activity Levels in Elderly Patients with Metastatic Thyroid Cancer

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Even though RAI therapy is a mainstay in the treatment of metastatic thyroid cancer, there is considerable controversy regarding the dose that can be safely administered without dosimetric determination of the maximal tolerated activity (MTA). Since dosimetry studies are not routinely available in most institutions, a fixed empiric dosing strategy is often used with administered activities ranging from 150 to 250 mCi. Based on our experience with dosimetry, we were concerned that this empiric dosing strategy may result in administered RAI activities that exceed our established safety limits in older patients with metastatic disease. Therefore, we retrospectively analyzed 484 hypothyroid dosimetry studies performed as part of routine clinical care in 335 patients. The MTA was less than 140 mCi in 2%, less than 200 mCi in 9%, and less than 250 mCi in 16%. Analysis of MTA values by age at time of dosimetry revealed little change in the MTA up until age 60 years when a significant decrease occurs. Empiric doses of 140 mCi rarely exceeded the MTA in patients less than 70 years of age. However, an empiric dose of 200 mCi would exceed the MTA in 14% of patients age 60-69, and 15% of patients aged 70-79 years. Furthermore, an empiric dose of 250 mCi would exceed the MTA in 23% of the patients aged 60-69, and 40% of patients aged 70-79 years. In summary, administered RAI activities less than 140 mCi rarely exceed the calculated MTA except in the very elderly. However, fixed doses of 200-250 mCi frequently exceed the calculated MTA in patients older than 60 years of age. Therefore, dosimetry guided RAI therapy is preferable to fixed-dose RAI treatment strategies in older patients with metastatic thyroid cancer.

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Program Number 178 Cancer

Distinct Localization Patterns of Activated Akt in Thyroid Cancer Correspond to Tumor Invasion and Ret Expression

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Overactivation of the Akt occurs in Cowden's syndrome and in sporadic thyroid cancers. In addition, RET/PTC oncoproteins signal in part through Akt, and Akt activation appears necessary for growth factor-mediated thyroid cell growth. Our purpose was to define the pattern of Akt activation in human thyroid tumors. We immunohistochemically examined 45 thyroid tissue samples [18 follicular adenomas (FA), 9 follicular carcinomas (FC), 13 papillary carcinomas (PC, 6 with nodal mestastases), and 5 poorly differentiated papillary carcinomas (PDPC)] using antibodies specific for activated (phosphorylated) Akt (pAkt, Ser473) and Ret (C19). Adjacent normal thyroid tissue served as an internal control for all cases. In normal thyroid, Ret was not expressed and pAkt was faintly detected in 2/45 cases. Six of 18 FAs (5 with minimal Ret expression) demonstrated low-levels of nuclear pAkt in subcapsular and peri-vascular regions. All 9 FCs expressed pAkt intensely in the nucleus and cytoplasm, but only in regions of vascular or capsular invasion suggesting that invasive cells were more likely to have Akt activation. Ret was faintly detected in 3/9 FCs. In PCs, cytoplasmic, but not nuclear pAkt was detected in 5/6 cancers with nodal metastases and 5/5 PDPC samples, but in only 2/7 PCs with no metastases. PCs with homogenous Ret expression were characterized by homogeneous cytoplasmic pAkt; however, the most intense staining was still localized to regions of local or vascular invasion. In conclusion, we have demonstrated that invasive thyroid cancer cells have high levels of Akt activation in vivo, and that activation of Akt may represent an early event in the invasive potential of follicular tumors. Cytoplasmic activated Akt appears to be associated with Ret overexpression in papillary cancers, suggesting signaling through Akt in vivo. Taken together, these data suggest a potential role for Akt activation in the invasive potential of thyroid cancers.

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Program Number 179 Cancer

Combretastatin A4 Phosphate Has Primary Antineoplastic Activity against Human Anaplastic Thyroid Carcinoma Cell Lines and Xenograft Tumors

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Anaplastic thyroid carcinoma (ATC) is a fatal malignancy whose clinical outcome is unaltered by current therapeutic modalities. A recent phase I clinical trial of combretastatin A4 phosphate (CA4) demonstrated a durable total response in an ATC patient. CA4 is a putative tumor vasculature angiolytic, tubulin-binding, agent derived from the African bush willow, Combretum caffrum. In order to discriminate primary antineoplastic effects from tumor antivascular activity, we evaluated CA4 cytotoxicity in eight human ATC cell lines and compared it to another agent with significant clinical activity, paclitaxel. CA4 displayed significant cytotoxicity against the ATC cell lines, comparable to that of paclitaxel, and these effects were longer lasting compared to the duration with paclitaxel. We further investigated the effects of CA4 on xenograft tumors from four ATC cell lines injected in athymic nude mice. Significantly lower tumor weights were observed in animals treated with CA4 compared to those injected with the vehicle alone. These results suggest that the antitumoral effects of CA4 can be consequent to possible primary antineoplastic effects besides the potential destruction of tumoral vasculature. This activity and potential dual mechanisms of action warrant further clinical evaluation of CA4 in therapeutic trials for anaplastic thyroid carcinoma.

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Program Number 180 Cancer

Effectiveness of I-131 in Destroying Metastatic Thyroid Cancer Lesions

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I-131 (RAI) has been a mainstay in the therapy of metastatic differentiated thyroid cancer (DTC) for over 40 years. The effectiveness of RAI correlates with the radiation dose delivered. Doses of 8.5 to 10 Gy are thought necessary to destroy an individual lesion (Maxon et al). We performed a retrospective analysis of a cohort of DTC patients with RAI-avid metastases (mets) who were treated with RAI at one medical center over a 4-year interval. Some patients were prepared for RAI by thyroid hormone withdrawal (THW) and some by recombinant human TSH (rhTSH). All pts were on a low-iodine diet prior to therapy and all had had a total thyroidectomy. Full dosimetry was performed in all pts in the same physiologic state that they were treated in. Responsiveness was defined as the percentage of patients who had complete resolution of RAI uptake in the region of interest on a 5 mCi whole body RAI scan performed one year later. The regional success rates following THW preparation were: 81.5% (thyroid bed; TB, n=124); 64.3% (cervical nodes; Cv, n=70); 33.3% (lung, n=54); 58.6% (mediastinum; med, n=29); and 4.5% (bone, n=22). The regional success rates following rhTSH preparation were: 84.3% (TB, n=70); 63.6% (Cv, n=44); 55.6% (med, n=27); and 21.4% (bone, n=14). There were no statistical differences in the response rates in any tissue between the THW and the rhTSH preparations. In summary, RAI is most effective at destroying thyroid remnants; it has moderate activity against loco-regional mets; it has limited effectiveness against lung mets, and is ineffective for bone mets. New therapeutic approaches are desperately needed for pts with lung and/or bone mets from DTC, as mets in these tissues are associated with significant morbidity and mortality.

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Program Number 181 Thyroid and Development

Microarray Analysis Reveals That the Transcription Factor NeuroD Is Responsive to Thyroid Hormone during Late Rat Brain Development

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Thyroid hormone (TH) plays an important role during late brain development. TH deficiency leads to deficits in brain maturation including aberrant cerebellar and hippocampal development. Elucidating the molecular basis of TH action in the developing mammalian brain requires identification of TH-responsive brain genes. To this end we have undertaken a gene discovery approach utilizing microarray methodology. Brains were harvested from postnatal day 10 (P10) euthyroid or hypothyroid rat neonates. TH effects on brain gene expression were determined by subjecting total RNA isolated from 4 hypothyroid and 4 euthyroid brains to microarray analysis. We constructed our array from 10,470 University of Iowa Brain Molecular Anatomy Project cDNA clones derived from mRNAs isolated from C57Bl6/J mouse brain. Our results revealed TH-regulated enhancement of several previously described TH-responsive brain genes; including myelin basic protein, proteolipid protein, BTEB, Na, K-ATPase and a neurofilament gene. Several genes not previously reported as TH-responsive were identified using this methodology. One of these genes, the basic helix-loop-helix transcription factor neurogenic differentiation factor 1 (NeuroD), plays an important role during late brain development. NeuroD null mice exhibit deranged cerebellar and hippocampal development (Genes and Development (1999) 13:1647-1652); a neuropathogenic phenotype similar to that associated with hypothyroidism. Quantitative, real time PCR revealed that NeuroD mRNA levels were approximately 2-fold upregulated by TH on postnatal day 10 but unaffected by TH status immediately after birth (P6) and in the young adult (P30 and P50). This transient responsivity to TH is observed in many TH-responsive brain genes. Additionally, acute treatment of hypothyroid rats with TH for 48 hours prior to tissue harvest resulted in 2-fold NeuroD upregulation in P10 and 12 rats suggesting that TH may directly regulate NeuroD expression. Thus, microarray analysis has revealed a gene that may directly mediate the functional effects of TH during late mammalian brain development.

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Program Number 182 Thyroid Hormone Action

Role of Thyroid Hormone Receptor Alpha (TRα) and Skeletal Muscle in Thyroid Hormone Thermogenesis

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The mechanisms whereby thyroid hormone increases thermogenesis remain elusive. Mice with deletion of TRα gene (TRα0/0) but not TRβKO mice have lower body temperature. We investigated the effect of a TRβ-selective ligand, GC1, or the deletion of TRα gene on thermogenesis (oxygen consumption, QO2) and mRNA (RT-PCR) of potentially thermogenic genes. Hypothyroid wild type (WT) mice treated with doses of T3 10 times the daily production rate for 8 days increased QO2 over the euthyroid level, whereas QO2 remained unchanged in mice given an identical regimen of equimolecular doses of GC1. T3- but not GC1-treated hypothyroid mice maintained their body temperatures after 6 hours of exposure to 10°C. Both GC1 and T3 induced liver mitochondrial glycerol 3-P (mGPD) activity and mRNA above the euthyroid level. While T3 normalized the expression of UCP2, UCP3 and mGPD in skeletal muscle, GC1 failed to do so. WT, TRα0/0 or TRβKO mice were rendered hypothyroid and treated for 4 days with T3 in doses equivalent to 5 times the daily production rate. UCP3 mRNA level was higher in skeletal muscle and heart of TRα0/0 ($P<0.05$) than in TRβKO or WT before T3, responded to this in all three genotypes but significantly more ($P<0.001$) in TRα0/0 mice. In contrast, UCP2 mRNA failed to respond to T3 in both heart and muscle of TRα0/0 mice, while it was normalized in WT and TRβKO mice. Liver mGPD mRNA equally responded to T3 in all three genotypes, but muscle mGPD mRNA responded less in TRα0/0 (50%;$P<0.01$). Conclusions: 1) Mice lacking TRα have a thermoregulatory defect, not a mere resetting of the hypothalamus; 2) muscle is an important site for TH thermogenesis; 3) UCP2 strictly requires TRα, while the TRα requirement of mGPD is tissue-specific; and 4) UCP3, without a specifc TR isoform requirement, may partially compensate for the thermogenic deficiency of TRα0/0 mice.

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Program Number 183 Thyroid Hormone Action

Differential Effects of 3,5,3'-Triiodo-L-Thyronine (T_3) on Metabolic Rate, Cholesterol, and Heart Rate in Cholesterol-fed Wild Type and TRalpha1 $^{-/-}$ Mice

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Thyroid hormone receptor beta (TRbeta) selective agonists selectively reduce cholesterol and increase metabolic rate with minimal tachycardia. The goal of this study was to determine the effects of T_3 on metabolic rate, heart rate (HR) and cholesterol in wild type (WT) and TRalpha1 $^{-/-}$ mice to further dissect the role of TRbeta in their control. The mice were cholesterol-fed for 2 weeks followed by 7 days treatment with T_3. Baseline HR and body temperature were lower in WT vs TRalpha $^{-/-}$ mice, while metabolic rate was slightly higher in TRalpha1 $^{-/-}$ mice (2697 ± 54 and 2885 ± 51 mL/O2/kg/h for WT and TRalpha1 $^{-/-}$ respectively). T_3 reduced cholesterol with similar potency in WT (ED50 = 29 nmol/kg/day) and TRalpha1$^{-/-}$ mice (ED50 = 35 nmol/kg/day). T_3 increased metabolic rate and HR in a dose-dependent manner in WT (ED15 = 34 nmol/kg/day for HR and ED5 = 7 nmol/kg/day for metabolic rate) and TRalpha1$^{-/-}$ mice (ED15 = 469 nmol/kg/day for HR and ED5 = 10 nmol/kg/day for metabolic rate), but with a significantly lower slope in TRalapha1$^{-/-}$ mice. The potency ratios show that TRalpah1$^{-/-}$ mice are 27-fold more selective for cholesterol lowering vs tachycardia and 10-fold more selective for 5-10% increases in metabolic rate vs tachycardia in response to T_3. The results suggest TRbeta is primarily responsible for the cholesterol-lowering effects of T_3 and that TRbeta activation can increase metabolic rate 5-10% increase with no tachycardia. This is consistent with studies using TRbeta selective agonists.

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Program Number 184 Thyroid Hormone Action

Altered Cardiac Phenotype in Mice Expressing the Dominant Negative PV Mutant of the Thyroid Hormone Receptor Beta

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The thyroid hormone mediated-effects upon the heart act through the thyroid hormone receptor alpha (TR-α) and beta (TR-β) gene products. Mice devoid of TR-α exhibit a hypothyroid cardiac phenotype. In contrast, mice lacking the TR-β have a normal cardiac phenotype. In order to determine the role of TR-β in the heart, we studied mice whose endogenous TR-β had been mutated to the dominant negative TR-β PV mutant found in RTH patients. Mice carrying the homozygous mutation are hyperthyroid, yet the heart rate in these animals compared with WT controls was decreased by 19% ($p<0.05$). When T3 levels in both mutant and wt were equalized to euthyroid levels, the TR-β PV heart rates were decreased by 37% ($p<0.05$) compared with control animals. The level of HCN2 mRNA was not altered in the euthyroid TR-β PV mice but HCN 4 levels were decreased by 45% ($p<0.05$), which could contribute to the observed bradycardia. We extended our studies to determine the contractile function of the hearts of TR-β PV and WT mice. There were no differences in contractile function in the hyperthyroid TR-β PV mice, however when the PV mutants were euthyroid, there was a 35% ($p<0.05$) decrease in developed LV peak systolic pressure, +dP/dT, and –dP/dT. These contractile differences could result from a 13% decrease in SERCA2 mRNA and a 235% ($p<0.05$) increase in myosin heavy chain-beta mRNA expression. Conclusions: 1. The decreased heart rates indicate that the TR-β PV is expressed in the conduction system of the mouse heart leading to bradycardia. 2. The contractile deficit indicates that the TR-β PV is expressed throughout the ventricle of the heart and can antagonize the effect of TR-α. 3. In contrast to the mice lacking TR-β, there is a significant cardiac phenotype due to the expression of the TR-β PV mutant receptor.

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T3 but Not the Thyroid Hormone Receptor Beta-selective Compound GC-1 Reduces Bone Mass of Normal and Hypoestrogenic Rats

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To investigate the role of thyroid hormone receptor beta (TRb) in mediating the osteopenic effects of T3, we studied the bone mass of intact and ovariectomized (OVX) adult rats chronically (64 days) treated with T3 (3 ug/100 BW day) or with an equimolar dose of GC-1, a TRb-selective thyromimetic compound. Treatment with T3 significantly decreased bone mineral density (BMD) in the hind body (HB= hind limbs+pelvis+second lumbar to fourth caudal vertebra segment), lumbar vertebrae (L2-L5), femur and tibia of both intact (10-15% from baseline) and OVX rats (4.2-7.7%). In contrast, no skeletal site was affected by treatment with GC-1 even though known GC-1 biological effects were clearly detected, i.e., reduction in serum TSH (-52% vs. control, $p < 0.05$) and serum cholesterol (-21% vs. control, $p < 0.05$). Histomorphometric analysis confirmed that trabecular bone was negatively affected by T3 but not by GC-1 treatment. In the T3-treated animals, the trabecular number, volume and thickness were, respectively, 31% ($p < 0.01$), 46% ($p < 0.05$) and 22% ($p < 0.01$) lower than in control rats. On the other hand, GC-1 significantly increased the apposition mineralization rate ~2-fold as compared to both controls and T3-treated animals ($p < 0.05$). T3 treatment decreased lean mass (LM) and fat mass (FM) in the HB of OVX rats, while GC-1 decreased FM in intact rats (60%, $p < 0.05$) and significantly impaired FM gain in OVX rats, but had no effect on LM. These results suggest that TR alpha and not TRb mediates T3-induced bone loss, illustrating the role of individual TRs in mediating selective actions of thyroid hormone. Suppression of TSH without promoting bone loss by selective stimulation of TRb may have therapeutic applications, such as in thyroid cancer patients.

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Program Number 186 Thyroid Hormone Action

Effect of Thyroid Receptor Beta Expression on the Contractile Phenotype of the Mouse Heart

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Thyroid hormone affects contractility through its interaction with alpha thyroid receptor (isoform 1) and beta thyroid receptor (isoform 1 and 2). The T3 receptor alpha1 (TRa1) represents two-thirds of cardiac TR and is the dominant receptor in the mouse heart. The dominant role of TRa1 is supported by diminished contractile function of the TRa-knock-out mouse; however, the contribution of the beta receptors (TRb) is not clear. It is also unresolved as to whether the dominant TRa effect is merely a consequence of receptor number or is isoform specific. To investigate the role of the beta receptor in the heart, cardiac function was examined in isolated Langendorff perfused hearts from beta receptor knock-out mice made euthyroid through treatment with a low iodide/PTU diet and thyroid hormone replacement. Cardiac function (as determined by +dP/dt , - dP/dt and peak systolic pressure) under both baseline conditions and increased workload was not different between TRb knock-out and wild type mice, indicating that TRa1 alone provides a sufficient amount of TR for contractile function under euthyroid conditions. The effect of TRb expression on cardiac function in mice lacking TRa1 was determined by the injection of adenovirus expressing beta thyroid receptor (isoform 1) directly into the free wall of the left ventricle 4 days prior to Langendorff perfusion. Treatment of TRa1 knock-out mice with adenovirus expressing TRb resulted in a 27% increase in the rate of relaxation (-dP/dt, $p<0.05$) and a 14% increase in systolic pressure development in comparison to TRa knock-out mice treated with an empty adenovirus. Together, these results indicate that although TRa1 provides sufficient TR to regulate the contractile phenotype in the mouse heart under normal conditions, increasing TRb expression in the absence of TRa can improve contractile function, suggesting that TR receptor quantity regardless of isoform significantly contributes to the cardiac phenotype.

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Program Number 187 Cell Biology

Targeting of Thyroglobulin to Transcytosis following Megalin-mediated Endocytosis: Evidence for a Preferential pH-independent Pathway

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Thyroglobulin (Tg) internalized from the colloid by megalin by-passes lysosomes and is transported intact across thyrocytes by transcytosis. Most of the intracellular mechanisms responsible for targeting to transcytosis are unknown, but in certain cases a role of pH has been established. Thus, ligands that enter lysosomes dissociate from their receptors due to the low lysosomal pH and are therefore degraded, whereas certain ligands that undergo transcytosis fail to dissociate because they bind to their receptors at acidic pH. Here we investigated the role of pH in megalin-mediated transcytosis of Tg. In solid phase assays, megalin bound to Tg at various pH values (range: 4-8), but binding was optimal at acidic pH (range: 4.5-6). We studied the effect of chloroquine (CQ) and ammonium chloride (AC), which increase lysosomal pH and inhibit degradation, on Tg endocytic pathways in FRTL-5 cells. Treatment of cells with CQ or AC did not affect binding and uptake of Tg, but, as expected, reduced T3 release from exogenously added Tg, used as a measure of Tg lysosomal degradation. In transcytosis assays we used FRTL-5 cells cultured on transwell filters, with megalin expression only on the upper surface of the layers. Following addition of Tg to the upper chamber and incubation at 37°C, transcytosed Tg was measured in fluids collected from the lower chamber. Treatment with CQ or AC increased Tg transcytosis, but only to a minimal extent (15-20%). The effects of CQ or AC and those of a megalin competitor (the monoclonal antibody 1H2) were not additive, suggesting that CQ and AC act on the megalin-mediated pathway. In conclusion, optimal pH for Tg binding to megalin is acidic and probably Tg does not dissociate from megalin in the lysosomal pathway. However, pH-dependence of binding accounts only minimally for transcytosis, which occurs because of other mechanisms of targeting.

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Program Number 188 Cell Biology

Increase of p66 Shc Expression in Proliferating Thyroid Cells: Its Regulation and Role in Thyrocytes

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Among growth factors for the growth of thyrocytes, TSH has been reported to have a major role in goitrogenesis. However, the traditional intracellular signaling pathway of TSH receptor by cAMP/protein kinase A (PKA) seems not to explain the whole mechanism of goitrogenesis. Recently it was reported that the expression of p66 Shc was increased by TSH stimulation in thyrocytes, suggesting that p66 Shc molecule may play a critical role in the transmission of the TSH-induced growth signals. We also reported that TSH increased the expression of p66 Shc via TSH receptor-Gs protein-adenylate cyclase-cAMP-PKA pathway in FRTL-5 cells (ENDO2002, SF). In this study, we tried to verify the regulation and role of p66 Shc in growth of thyrocytes. The expression of p66 Shc was increased in the tissues of human thyroid diseases including adenomatous goiter, adenoma, Graves' disease, and papillary thyroid cancer, while increased expression was not found in normal thyroid tissues. On the other hand, IGF-1 and EGF increased tyrosine phosphorylation of p66 Shc and insulin increased serine phosphorylation, which effects were amplified by pretreatments of TSH. Similar results were found in the 3H thymidine uptake assay. When we compared the amount of p66 Shc expression induced by individual TRAb from 130 Graves'disease patients with clinical characteristics, TSH receptor stimulating activity and goiter size showed a weak correlation. In summary, the TSH or TRAb-induced p66 Shc expression seems to mediate the responses to the stimulation of growth factors by amplifying their effects, and may play an important role in goitrogenesis.

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Program Number 189 Cell Biology

Regulation of Cellular Prion Protein (PrP^c) mRNA Expression by TSH in Human Thyroid Follicles

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Cellular prion protein (PrP^c) is a cell-surface glycosyl phosphatidyl-inositol-anchored protein which is extensively expressed on the neural cell membrane, and may function as a neural cell receptor or as a cell adhesion molecule directing and/or maintaining the architecture of the nervous system. Using cDNA microarray which can analyze 2400 genes in a single run, we demonstrated that TSH increased more than 100 genes (THYROID, in preparation). Interestingly, PrP gene was strongly expressed and its expression levels were increased 3-fold by TSH. Therefore, we studied PrP expression levels in cultured human thyroid follicles derived from neurologically normal patients (Graves' disease) by Northern blot analyses.
Thyroid follicles, prepared by digestion with collagenase and dispase, were cultured in F-12/RPMI-1640 medium supplemented with 0.2% fetal bovine serum, 10^{-8}M KI, and bTSH (1-100 μU/ml) in culture dishes which had been coated with agarose. After appropriate periods, total RNA was extracted, and expression levels of PrP mRNA were determined by Northern blot hybridization, using ^{32}P-labelled human PrP cDNA.
bTSH time- and dose-dependently (1-100 μU/ml) increased the steady state levels of PrP mRNA expression. Dibutylyl-cAMP and phorbol ester and A23187 also increased the PrP expression levels. However, iodide did not modulate mRNA expression levels at 10^{-5} M, at which concentration thyroid hormonogenesis was almost completely suppressed.
These findings suggest that PrP mRNA is relatively abundantly expressed in human thyroid follicles, and that TSH stimulates PrP mRNA expression in thyrocytes, as nerve growth factor does in neurons. However, physiological roles of PrP^c in endocrine tissues remain unknown.

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Program Number 190 Cell Biology

The High Selenium Content of the Thyroid Gland Is due to the Expression of Several Types of Selenoproteins

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The thyroid gland is among the organs with the highest selenium content, and several diseases associated with selenium deficiency, such as Kashin-Beck disease and myxedematous cretinism, also involve the thyroid gland. This suggests that adequate selenium supply is required for thyroid function. During thyroid hormone biosynthesis, the thyroid gland produces high amounts of H2O2. Efficient protection against its potentially toxic effects may be provided by selenoproteins such as glutathione peroxidases (GPx), thioredoxin reductases (TrxR), and selenoprotein P (SeP). Their expression was studied in goiter samples and thyroid cell lines. Using a multiple tissue Northern array for expression analysis, we found that the thyroid gland expressed high GPx and TrxR mRNA levels, whereas SeP mRNA was only moderately expressed (14 % signal intensity as compared to liver). GPx enzyme activity was detected in cell lysates and conditioned media of the thyroid cell lines FRTL-5 (normal, rat), FTC-133, FTC-238 (human follicular thyroid carcinoma), and XTC (human Huerthle cell carcinoma). GPx activities ranged from 8.9 to 30.3 nmol NADPH oxidized per min and mg protein. In situ hybridization studies on goiter ultrathin sections revealed expression of GPx mRNAs in thyrocytes. TrxR enzyme activity was observed in cell lysates and conditioned media of the thyroid cell lines mentioned above and ranged from 3.0 to 5.3 mU per min and mg protein. SeP was detected by Western blot in thyroid carcinoma cell lines, both within cells (FTC-133/-238, HTh74, C643) and secreted into the culture medium (XTC). Thus, the high selenium content of the thyroid gland is explained by the expression of a broad spectrum of selenoproteins. These may indeed be involved in the regulation of H2O2 availability and the scavenging of excess H2O2, thereby preventing intracellular iodination, membrane peroxidation, and mutagenic oxidative DNA damage and thus ensuring proper thyroid function. - Supported by DFG.

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Program Number 191 Cell Biology

Stress-inducible hSNK Gene Expression in Thyroid Follicular Cells

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The question of the biological relevance of the human homologue of mouse serum-inducible kinase, hSNK, was addressed in this study. hSNK belongs to the polo-like kinase (PLK) family whose members exert their function at several stages of mitosis. Using select-PCR differential display, we demonstrated that hSNK expression was rapidly up-regulated after the irradiation of primary human thyrocytes. Characterization of hSNK promoter structure revealed that activation elements including the TATA-like motif and GC box resided within first 122 nucleotides upstream of an analogous transcription start site. Previously, an inducible enhancer between nucleotides -148 to -122 containing p53RE motif was identified. Radiation and other stresses (UV, hydrogen peroxide, serum starvation) induced the increase of hSNK mRNA levels in ARO cells. The up-regulation of hSNK expression was dependent of the presence a positive regulatory element located between nucleotides -85 and -65 of the promoter identified as a typical cAMP response element (CRE). Deletion of this fragment significantly decreased hSNK promoter activity of the 5'-122 construct upon the challenge by virtue of inability to bind CREB and CREM. As cAMP and hydrogen peroxide are known to modulate TSH effects on thyroid follicular cell proliferation, differentiation and function, and taking into account the distinctive features of hSNK regulatory region, we speculate that hSNK expression and function may comprise thyroid-specific events affecting cell fate following the stress and/or stimulatory signals.

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Program Number 192 Cell Biology

Expression of Tumor Necrosis Factor-α in FRTL-5 Rat Thyroid Cells

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Series of studies have demonstrated that various cytokines are involved in the pathogenesis of autoimmune thyroid disease (AITD). It is clear that thyroid cells produce such cytokines as type I interferon (IFN), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). While IL-6 production is much better characterized in the thyroid, the regulation of TNF-α production remains largely unknown. In the present study, we examined the mechanism of TNF-α gene expression using cultured thyroid cells. [Methods] FRTL-5 rat thyroid cells were cultured in Coon's modified Ham's F-12 medium supplemented with 5% calf serum and five or six hormone mixture. TNF-α gene expression was examined by RT-PCR. Phosphorylation of iκB or p42/p44 mitogen-activated protein kinase (MAPK) was evaluated by immunoblotting. [Results] Stimulation of FRTL-5 cells with lipopolysaccharide (LPS) or TNF-α resulted in an increase in TNF-α mRNA levels. However, IFN-γ, synthetic double-stranded RNA or DNA, or amiodarone did not induce TNF-α gene expression. IFN-γ enhanced TNF-α- or LPS-induced TNF-α gene expression. Addition of TNF-α or LPS led to phosphorylation and degradation of iκB in FRTL-5 cells. Phosphorylation of p42/p44 MAPK was not induced by TNF-α or LPS. [Conclusions] TNF-α and LPS induce TNF-α gene expression through activation of NFκB in thyroid cells. Thus, our results suggest that TNF-α produced by thyrocytes may modulate thyroid function and activity of infiltrating mononuclear cells such as lymphocytes and macrophages in AITD.

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Program Number 193 Thyroid and Development

Impaired Word Recognition Abilities in Children with Congenital Hypothyroidism: An Event-related Potential Study

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Thyroid hormone (TH) is essential for neural structures underlying memory and animals that lack TH show memory impairments and damage to neural structures important for learning and memory (e.g., hippocampus, posterior cortex). Children with early-treated congenital hypothyroidism (CH) achieve normal intelligence but show selective deficits on memory tasks. Impairments in short-term recognition of visual stimuli, memory for places, event-related memory, and associative learning are typically seen and are also consistent with damage in hippocampus and posterior cortex. In this study, we used electrophysiological techniques to evaluate recognition memory in 9 children with CH (11-13 years) and 9 matched controls. Event-related potentials (ERPs) were recorded while children viewed a sequence of 120 words, 40 of which repeated immediately, 40 repeated after a 5-item lag, and 40 were new. Children pressed different keys for "new" versus "old" stimuli. Groups did not differ in accuracy and reaction time or in latency for any electrodes. However, a Group X Condition interaction at the left-side electrode over the parietal lobe (P3) was observed in the immediate-repeat condition ($p<.005$). This reflected a positive amplitude shift for repeated versus new items, which is known as the repetition effect and is thought to signify recognition. Only controls showed this effect and it was not evident in children with CH. These results suggest that despite equivalent levels of behavioral recognition, the neurophysiological response of children with CH to repeated words is dampened and may explain some of the memory difficulties seen in this population.

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Program Number 194 Thyroid and Development

Construction of a Subtraction Hybridization Library for Identification of Differentially Expressed Genes in Thyroid Dysgenesis

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Thyroid dysgenesis is the most common cause of congenital hypothyroidism (CH). In 65% of CH patients the thyroid development process fails resulting in thyroid tissue at the base of the tongue or in any position along the thyroglossal tract. Genetic defects in any of the essential steps in thyroid development may result in ectopic thyroid. In higher eukaryotes, biological processes such as cellular growth and organogenesis are mediated by programs of differential gene expression. There is a consensus among thyroidologists that the syndrome of ectopic thyroid gland is the final result of the action of many genes (polygenic disorder). One of the approaches to investigate differential expressed genes is the suppression subtractive hybridization (SSH) to generate subtractive cDNA libraries. This methodology has been used with success to study gene expression in osteocytes compared with osteoblasts and in retina following optic nerve injury. It was also used to identify 17 known genes not previously recognized as differentially regulated by the sex-specific GH pattern. To investigate the genes involved in lingual thyroid gland we constructed a subtraction library. We compared lingual thyroid (obtained at surgery) with normal thyroid tissue using total RNA from normal tissue as the tester and total ectopic RNA as driver. This forward library was constructed to identify the genes that are not expressed in the ectopic thyroid. To synthesize de cDNA and to construct the subtraction library we used the Smart PCR cDNA synthesis kit and the PCR-select cDNA subtraction Kit (Clontec). Now the library is being analyzed by hybridization in macroarrays. After the identifications of differential expressed genes, their expression will be evaluated in both lingual and normal thyroid tissues.

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Program Number 195 Thyroid and Development

Area-specific Effects of Hypothyroidism on Intracellular Thyroid Hormone Levels in Developing Chicken Brain

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Pre- and perinatal hypothyroidism have detrimental effects on brain development and may lead to lifelong defects. In accordance with its essential need for thyroid hormones (TH), the developing brain seems to maintain relatively high intracellular TH levels even when circulating levels are low. We induced hypothyroidism in chick embryos by injecting methimazole at the beginning of the third (final) week of development. Plasma and tissue samples, including telencephalon, cerebellum, diencephalon, optic lobes and brain stem were collected daily. When comparing intracellular T4 and T3 levels in control animals, it was clear that ontogenic profiles differed according to the brain area. Highest T4 levels were reached in telencephalon and brain stem but they remained in general below plasma T4 levels (pmol/g or ml). For T3, on the contrary, all levels were high compared to plasma levels, especially at the transition from allantoic to lung respiration, when a sharp peak was found in all areas. This peak was reduced by mild hypothyroidism and totally abolished by severe hypothyroidism, which also prevented hatching. T4 levels in most brain areas decreased with more severe hypothyroidism, but there was a gradual increase in tissue/plasma T4 ratio. Telencephalon reacted differently compared to other brain areas. The sharp increase in T4 on day 18 was maintained even in severe hypothyroid embryos, resulting in very high tissue/plasma T4 ratios. In spite of this, T3 levels in telencephalon of hypothyroid embryos were reduced as in other brain areas suggesting that intracellular T4 was not converted to T3. This is unlike the situation in brainstem, where the sudden increase in intracellular T4 at the end of development in severe hypothyroid animals was accompanied by a similar increase in T3. Our results illustrate that hypothyroidism can influence intracellular TH levels and hence cell development differently according to the specific brain area.

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Program Number 196 Thyroid and Development

Phosphorylation of Heat Shock Protein 90 by TSH in FRTL-5 Thyroid Cells

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Although TSH acts via protein kinase A (PKA) and protein kinase C (PKC), the phosphorylation products of TSH action have not been fully identified. To explore this further, FRTL-5 thyroid cells were exposed to bTSH (10 μU/ml) for 30 min prior to 2D electrophoresis of the total proteins. Initial studies revealed that TSH-induced an acidic isoelectric shift in a prominent 90 kDa protein. Subsequent mass spectroscopy identified this 90 kDa protein to be heat shock protein 90 (hsp 90). Further analysis by Western blotting using a monoclonal antibody to hsp 90 confirmed the identity of the protein target. Preliminary 2D studies using forskolin (1 μM, 30 min) or phorbol myristate acetate (100 nM, 30 min) indicated changes in the phosphorylation states of a 90 kDa protein, but distinct from that produced by TSH. Exposure to either PKA inhibitor (H-89) or the PKC inhibitor (Gö 6976) altered the pattern of the 90 kDa protein induced by TSH in 2D electrophoresis. In summary: 1) TSH causes phosphorylation of hsp 90 in FRTL-5 thyroid cells; 2) TSH phosphorylation of hsp 90 could be mediated by both PKA or PKC pathway(s). We conclude that hsp 90 may be an important intracellular mediator of TSH action possibly involved in thyroglobulin assembly.

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Program Number 197 Thyroid and Development

Role of Type III Iodothyronine Deiodinase (D3) for Human Brain Development

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Strictly regulated thyroid hormone levels are required for normal brain development. Deiodination is a key process in this regulation: D1 and D2 catalyze the conversion of the prohormone T4 to the receptor-active T3; D3 catalyzes the degradation of T3 and the conversion of T4 to rT3. D1 and D2 activities are undetectable to low in human fetal brain, whereas D3 activity is maximal in the fetal stage. To study the role of D3 in the regulation of intracellular thyroid hormone levels in the developing human brain, we determined D3 activity and T3, T4 and rT3 levels in different fetal and post-natal brain regions at 13-42 weeks post-menstrual age. D3 activity showed a consistent pattern of distribution at all post-menstrual ages, decreasing in the order (mean±SEM in fmol/min/mg protein): cerebellum (64.01±8.46) >> midbrain (32.38±4.75) ~ basal ganglia (31.86±7.22) > brain stem (22.16±2.57) > spinal cord (18.70±2.86) > hippocampus (14.57±5.85) >>> germinal eminence (2.28±0.45) > choroid plexus (1.09±0.17) > cerebral cortex (0.46±0.10). D3 activities tended to decrease with age in cerebellum, brain stem and spinal cord; in these regions T3 was low (0.41-0.81), and T3 and T4 increased with age. Highest T4 (14.80±1.77) and rT3 (3.01±0.33) were found in the choroid plexus, T4 being >3-fold higher than in any other brain region, whereas T3 was low (0.78±0.11). Comparing the different brain regions for thyroid hormone ratios and D3 activity, we found a positive correlation between D3 and rT3/T3 (r=0.81) and rT3/T4 (r=0.89). The high D3 activities found in several human brain regions together with the good correlations between D3 activity and thyroid hormone ratios suggest that D3 is important in the regulation of intracellular thyroid hormone levels in the developing brain. Since D3 tends to decrease with age, D3 may protect the various brain regions from undue T3 until differentiation is required.
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Program Number 198 Thyroid Hormone Metabolism

Type I Iodothyronine Deiodinase (D1) Splice Variants in Human Liver

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Introduction. The human D1 gene is 17.5 kb long, 2.2 kb of which is distributed over 4 exons. Exon I codes for the 5' UTR and amino acids (AAs) 1-112; exon II for AAs 113-160, including selenocysteine (Sec), exon III for AAs 161-227, and exon IV for AAs 228-249 and the 3' UTR. Alternative splicing may have important consequences for expression of diverse functional gene products. Alternative splicing has been documented in D2 but not in D1. We have now studied the possible existence of D1 splice variants in human liver. Methods. Total RNA was isolated from 8 human livers and RT-PCR was performed using primers located over the translation start and stop codons. Agarose gel electrophoresis showed multiple bands ranging from 400-750 kb. The PCR products were AT cloned and sequenced. Results. Six D1 variants (D1a-f) were identified: D1a represents wild-type D1; D1b lacks part of exon I (AAs 48-112); both D1c and D1f lack a smaller part of exon I (AAs 78-112), while D1c also lacks exon III; D1e only lacks exon III; and D1d lacks exon II. All splicing events follow the gt-ag consensus. Of hD1e 2 ESTs are listed in GenBank; all other variants are novel. All variants code for shorter proteins, 2 of which (b,e) still contain the catalytic Sec residue. Conclusions. In human liver the D1 transcript undergoes alternative splicing, resulting in at least 6 mRNA variants. The possible function and regulation of this process remains to be determined.

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Program Number 199 Thyroid Hormone Metabolism

Demonstration of Dose Linearity In Vivo between Different Strengths of Sodium Levothyroxine Tablets

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Treatment of thyroid disorders with levothyroxine (LT4), e. g., hypothyroidism, aims at constant LT4-blood levels. Constant release and bioavailability of LT4 by commercial sodium levothyroxine tablets over a broader range of strengths is desirable. The aim of the study was to prove dose linearity between various doses (0.05, 0.1, 0.15, and 0.2 mg) of sodium levothyroxine tablets (Berlthyrox® / BERLIN-CHEMIE AG, Germany) in vivo. In four different studies three tablets of each strength were administered once daily to healthy male volunteers (n=68) over a period of seven days resulting in a daily dose of 0.15, 0.3, 0.45, and 0.6 mg LT4. The primary pharmocokinetic parameter for demonstration of dose linearity between Berlthyrox® 0.05, 0.1, 0.15, and 0.2 mg was the AUC (0-24) of total T4 after repeated drug administration on day 7 in all studies. Furthermore, mean values of both AUC (0-24) of T4 on day 1 after single dose as well as of Cmax of T4 after single and repeated doses were considered. Both the Kruskal-Wallis as well as the F-tests tested the homogeneity of the four strength groups. Mean values of AUC (0-24) and of Cmax for single and repeated dosing were adjusted for T4-baseline (predose) values. Analysis of covariance was performed after considering the difference in T4 baseline values. A linear relationship between dosage and AUC (0-24) of total T4 could be demonstrated, particularly after repeated administration. Although slightly less marked after single dosing, linearity was indicated for Cmax. Further regression analyses considering AUC (0-24) and Cmax in relation to the individually calculated per kilogram body weight doses supported the findings. In conclusion, dose linearity between the tested strengths of Berlthyrox® sodium levothyroxine tablets was clearly demonstrated.

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Program Number 200 Thyroid Hormone Metabolism

Is the Low T3 State a Crucial Factor Determining the Outcome of CPB Patients? Evidence from a Clinical Pilot Study

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The cardiovascular system is an important target for thyroid hormones. The present study evaluates the changes affecting thyroid hormone metabolism during and a few days after cardiopulmonary by-pass (CPB) and their relationship with post-operative outcome of patients. Thirty-three patients were enrolled in the study, their thyroid hormone profiles were determined at 13 sampling points during the surgery and for a few days afterwards. Serum total triiodothyronine (T3) and free-triiodothyronine (FT3) concentrations significantly decreased after surgery ($p<0.001$) and they remained significantly low until the end of the study. Free-thyroxine (FT4) and thyroxine (T4) significantly dropped immediately after surgery ($p<0.05$ for FT4, $p<0.001$ for T4) but they rapidly returned to baseline values. Serum reverse T3 (rT3) remarkably increased after surgery ($p<0.001$) and remained significantly higher than baseline value throughout the study. A relevant finding was that the days of hospitalization inversely correlated with T3 and FT3/FT4 ratio during the post-operative period ($p=0.03$) or the rapidity of the recovery of T3 ($p=0.03$). Our data strongly suggest a prolonged reduction of T4 to T3 conversion in patients undergoing cardiac surgery, encourage T3 replacement therapy in these patients and point at the recovery period as the most critical for a successful T3 substitutive therapy.

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Program Number 201 Thyroid Hormone Metabolism

Regulation of Type III Iodothyronine Deiodinase Expression in Human Cell Lines

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Type III iodothyronine deiodinase (D3) catalyzes the inner ring deiodination of the active hormone T3 to 3,3'-T2 and of the prohormone T4 to rT3. The enzyme is highly expressed in brain, placenta, pregnant uterus and fetal tissues, and is considered to play an important role in regulating thyroid hormone bioavailability during fetal development. We examined the regulation of D3 activity and mRNA expression (RT-PCR) in human cell lines. Significant D3 activity was found in ECC-1 endometrium carcinoma cells, which was increased 9-fold by a 6-h incubation with 0.1 microM TPA (a pkC-activator). Whereas TPA also significantly stimulated D3 activity in MCF-7 breast cancer cells and in WRL-68 embryonic liver cells and slightly stimulated D3 activity in SH-SY5Y neuroblastoma cells, no effect of TPA on D3 activity or mRNA level was found in BON carcinoid cells. A 48-h incubation with 3 microM all-trans-retinoic acid (tRA) or 9-cis-RA increased D3 activity 2-3-fold in ECC-1 and MCF-7 cells. In contrast, D3 activity was markedly decreased in tRA- or 9-cis-RA-treated SH-SY5Y cells. D3 activities correlated well with D3 mRNA levels in the different cell lines, indicating pretranslational regulation of D3 expression. No significant D3 activity or mRNA expression was found in JAR-, JEG-3- and BeWo choriocarcinoma, Ishikawa endometrium carcinoma, U373-, U87- and CCF-STTG1 astrocytoma, and HepG2 hepatocarcinoma cells. Interestingly, just 1 kb upstream of the D3 transcription start site, on the strand opposite to the D3 gene, a gene encoding an uncharacterized 14 kDa protein (GI14754294) is located. We found that mRNA levels for this gene and D3 are strongly correlated. In conclusion, the regulation of D3 expression is cell type-specific and ligand-dependent, and the expression of the genes encoding D3 and the 14-kDa protein seems to be regulated by the same promoter.

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Program Number 202 Thyroid Hormone Metabolism

Metabolic Effects of Targeted Expression of Type 2 Iodothyronine Deiodinase (D2) to Rodent Liver

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D2 plays a major role in thyroid hormone (TH) metabolism by converting T4 to the active hormone, T3. Indirect evidence suggests that T3 derived from D2 is targeted to the nucleus of tissues where it is expressed. To test this hypothesis, we developed a transgenic (T) mouse model which expresses D2 in liver, a tissue where D2 is not normally found, and examined it for indices of hepatic TH action. In T mice high levels of D2 transgene mRNA and activity were expressed in the liver (activity: ~200 fmols/min.mg protein; units), with lower levels in the kidney (~1.7 units), and none in other T tissues or in organs from non-transgenic littermates (non-T). Enhanced conversion of T4 to T3 in the T mice was evidenced by a 2-fold increase in serum T3 (T: 54.6±4.2 ng/dl; non-T: 27.2±1.8; $p<0.001$) and a 1.6-fold increase in hepatic T3 content (T: 2.24±0.23 ng/g; non-T: 1.33±0.15; $p<0.005$). However, TH action in the liver of T mice was not enhanced as judged by the expression of four TH-responsive genes: type 1 deiodinase, S14, BCL3, and G6P. In contrast, both T and non-T mice injected with T3 had significant increases in these parameters. The serum T4 level was markedly diminished in T mice (T: <0.25 microg/dl; non-T: 3.21±0.20; $p<0.01$). That this was due to enhanced clearance and/or metabolism of T4 was supported by the finding of a normal TSH level and a markedly increased rate of disappearance from plasma of intravenously administered [125I]T4. In summary, expression of D2 in the rodent liver results in enhanced T4 to T3 conversion, but no increase in TH action in this organ. Thus, T3 derived from D2 is not specifically targeted to the nucleus. Rather, other tissue-specific factors appear to be involved in determining the fate of T3 formed in cells.

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Program Number 203 Thyroid Hormone Metabolism

Substitution of Cysteine for Selenocysteine in the Catalytic Center of Type III Iodothyronine Deiodinase Reduces Catalytic Efficiency and Alters Substrate Preference

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Thyroxine (T4) undergoes enzymatic outer ring deiodination (ORD) in peripheral tissues to T3 catalyzed by the deiodinases type I and type II, as well as inner ring deiodination (IRD) to rT3 catalyzed by type III deiodinase (D3). In fact, D3 is the major T4 and T3 inactivating enzyme. The D3 protein is a selenoprotein, containing a selenocysteine residue (SeC) in the catalytic center. The present studies were undertaken to elucidate the functional role of the SeC residue in detail. COS cells were transfected with expression vectors encoding the human D3 wild-type protein, D3 Cys protein (cysteine substituted for SeC) or D3 Ala protein (alanine substituted for SeC). Kinetic analysis was performed on homogenates in the presence of 10 mM dithiothreitol as reducing cofactor. The Km for T3 increased 5-fold for the D3Cys protein compared to D3 wt protein. In contrast, the Km for T4 increased about 100-fold compared to wt D3. The D3Ala protein was inactive. Semi-quantitative immunoblotting of homogenates with a D3 antiserum revealed that 40- to 50-fold higher quantities of D3 Cys and D3 Ala protein are expressed relative to D3 wt protein. Using this estimate of the relative expression levels we calculated that the substrate turnover number of D3 Cys enzyme is 2-fold reduced for T3 and 5-fold reduced for T4 deiodination compared to D3 wt enzyme. Experiments using intact transfected COS cells expressing D3 wt or D3 Cys enzyme showed that the D3 Cys enzyme is also active under in situ conditions. In conclusion, substitution of cysteine for selenocysteine in the catalytic center of D3 causes only a minor decrease in substrate turnover number. The main difference between D3 Cys and D3 wt enzyme is the strongly decreased affinity of the former for T4.

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Program Number 204 Thyroid Hormone Metabolism

Structure-Activity Relationships for Iodothyronine Deiodination by Cat Type I Iodothyronine Deiodinase

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Previous studies with rat and cat liver microsomes have indicated important differences in iodothyronine substrate specificity between cat and rat type I iodothyronine deiodinase (D1), in particular with regard to outer ring deiodination (ORD) of rT3 (Foster et al., J Mol Endocrinol 24: 119-126). The aim of the present study was to analyze in detail the reason(s) for the very slow deiodination of rT3 by cat D1 in comparison to rat D1. The cat D1 coding sequence (CDS) was cloned by RT-PCR on cat liver total RNA. Sequencing yielded a deduced amino acid sequence showing 83% overall identity with human D1 and 78% with rat D1. Cat D1 protein was expressed in COS cells after transfection of an expression vector construct containing the cat D1 CDS linked to the rat D1 SECIS element. Kinetic analysis was performed on homogenates in the presence of 10 mM dithiothreitol as reducing cofactor. The Km for rT3 ORD was 11 microM for cat D1 versus 0.2 microM for rat D1. The catalytic efficiency, as reflected in the Vmax/Km ratio, was 100-fold reduced for cat D1 compared to rat D1 enzyme (2 versus 200). Comparison of the primary amino acid sequence of rat versus cat D1 revealed several striking differences, of which the Phe65Leu - Phe66Tyr substitutions were investigated in more detail. By site-directed mutagenesis the cat D1 enzyme (Leu60Tyr61) was changed to Phe60Phe61, Phe60Tyr61, Tyr60Tyr61, Leu60Leu61, and compared to rat D1 (Phe65Phe66) for rT3 ORD. The Vmax/Km ratios (in parentheses) were: wild-type cat D1 (2), cat D1 Phe60Phe61 (4), cat D1 Phe60Tyr61 (6), cat D1 Tyr60Tyr61 (inactive), cat D1 Leu60Leu61 (inactive) and rat D1 (200). In conclusion, the poor deiodination of rT3 by cat D1 is only partially improved by substitution of leucine residue 60 by phenylalanine as in rat D1.

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Program Number 205 Thyroid Hormone Metabolism

Thyroid Hormone Metabolism in a Transthyretin-null Mouse Strain Exposed to Conditions of Increased Hormone Demand

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Transthyretin (TTR) is synthesized by the liver and the choroid plexus and acts as the major plasma and cerebrospinal fluid carrier for thyroxine (T4) and retinol. Despite the 50% decrease in total T4 plasma levels, mice lacking TTR have unaltered free T4 levels and total and free triiodothyronine (T3) hormone levels. Further studies on this TTR-null mice revealed that, under normal physiological conditions, TTR does not seem to be essential for the normal homeostasis of thyroid hormones, and for T4 to reach the brain and other tissues. Therefore it was suggested that TTR could play a buffer/storage role in conditions of increased or decreased hormone demand. In order to test this hypothesis we induced a condition of increased hormone demand by exposing TTR-null and control animals to cold (4ºC) during one month. Exposure to cold triggers thermogenesis for which thyroid hormones are required. TTR-null mice resisted as well as the control mice to the imposed cold environment. Body weight, food intake and plasma free fatty acids content variations did not differ between the two groups of animals. These data indicate that despite the low levels of circulating T4, the free T4 plasma levels in the TTR-null mice seem to be sufficient to fulfill the increased need of thyroid hormones during acute thermogenesis. We are presently assessing parameters of thyroid hormone metabolism and thermogenesis, specifically: thyroid hormone fluid and tissue content; the expression of key enzymes in thermogenesis and in thyroid hormone metabolism (uncoupling protein 1, deiodinases type I and II, and UDP-glucuronosyltransferase). This will allow us to conclude whether TTR is required in conditions of increased hormone demand.

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Program Number 206 Thyroid Hormone Metabolism

Type I Iodothyronine Deiodinase Protein in Normal and Hypothyroid Chicken Brain

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Thyroid hormones play a major role in cellular maturation and brain development in general. They influence different processes of cell migration, neuronal differentiation, synapse formation and myelinisation. In rat, it is well described that situations of hypothyroidism provoke a disturbance in cellular migration and thus in the generation of productive interneuronal connections. Although no type I deiodinase (D1) activity could be detected in the chicken brain, an alkaline phosphatase staining of brain slices of 1-day-old chickens, using a polyclonal D1 antiserum, revealed an intense colouring in the internal granular cell layer of the cerebellum. To verify the specificity of this D1 antiserum, the effect of methimazole (MMI)-induced hypothyroidism on the level of D1 protein staining in the chicken cerebellum was investigated. Therefore, embryos were injected on day 14 of incubation with either saline or MMI (0.5 mg or 2 mg). An anti-D1 staining was performed on brain slices of 17- and 19-day-old control and hypothyroid animals. Staining of both the external (EGL) and internal (IGL) granular cell layer of the cerebellum was obtained in control animals, while the colouring diminished and even disappeared with more severe hypothyroidism. This result was in agreement with the expected downregulation of D1 activity in conditions of hypothyroidism. As described above, staining with anti-D1 of 1-day-old control chickens only showed a positive signal in the IGL. The reason for this was further investigated using a histochemical staining. This indeed revealed that the migration of mature granular cells from the outer germinal layer towards the inner granular layer was further progressed in 19-day-old compared to 17-day-old control embryos. In hypothyroid animals however, this process was apparently delayed. The external cell layer consisted of more layers and the cell packing was denser in these animals compared to control animals.

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Program Number 207 Thyroid Hormone Metabolism

A Comparative Analysis of Transferred Metabolites in Maternal Compartment following Fetal Infusion of 125I-T3 or -T4 in Sheep

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We showed 3,3'-diiodothyronine-sulfate (T2S) was the major metabolite in maternal urine(MU) after infusing [125I-3'],3,5-T3 (T3*) via fetal vein (Ped Res 50:358, 2001). In this study, we compared radioactive metabolites in MU following fetal infusion of [125I-3',5'],3,5-T4 (T4*) with those following T3* infusion. In pregnant ewes with singleton fetuses, hysterotomy and catheterization of the fetal femoral vein were performed at 131±2 days of gestation. One week later, an iv bolus of 0.25 mCi T3*(N=4) or T4* (N=4) diluted in saline was given. Samples of maternal serum (MS), MU, and fetal serum (FS) were collected hourly for 4 hours. Metabolites were identified by HPLC and specific antibodies. At 4 hours, mean cumulative radioactivity in MU was 16.9% of infusion dose after T3* but only 0.99% after T4*. T2S was the major metabolite in MU after fetal infusion of either T3* or T4*. After T3*, T2S comprised 42% of urinary radioactivity in the first hour, falling to 20% in the fourth hour. After T4*, T2S comprised 11% of urinary radioactivity in the first hour and rose to 15% in the fourth hour; free radioiodide essentially accounted for the rest. MS contained negligible radioactivity in all studies. In FS, after T3* infusion, T3* radioactivity fell from 83.6% at 1 hour to 11.1% at 4 hours while T2S radioactivity (the major metabolite) rose from 6.4% to 40.1%. In contrast, in FS after T4* infusion, T4 radioactivity decreased slowly from 91.7% at 1h to 87.4% at 4 hours, while rT3* and T3* radioactivity (major metabolites) increased respectively from 1.0% and 0.8% at 1 hour to 3.2% and 1.4% at 4 hours. Meanwhile, T2S radioactivity was 0.14% at 1h and 0.76% at 4 hours. In conclusion: Circulating T3, from thyroid secretion or T4 monodeiodination, is rapidly converted to T2S in fetus and is then transferred from fetus to ewe, and its formation and transport provide an important mechanism for maintaining a low T3 state in the fetus. These findings support the hypothesis that a portion of T2S in maternal urine during pregnancy is of fetal origin and maternal urinary increments of T2S may reflect fetal production of T3 and T4.

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Program Number 210 Iodine Uptake and Metabolism

Dose-Response Relationship of Perchlorate and Human Health Effects

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Perchlorate inhibits the uptake of iodine by the sodium iodide symporter (NIS) on the thyrocyte basolateral membrane. This mechanism of action underlies over 50 years of medical use for the treatment of hyperthyroidism. Perchlorate was recently found to be an environmental contaminant in drinking waters at levels above the new (April 1997) detection limit of 4 ug/L. Subsequently, occupational, epidemiological, and volunteer studies have been conducted to identify the dose-response relationships for perchlorate and to compare them with hypothesized exposures from environmental contamination. The medical literature demonstrates toxicity at exposure levels of 1-2 grams perchlorate/day. Doses of 900 mg/day will capture control of the thyroid, and doses of about 85 mg/day (minimum 40 mg/day) will maintain control. Occupational studies with employees absorbing 34 mg/day show no adverse effect on thyroidal health, including no change in either serum T4 or TSH levels. Benchmark dose modeling predicates effects on T4 at doses above 45 mg/day and on TSH at doses above 57 mg/day. Volunteer studies show a log-linear relationship between perchlorate dosages of 0.5-35 mg/day and inhibition of iodine uptake. These studies indicate a threshold dosage of 0.45 mg/day for thyroidal iodine uptake inhibition. Epidemiological studies show no adverse effect on neonatal or schoolchild health at contamination levels of 6-120 ug/L. Thyroidal diseases, neurobehavioral diseases of childhood, and thyroid cancer associations with environmental perchlorate exposures less than 24 ug/L have been sought and not found. The no-adverse-effect level (decrease in T4) for perchlorate is found to be 45 mg/day or 0.6 mg/kg/day. The no-effect level (inhibition of iodine uptake) for perchlorate is found to be at 0.45 mg/day or 0.006 mg/kg/day. This no-effect level of 450 ug/day would be equivalent to a drinking water level of 225 ug/L and can serve as a basis for establishing a regulatory level.

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Program Number 211 Iodine Uptake and Metabolism

Two-Week Low Iodine Diet Is Necessary for Adequate Outpatient Preparation for 131-I Thyrogen Scanning in Patients Taking Levothyroxine

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For over a quarter of a century, clinicians have believed excessive dietary iodine intake reduces the efficacy of therapeutic and diagnostic radioiodine uptake studies. We evaluated the utility of a self-managed, low iodine outpatient diet (<50 μg/day) designed to sufficiently decrease total body iodine in preparation for 131-I scans. With the development of recombinant TSH, the use of thyrogen-stimulated 131-I uptake studies have become a preferred method, because patients do not have to suffer the hypothyroid symptoms associated with thyroid hormone withdrawal in the thyroprivic studies. Thyrogen studies permit patients to continue on their thyroid replacement hormones before and during the studies. However, levothyroxine is a significant source of dietary iodine. Measuring urine iodine to creatinine ratios (I/Cr), a reflection of total body iodine, we evaluated the diet for one- and two-week periods before thyroprivic or thyrogen scans to determine efficacy of our diet with and without levothyroxine. Patients following the diet for two weeks (n=27) before a thyroprivic 131-I scan attained an iodine deficient state (I/Cr <50 μg/g) in 78% of the patients. In thyrogen studies, a one-week low iodine diet period (n=22) with levothyroxine adequately prepared (I/Cr <100 μg/g) only 41% of the patients, whereas two-weeks on the diet (n=24) with levothyroxine adequately prepared 71% of the patients. One week of dieting while taking levothyroxine was insuffiecient to attain an iodine deficienct state. However, two weeks diet preparation with levothyroxine made 21% of patients iodine deficient. Extending the diet period to two weeks yielded urinary iodine contents which correlated increasingly with levothyroxine as the dietary iodine source. In conclusion, our simple, self-managed, low iodine diet used in an outpatient setting effectively makes patients iodine deficient. Though less efficacious when taking levothyroxine, this diet adequately reduces total body iodine for thyrogen-stimulated 131-I radioiodine uptake scans when followed for two weeks.

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Program Number 212 Iodine Uptake and Metabolism

Differential Action of Iodine on Mitochondria from Human Tumoral and Extratumoral Tissue in Inducing the Release of Apoptogenic Proteins

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Iodide is actively concentrated in the thyroid gland for thyroid hormone (TH) biosynthesis, and to a certain extent in breast for function that is still not clear. Excess iodine induces apoptosis in thyrocytes and mammary cells. The mechanism, however, is poorly understood. Mitochondria play a pivotal role in apoptosis and also provide a favorable environment for the organification of iodine. We investigated the direct interaction of iodine and breast mitochondria vis-a-vis its role in the initiation of apoptosis in vitro. We observed that mitochondria isolated from the tumoral (TT) and extratumoral tissue (ET) from human breast display significant uptake of iodine. Mitochondrial proteins were found to be preferentially iodinated in ET mitochondria, whereas the extent of organification was low in TT. Treatment with iodine resulted in increased permeability transition (PT) in mitochondria from TT, whereas it decreased in ET mitochondria. Iodine-induced release factor(s) other than cytochrome c from tumor mitochondria were found to initiate apoptosis in vitro, while those from ET mitochondria were non-apoptogenic. The release of cytochrome c, the principal initiator of apoptosis, observed at basal level in ET mitochondria was inhibited by iodine. We for the first time report that iodine acts differentially on mitochondria to induce apoptogenic proteins from tumor tissue and has a protective effect on extratumoral tissue from human breast.

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Program Number 213 Iodine Uptake and Metabolism

Sustained Bio-contamination of Thyroid Glands among Wild Deer from Nuclear Reprocessing

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For the past 50 years, a 310-square-mile area in South Carolina, referred to as the Savannah River Site, has been allocated for reprocessing nuclear fuel to extract plutonium, (Environmental Report for 2000, WSRC-TR-2000-00330 Aiken, SC 29808). The process requires separation of many radioactive fission-products, including 137-Cs, metabolized like potassium, and 129-I which concentrates in thyroids. If fission-products accumulate in a reactor for 2 years, there is seven times more 137-Cs than 129-I but the half-life of 129-I is > half million times longer than the 30 year half-life of 137-Cs. By measuring these isotopes in indigenous thyroids, an index of bio-contamination can be derived. During the past 16 years we measured 129-I and 137-Cs in deer thyroid and muscle, collected at Savannah River Site from road kills and hunts. Median 129-I in thyroids has ranged 0.05 to 0.09 Bq/g with sample maxima of 100 to 1000 times > median, from central areas. Median 137-Cs in thyroids ranged 0.05 - 0.12 Bq/g and 137-Cs in muscle averaged 2 times more than in thyroids. Maximal thyroid 137-Cs lacked high levels found for 129-I. For the past 9 years median 129-I in thyroids ranged 0.02 - 0.04 Bq/g; half as high as 10 years ago. Since year 2000, medians of both 137-Cs and 129-I in thyroids have been 0.02-0.04 Bq/g but 129-I in thyroids from central areas have continued to be 100 to 1000 times greater than the medians. In 1995 iodized salt blocks were placed in central compartments to dilute radio-iodine; they were ineffective. In 2001, medians of both isotopes in thyroids were 25-50% of 1985 - 1990 values. The isotope intake may have been reduced by adsorption in clay, washed into drainage or reduced by other processes.

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Program Number 214 Thyroid Imaging

Role of Neck Ultrasonography in the Follow-up of Children Operated on for Thyroid Papillary Cancer

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The aim of the study was to evaluate the role of neck ultrasonography (NU) in comparison with 131I whole body scan (WBS) and circulating thyroglobulin (Tg) measurement in the follow-up of children with thyroid papillary cancer, previously submitted to total thyroidectomy, for the diagnosis of neck lymph node metastases (LNM).
Forty-five children were consecutively examined. NU and WBS were performed blindly by different operators. NU and diagnostic WBS were concordant about the presence (N° 12 patients) or the absence (N° 23 patients) of LNM in 35 patients. Diagnostic WBS revealed the presence of LNM in 6 cases not detected by NU. While NU was positive in 3 cases negative at diagnostic WBS but confirmed by post-131I therapy WBS. One patient had at NU suspicious neck lymph nodes not confirmed by diagnostic or post therapy WBS; this case was considered as a false positive result of NU. All children had palpable angulo-mandibular and/or laterocervical lymphnodes. Among the 15 patients who presented at neck ultrasonography images suspected for LNM confirmed by diagnostic or post-therapy WBS, the LNM resulted palpable on physical examination in 5/15 (33%). NU and Tg were concordant about the presence (N°10) or the absence (N°19) of LNM in 29 patients. Tg was elevated in 10 patients with negative NU (7 had also lung and/or mediastinic LNM). Tg was undetectable in 5 patients in whom the presence of LNM was confirmed by NU and diagnostic and/or post therapy WBS. The last one case was that considered as a false positive result of NU.
In conclusion, our study in children demonstrates that NU can detect LNM which are not suspected by palpation, diagnostic WBS or serum Tg determination. Furthermore, NU can locate the anatomical site of the LNM.

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Program Number 215 Thyroid Imaging

A Survey on the Utilization of Thyroid Ultrasound in the Clinical Endocrinology Training Programs

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Thyroid nodules present a common diagnostic challenge in endocrine practice. Thyroid ultrasound (US) provides the best anatomic information about the thyroid gland and nodules. During the past decade, thyroid US has become increasingly popular for diagnostic purposes. Recently, at the Endocrine University course, "Technology for Endocrine Fellows-in-Training", 134 second- and third-year fellows representing 70 programs across the United States participated in a 2-day didactic and practical thyroid US certification workshop. A survey was conducted to ascertain the utilization of thyroid US in endocrinology training programs. 110 responses (82%) were obtained. 40% of the respondents were from the Northeast, 29% from the Midwest, 13% from the South, 10% from the Southwest, and 8% from the West Coast. 85% identified their primary clinical setting as academic/university, 6% academic/community, 6% VA/government-affiliated or strictly in a community setting (3%). 45% declared their secondary clinical training setting as academic/university, 25% as VA/government-affiliated, 15% as academic community, 12% as community, and 3% as "other". Only 16% of the respondents acknowledged that they routinely utilize US “as an extension of the physical examination” during thyroid consultations. 23% utilize ultrasonography for localization and assessment of thyroid nodules (cystic vs. solid) prior to performing the FNA. 20% routinely perform US-guided FNA of the thyroid nodule. A surprising 64% reported that they currently do not utilize thyroid US in any of the aforementioned evaluations. Demographics seemed to have no bearing on the practice or utilization of thyroid US in endocrinology training programs. The majority (95.5%) stated that the thyroid US certification course would have an impact in changing their future practice, especially for the evaluation and management of nodular thyroid disease. In conclusion, this survey highlights the need for improvement in and appropriate use of thyroid US technology by clinical endocrinology fellows in training programs.

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Program Number 216 Thyroid Imaging

Ultrasonographic Classification of Nodules with Liquid Content: Correlation with Cytological and Histological Findings

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Thyroid ultrasound (US) is the most sensitive diagnostic imaging technique in the evaluation of thyroid nodule. Its widespread use over the past few years has contributed to the higher detection of hidden thyroid nodules. The objective of this study was to determine whether the presence of cystic degeneration and the pattern of distribution of liquid content in the nodule could differentiate benign from malignant thyroid nodules. We compared the ultrasonographic characteristics of these nodules with the cytological and histological findings. Since 1997, we have examined 2460 patients with thyroid nodules referred to the Thyroid Unit of the University of Sao Paulo Medical School. In all of them, an ultrasonographic examination followed by ultrasound guided fine needle aspiration biopsy (US-FNAB) was carried out. Two hundred sixty-eight patients underwent thyroidectomy. Forty-one patients (mean age: 44 years, range: 14-69 years) had nodules with liquid content on US (6 male and 35 female). We classified the nodules according to the distribution of liquid content in 3 patterns: A) mixed or complex nodule that looks like a sponge (17 patients, 41.4%); B) solid nodule with central cystic degeneration (20 patients, 48.8%); C) cyst with a solid mass arising from the cystic wall (4 patients, 9.8%). Thyroid cancer was found in 1/17 (5.9%) patients with type A pattern, 9/20 (45.0%) with type B, and 3/4 (75.0%) with type C. Six patients (30.0%) with type B had an indeterminate cytology pattern. We conclude that most mixed nodules (type A) are benign. Solid nodules with central cystic degeneration (type B) and cysts with solid mass in the cystic wall (type C) had a certain risk for malignancy.

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Program Number 217 Thyroid Imaging

Myocardial Doppler Imaging in Hyperthyroidism

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A new color Doppler myocardial imaging (DMI) system with high spatial and temporal resolution has been recently developed that allows quantifiable echocardiographic evaluation of left ventricular (LV) function. The purpose of this prospective study was to determine whether regional myocardial velocities could be accurately and reproducibly measured in thyrotoxicosis by using DMI. Seventeen patients (median age 43 years, 14 females) with untreated Graves' hyperthyroidism were included. Studies were performed during thyrotoxicosis, after one week of propranolol monotherapy (0.16 g/day), and after restoration of euthyroidism. Thirty healthy volunteers served as controls (34 years). All gray scales and color Doppler images were obtained simultaneously in real time by using an available Vingmed ultrasound machine (Horten, Norway). Systolic and diastolic velocities using the standard-16-segment-model of the LV were measured. Compared to controls, myocardial systolic color Doppler velocities were markedly increased in all segments in untreated thyrotoxicosis, e.g., mean (SD) 7.9 (2.0) vs. 6.7 (1.1) cm/s for the seventh segment, p=0.007. After restoration of euthyroidism, myocardial velocities strongly declined in most basal segments, e.g., no. 10: 7.4 (2.4) vs. 6.5 (2.3) cm/s, p=0.013; no. 13: 7.8 (1.2) vs. 6.7 (1.3) cm/s, p=0.013; no. 16: 7.4 (2.4) vs. 5.9 (1.6) cm/s, p=0.05. Marked diastolic changes were not observed. Propranolol therapy decreased heart rate (median value 96 vs. 77 beats/min, p=0.039), but neither significant change of systolic contraction nor of diastolic velocities was noted during beta blockade. Thus, for the first time, a myocardial Doppler imaging system provided accurate and reproducible quantification of segmental LV circumferential and longitudinal contraction in thyrotoxic patients. Extended studies are now being performed during exercise and in subjects with subclinical thyroid disease.

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Program Number 218 Thyroid Imaging

Technical Error: Another Cause of an Inappropriately Low Radioactive Iodine Uptake in Hyperthyroidism

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Several conditions are associated with abnormally low radioactive iodine uptakes. Categorizations encompass non-pathological causes, secondary to iodide administration, as well as problems related to the tracer and pathological states due to subacute thyroiditis, thyrotoxicosis factitia, and struma ovarii. A non-pathological cause not often recognized is the technical error in the uptake calculation of radioactive iodine. Uptake is usually calculated as ([net thyroid counts]/[standard counts x dilution factor- background counts]) x 100. A tracer dose is administered orally to the patient and an aliquot is kept aside as a "standard". At 24 hours, the net thyroid count and the "standard" count are obtained to calculate the percentage thyroid uptake. Spurious thyroid uptake values may result if the wrong "standard" is inappropriately used and counted. A 36-year-old female with thyrotoxicosis presented with a TSH<0.01 (nl, 0.13-4.6), fT4 4.94ng/dl (nl, 0.78-2.19) and fT3 12.7ng/dl (2.77-5.27 pg/ml). She had a diffuse, symmetrical thyroid scintigram and an inappropriately low 24-hour uptake of 16% (nl, 10-35%). Iodide excess was excluded as a cause for the low uptake of radioactive iodine. Review of the record of uptake calculation revealed a technical error. The content of the wrong "standard" was counted. When the correct "standard" was counted and used for uptake calculation, it was 49%. It is important for clinicians to realize that an error in the uptake calculation by using a different "standard" is a cause of a falsely low uptake value. This is an easily avoidable error in the management of a patient with thyrotoxicosis.

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Thyroid Hemiagenesis Associated with Hürthle Cell Carcinoma

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Introduction: Thyroid hemiagenesis' prevalence is estimated to be around 1: 2300. So far, more than 250 cases were reported in the literature. However, its real prevalence is unknown and probably understimated. The absence of the left thyroidal lobe occurs in 80% of the cases and the absence of the istmus in 50%. Women represent 75% of the cases and men 25%. Other thyroid diseases have been described in association with thyroid hemiagenesis: Graves' disease, thyroiditis, hypothyroidism, toxic adenoma, benign adenoma and carcinoma. Nevertheless, there is no mention of Hurthle cell carcinoma in association with thyroid hemiagenesis in the literature. It is known that Hurthle cell tumors are relatively rare, accounting for 5% to 10% of the thyroid tumors. Case History: An euthyroid 68-year-old woman had a palpable thyroidal nodule. The ultrasonography showed absence of the left lobe and istmus, and the presence in the right lobe of a solid, solitary, hypoechoic nodule with irregular borders and with a small content of liquid, measuring 5.5 x 3.0 x 4.8 cm (volume 41.2 mL). The cytology was suggestive of Hurthle cell neoplasm. The technetium-99m pertechnetate scintigraphy findings were consistent with left lobe hemiagenesis. The patient was submitted to total thyroidectomy and the histology confirmed Hurthle cell carcinoma. Discussion: Ultrasonography is the key investigational tool for the diagnosis of thyroid hemiagenesis. Fine-needle aspiration biopsies, thyroid function tests, and scintigraphies are also useful to indicate other diseases within the remaining lobe or to visualize ectopic thyroid tissue. Considering that the use of thyroidal ultrasound has increased continuously in clinical practice, we expect that the presence of thyroid hemiagenesis and its associated pathologies will become more frequent.

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Friday, October 11, 2002
Short Call Presentations

8:45 am

Advanced Bone Formation in Mice with Resistance to Thyroid Hormone

P.J. O'Shea[1], C.B. Harvey[1], H. Suzuki[2], M. Kaneshige[2], K. Kaneshige[2], S.-y. Cheng[2], G.R. Williams[1]
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Thyroid hormone (T3) regulates bone turnover and mineralization in adults and is essential for skeletal development during childhood. Hyperthyroidism is an established risk factor for osteoporosis. Nevertheless, T3 actions in bone remain poorly understood. Patients with resistance to thyroid hormone (RTH), due to mutations of the T3-receptor β (TRβ) gene, display variable phenotypic abnormalities, particularly in the skeleton. To investigate the actions of T3 during bone development, we characterized the skeleton in TRβPV mutant mice. TRβPV mice harbor a targeted RTH mutation (C-insertion at codon 448 of TRβ) and recapitulate the human condition. A severe phenotype, that includes short stature, was evident in homozygous $TR\beta^{PV/PV}$ animals. Accelerated growth *in utero* was associated with advanced ossification in the ribs, vertebrae, limbs and facial bones. Advanced endochondral bone formation resulted in early formation of secondary centers of ossification, premature quiescence of the growth plates, increased bone mineralization and post-natal growth retardation. Advanced intramembranous ossification resulted in premature fusion of the cranial sutures and craniosynostosis. *In situ* hybridization demonstrated increased expression of fibroblast growth factor receptor-1, a T3-regulated gene in bone, in $TR\beta^{PV/PV}$ growth plate chondrocytes and osteoblasts. Thus, $TR\beta^{PV/PV}$ mice exhibit a thyrotoxic skeletal phenotype with typical features of juvenile hyperthyroidism. Heterozygous $TR\beta^{PV/+}$ mice display a milder intermediate phenotype with normal growth but evidence of advanced ossification *in utero* and later onset of increased bone mineralization. The skeletal phenotypes of homozygous and heterozygous TRβPV mutant mice correlate with the severity of impairment of the pituitary-thyroid axis and with the degree of elevation of circulating hormone levels. In contrast to the pituitary, the skeleton displays increased sensitivity to elevated T3 concentrations in RTH. Together with data from TR-null mice, these studies establish that TRα is the major functional TR in bone *in vivo*.

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Generation of Thyroid Stem Cells Expressing the TSH Receptor

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Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of blastocysts that have the potential to undergo differentiation into all cell lineages. Here, we report that embryoid bodies (EBs) generated through the differentiation of mouse ES cells express a panel of genes indicative of thyroid development. While undifferentiated ES cells were found to express the stem cell marker Oct4, they did not express detectable levels of any known thyroid genes. By day 8 of differentiation, the EB-derived cells expressed a panel of gene markers traditionally associated with thyroid progenitor cells including thyroid transcription factors Pax8, TTF1, TTF2 as well as the TSH receptor, the sodium-iodide symporter, thyroperoxidase, and thyroglobulin as determined by RT-PCR. To further investigate thyrocyte development in the EBs, cells were grown on chamber slides and stained with antibodies against TSH receptor, Pax8 and TTF2. We confirmed TSH receptor expression in patches of EB outgrowth cells with hamster antiserum containing potent TSH receptor antibody of the stimulating variety. The expression of both Pax8 and TTF2 were also observed in the nuclei of small groups of differentiated cells. The addition of bFGF, BMP4, HGF and TSH to the developing EBs did not significantly alter the pattern of genes expressed suggesting that thyroid development was regulated by additional molecules. These results indicated that the process of early embryoid body development was associated with a programmed commitment to thyroid marker display. The differentiation of mouse ES cells into thyrocyte-like cells expressing the TSH receptor and other thyroid-specific genes sets the stage for the development of in vitro models for the study of human thyroid diseases.

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Subunits of TFIIH Are Temporally Recruited to Thyroid Hormone Response Elements (TREs) and Act as Coactivators for Thyroid Hormone Receptors (TRs)

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Temporal recruitment of co-activator complexes to TREs is likely an important feature of T3-mediated transcription. Indeed, it appears that steroid receptor co-activators (SRCs) and vitamin D receptor interacting proteins/thyroid hormone receptor associated proteins (DRIPs/TRAPs) are recruited by TRs to TREs of target genes, and facilitate histone acetylation and recruitment of RNA pol II. TFIIH is a multi-subunit complex involved in both transcription and DNA repair. Recently, several subunits of TFIIH have been shown to interact with nuclear hormone receptors. To investigate the potential role of TFIIH in T3-mediated transcriptional activation, we first examined TR interaction with individual TFIIH subunits in a yeast-two hybrid system. Among the nine subunits of TFIIH studied, only p62 subunit interacted with TRβ in a ligand-dependent manner. GST pull-down studies confirmed these results. Co-transfection studies with TSA201 cells showed that p62 increased T3-mediated transcription, which could be further enhanced when p62 and p44 (another TFIIH subunit) were co-transfected simultaneously. To examine whether p62 and p44 were recruited to the TREs of target genes *in vivo*, we performed chromatin immunoprecipitation (ChIP) assays in GH3 pituitary cells, which contain endogenous TRs. Using specific antibodies as well as primers that amplify TREs within the promoters of target genes such as SERCA, PEPCK, and CYP7, we showed that TRβ, SRC-1, GRIP-1, p62, and p44 were rapidly recruited to TREs (within 30 minutes) after ligand addition. SRC-1 and GRIP-1 remained on the TREs for approximately two hours; however, p62, p44 and RNA pol II were present even after six hours. In summary, our findings show that p62 is a novel coactivator involved in T3-mediated transactivation, and that at least some components of TFIIH may contribute directly to transactivation by sequential and temporal recruitment to TREs.

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Classification of Follicular Thyroid Tumors by Molecular Signature: Results of Gene Profiling

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Purpose. Thyroid nodules are common, with a lifetime risk of developing a clinically significant thyroid nodule of 10% or higher. Preoperative diagnosis was greatly enhanced by the introduction of fine needle aspiration (FNA) in the 1970s, but there has been little advancement since that time. Discrimination between benign and malignant follicular neoplasms is currently not possible by FNA, and can even be difficult after full pathologic review. The purpose of these studies is to identify genes expressed in follicular adenomas and carcinomas of the thyroid that will permit molecular differentiation of these neoplasms. **Experimental Design.** Gene expression patterns of 17 thyroid follicular tumors were analyzed by oligonucleotide array analysis. Gene profiles for follicular adenomas and carcinomas were identified, and the two groups were compared for differences in expression levels. The differentially expressed genes were used to perform a hierarchical clustering analysis training set. Five follicular tumors with diagnosis undisclosed to the investigators and two minimally invasive carcinomas were entered into the cluster analysis as a test set to determine if diagnosis by gene profile correlated with that obtained by pathologic evaluation. **Results.** Thyroid follicular adenomas and carcinomas showed strikingly distinct gene expression patterns. The expression patterns of 105 genes were found to be significantly different between follicular adenoma and carcinoma. Many uncharacterized genes contributed to the distinction between tumor types. For five follicular tumors for which the final diagnosis was undisclosed, the clustering algorithm gave the correct diagnosis in all five cases. **Conclusions.** Gene profiling is a useful tool to predict the molecular diagnosis of follicular thyroid tumors. Genes were identified that reliably differentiate follicular thyroid carcinoma from adenoma. This study provides insight into genes that may be important in the molecular pathogenesis of follicular thyroid tumors, as well candidates for preoperative diagnosis of follicular thyroid carcinoma.

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Thyroid-specific Expression of Interferon-gamma Suppresses Experimental Autoimmune Thyroiditis

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The role of IFN-gamma in the pathogenesis of autoimmune disease is controversial, being described as pro-inflammatory in some studies and anti-inflammatory in others. This study addresses the role of IFN-gamma in thyroglobulin-induced autoimmune thyroiditis in NOD.H-2h4 mice at both systemic and organ-specific levels. Blockade of systemic IFN-gamma with monoclonal antibody enhanced disease and increased IgG1 responses. To determine the contribution of local expression of IFN-gamma, we derived NOD.H-2h4 transgenic mice overexpressing IFN-gamma in a thyroid-restricted manner. Thyr-IFN-gamma transgenic mice showed upregulation of MHC class II on thyrocytes, but did not develop spontaneous thyroiditis. Upon immunization with thyroglobulin, transgenic mice exhibited milder disease and reduced IgG1 responses compared to wild type, but no detectable change in serum IFN-gamma levels. This study supports a disease-limiting role of IFN-gamma in autoimmune thyroiditis. Furthermore, it provides the first evidence that local IFN-gamma activity in the thyroid is sufficient for disease suppression.