

Biomarkers in Thyroid Neoplasia

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GENETIC MARKERS IN DIFFERENTIATED THYROID CANCER

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Follicular cell-derived thyroid cancer (FCDTC) accounts for about 90% of all endocrine cancer, producing approximately 20,000 new cases and 1,200 deaths annually in the United States. The three distinct histotypes papillary thyroid carcinoma (PTC), follicular cell carcinoma (FTC), and anaplastic thyroid cancer (ATC) appear to arise from the same parent cell-type and retain varying degrees of differentiated follicular function, including iodine concentration, TSH response, and, in some cases, production of T3, T4 and thyroglobulin. However, each histotype exhibits unique biological behavior, pattern of spread, and configuration of genomic alterations. These cancers exhibit extremes of malignancy, from PTC in young adults with near normal life-expectancy to ATC (median life expectancy = 5 months). FTC and Hürthle cell carcinoma (HCC), an oxyphilic variant with increased numbers of mitochondria, which can exhibit either the FTC or PTC phenotype, have intermediate malignant potential, and unique patterns of metastatic spread. In the U.S. the incidence rates are PTC > FTC >> ATC. The major established risk factor is ionizing radiation exposure to the head/neck region during childhood. Genetic factors, including Cowden's and Gardner's syndromes, familial PTC, and dysmorphogenesis, may contribute to rare cases of PTC and FTC. Iodine deficiency favors FTC development.

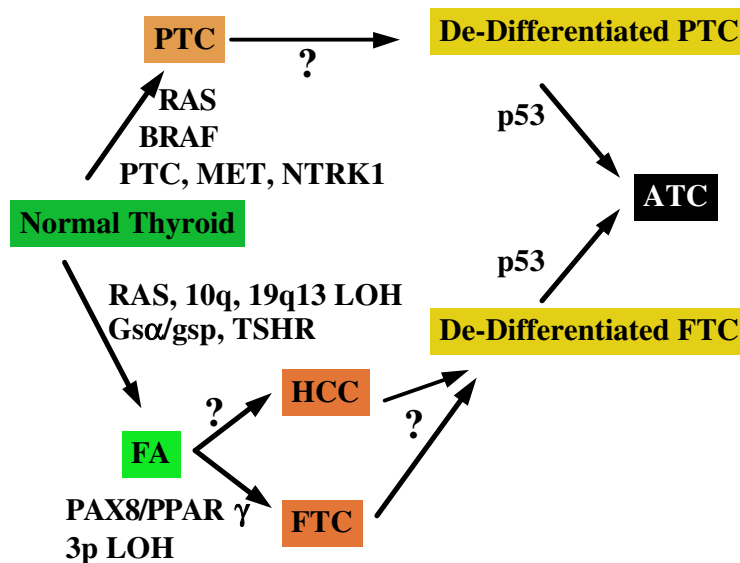
Most thyroid carcinomas present as palpable thyroid nodules, of which 80 - 95% are benign hyperplastic nodules. Most of the true neoplasms are benign follicular adenomas (FA) with less than 10% representing thyroid carcinomas. Since thyroid nodules are found in 30-60% of the population, the task of identifying malignant nodules is formidable. Fine needle aspiration biopsy is reliable in identifying PTC, but FA is indistinguishable from FTC except by careful histologic evaluation after surgical removal.

Most patients with PTC and FTC have an excellent prognosis after treatment, with cause-specific mortality less than 25% at 10 yr. Recurrence, particularly in regional lymph nodes, is common (10 - 30% rate), leads to substantial morbidity, and may be associated with premature mortality. Prognostic scoring systems to identify individuals at highest risk of recurrence/mortality agree on the importance of patient age (older = worse prognosis), and gender (male = worse prognosis). Significant tumor-related factors are histotype (worse survival in FTC than PTC), primary tumor size, and local and distant spread. Nevertheless, these systems misclassify a number of patients, leading to over-treatment of low-risk thyroid cancer patients and treatment-related morbidity. Also, some high-risk individuals may not be recognized, and so remain under-treated.

Improved understanding of the genetic events associated with thyroid carcinogenesis and progression to more aggressive forms, may lead to the identification of more reliable tumor-specific prognostic markers. Such markers might lead to improved tumor stratification, which could identify those tumors with the riskiest outcome and requiring the most aggressive treatment. The current discussion will focus on efforts to discern such markers.

Currently it is thought that PTC and FTC arise independently of one another, whereas there is some evidence to suggest a progression from FA to FTC. Figure 1 depicts a summary of the known or suspected molecular

Figure 1. Thyroid Cancer Progression



events that lead to the genesis of PTC, FA and FTC, and finally ATC.

Genomic rearrangements that result in activation of the *RET* proto-oncogene were the first recognized molecular events found to be common in PTC, but not FTC, particularly in individuals exposed to ionizing radiation. *RET* is a member of the transmembrane tyrosine kinase family and is normally expressed in thyroid C cells. Its expression in follicular cells results from the genomic rearrangement which fuses the promoters of follicular cell-specific genes such as *ELE1* or *H4* to the tyrosine kinase domain of *RET*. Activating mutations involving *MET* and *NTRK1* have also been found, but occur much less frequently. *RAS* mutations have been detected in PTC, but are more commonly detected in FA and FTC, and have been thought to be among the earliest events in cancer progression. More recently somatic mutation of the *BRAF* gene has been found to be an even more common genetic event accompanying the development of PTC. Current evidence indicates that mutations affecting *RAS*, *BRAF* and *RET* are non-overlapping in individual PTC. However, each of these genes functions in a common signaling pathway, providing a unifying hypothesis of tumor development in the case of PTC. Interestingly, since *RAS* mutations are more frequent in FA and FTC, but lack *BRAF* mutations, *RAS* most likely interacts with other downstream effectors to initiate FA/FTC. While these are considered to be initiating events that in themselves are not capable of sustaining tumor growth, the molecular events that lead from an oncogene-activated cell to clinically overt PTC are not known.

RAS, *Gsa/Gsp* and *TSH* receptor mutations as well as less defined loss of heterozygosity (LOH) sites on chromosomes 10q and 19q13 have been implicated in the development of FA. More recent work has indicated that a chromosomal rearrangement resulting in a fusion gene between the thyroid-specific transcription factor *PAX8* gene (2q13) and the *PPARγ* gene (3p25) may be involved in FA to FTC progression. This rearrangement is found infrequently in FA (<10%). Paradoxically, in our studies *PAX8/PPARγ* gene expression appears to occur most frequently among the most well-differentiated tumors, suggesting that its expression may be lost as the tumors progress to more undifferentiated and aggressive types. Additional LOH at the 3p locus has suggested the existence of one or more tumor suppressor genes on this chromosome that also may be involved in FA to FTC progression.

The development of ATC is thought to proceed from either PTC or FTC and is associated with mutations and/or loss of the *p53* gene as well as other chromosome rearrangements, possibly involving chromosome 17p.

To date none of the known markers of thyroid tumor development have been used as a routine clinical test. The most useful marker must be rapidly assessed from a small amount of tumor tissue, ideally a fine needle aspirate that can be assessed prior to the initiation of treatment. The need for new markers is perhaps most desirable to distinguish benign FA from FTC. However, it remains questionable whether a single marker will suffice for such purpose. One approach would be to screen with markers that measure the state of differentiation of the tumor, such as thyroid peroxidase or other thyroid-specific products (Table 1). High expression of these markers would signal a well-differentiated and possibly benign tumor. Absence of carcinoma would then require a second test to discern the absence of de-differentiation markers or markers that have been implicated in tumorigenesis (Figure 1 and Table 1). Such a test would likely require a panel of candidates due to the heterogeneity of mechanisms that can lead to carcinogenesis. In our own efforts to study the utility of screening for FTC using the *PAX8/PPAR γ* fusion gene we performed immunohistochemistry with a *PPAR γ* -specific antibody indicated positive staining for *PPAR γ* was more common amongst FTC than FA (57% versus 13%, $p < 0.0001$), yielding a positive predictive value for

Table 1: Markers that have been studied for the detection of benign and malignant thyroid cancers.

Benign or Early Stage Thyroid Carcinoma Markers	Early and Late Stage Thyroid Carcinoma Markers
Thyroid Peroxidase (TPO)	RET/PTC
Thyroglobulin (Tg)	RAS
TSH Receptor (TSHR)	BRAF
Na Iodide Symporter (NIS)	<i>PAX8/PPARγ</i>
TTF-1	Mucin (Muc1)
	Proliferating Cell Nuclear Antigen (PCNA)
	Leu-M1 Antigen
	<i>p53</i>
	DNA methylase
	Teleomerase
	Focal Adhesion Kinase (FAK)
	Galectin-3
	Ki-67 (MIB1)
	Oncofetal Fibronectin

the diagnosis of FTC of 88.6%. However, due to the loss of expression of the fusion gene during dedifferentiation, presumably due to the loss of *PAX8* promoter activity, the negative predictive value was only 54%, yielding a false negative rate of 42.5% in FTC. Consequently, this test would require a careful intra-operative histological analysis to establish the presence or absence of tumor invasion and de-differentiation to be useful. This study underscores the potential problems in relying on screening tools that are dependent on marker expression, since this may change during the course of tumor progression.

The future development of thyroid markers will rely on increased knowledge of the molecular genetic events that promote tumorigenesis. Efforts to discern markers from LOH analysis has been hampered by the inability to associate the regions of LOH with specific genes, that can be demonstrated to be involved in tumorigenesis. One possible source of potential markers may be the analysis of gene expression array data. Limited data is available for PTC;

however no systematic attempts to develop clinical markers from these data have yet emerged. In the case of FTC the increased frequency of seemingly wide-spread genomic disarray may complicate this approach, as the mosaic of genomic rearrangements may lead to heterogeneous expression patterns that hinders the search for valid markers. The genetic rearrangements that result in activated oncogenes that occur in both PTC and FTC are unique to solid tumors, as they have heretofore been confined to tumors of hematopoietic lineage. Consequently, knowledge of predisposing events that give rise to these highly selective chromosomal rearrangements may provide unique markers. Clearly, the development of unique prognostic and diagnostic markers to distinguish the multiple forms of FCTDC will demand significant continuing research.

Another area that is currently being explored is the development of markers of thyroid cancer recurrence that would serve as early detection systems. Assays of circulating cells that express thyroid cell differentiated gene products such as thyroglobulin mRNA have been examined. Other differentiated gene products could also be proposed (Table 1). While these may have utility to discern recurrent differentiated tumors, they will not be effective to discern de-differentiated tumors. In this regard approaches that depend on the assessment of DNA-dependent variables and not mRNA- or expression-dependent variables could be useful. An example might be to screen patients who had primary tumors with known mutations of oncogenes such as *BRAF* for the presence of this mutation in circulating cells. Thus even in *BRAF*-dependent PTC that had recurred, progressed, and even lost the expression of the oncogene could be detected by this type of assay. The utility of these approaches remain for future research to ascertain.

Selected Reading:

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