Lewis E. Braverman Award Lecture

Enhancing Iodide Transport in Thyroid & Breast Cancer

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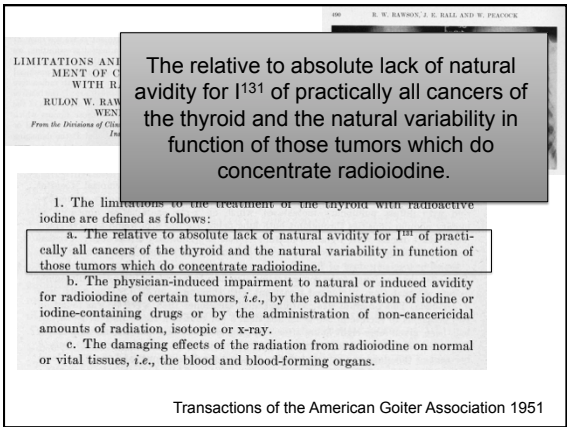
No Disclosures

Learning Objectives

- Describe the pathways that regulate iodide uptake in normal tissue.
- Understand the mechanisms for reduced iodide uptake in thyroid and breast cancers.
- Describe the approaches to augment NIS expression in cancer.
- Evaluate the potential for iodide uptake augmentation in cancer therapy.

Enhancing Iodide
Transport in Thyroid & Breast Cancer

- Introduction to the sodium/iodide symporter (NIS).
- Relative role of NIS gene expression and membrane insertion on iodide uptake.
- Targeting the PI3K-AKT-mTOR signal transduction pathways to augment NIS expression and iodide uptake in thyroid and breast cancer.
- Modifying NIS membrane insertion to enhance iodide uptake in thyroid and breast cancer.



The relative to absolute lack of natural avidity for I^{131} of practically all cancers of the thyroid and the natural variability in function of those tumors which do concentrate radioiodine.

1. The limitations to the treatment of the thyroid with radioactive iodine are defined as follows:

a. The relative to absolute lack of natural avidity for I^{131} of practically all cancers of the thyroid and the natural variability in function of those tumors which do concentrate radioiodine.

b. The physician-induced impairment to natural or induced avidity for radioiodine of certain tumors, *i.e.*, by the administration of iodine or iodine-containing drugs or by the administration of non-cancericidal amounts of radiation, isotopic or x-ray.

c. The damaging effects of the radiation from radioiodine on normal or vital tissues, *i.e.*, the blood and blood-forming organs.

Transactions of the American Goiter Association 1951

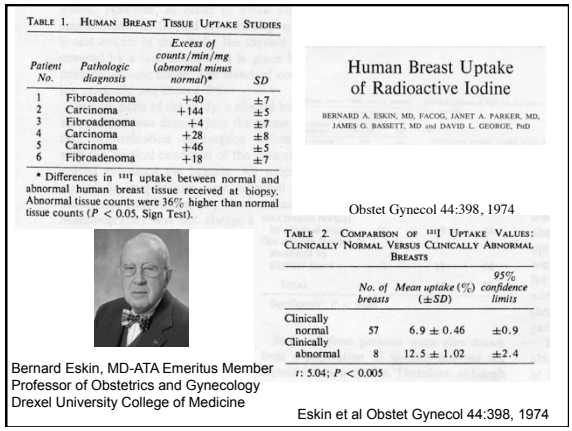


TABLE 1. HUMAN BREAST TISSUE UPTAKE STUDIES

Patient No.	Pathologic diagnosis	Excess of counts/min/mg (abnormal minus normal)*	SD
1	Fibroadenoma	+40	±7
2	Carcinoma	+144	±5
3	Fibroadenoma	+4	±7
4	Carcinoma	+28	±8
5	Carcinoma	+46	±5
6	Fibroadenoma	+18	±7

* Differences in ^{131}I uptake between normal and abnormal human breast tissue received at biopsy. Abnormal tissue counts were 36% higher than normal tissue counts ($P < 0.05$, Sign Test).

Human Breast Uptake of Radioactive Iodine

BERNARD A. ESKIN, MD, FACOG, JANET A. PARKER, MD, JAMES G. BASSETT, MD and DAVID L. GEORGE, PhD

Obstet Gynecol 44:398, 1974

TABLE 2. COMPARISON OF ^{131}I UPTAKE VALUES: CLINICALLY NORMAL VERSUS CLINICALLY ABNORMAL BREASTS

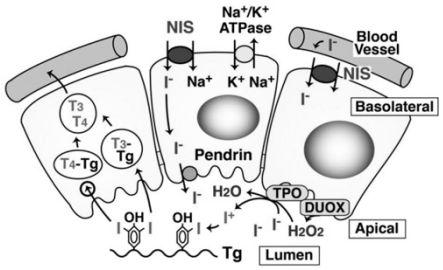
	No. of breasts	Mean uptake (%) (±SD)	95% confidence limits
Clinically normal	57	6.9 ± 0.46	±0.9
Clinically abnormal	8	12.5 ± 1.02	±2.4

t: 5.04; $P < 0.005$

Bernard Eskin, MD-ATA Emeritus Member
Professor of Obstetrics and Gynecology
Drexel University College of Medicine

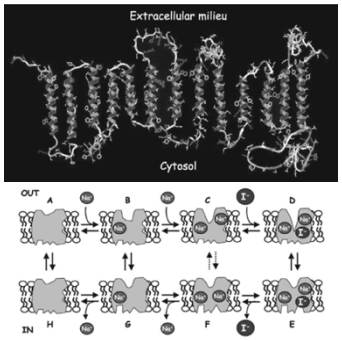
Eskin et al Obstet Gynecol 44:398, 1974

Iodine Transport in the Thyroid Follicle



Kogai and Brent Pharm & Therap 135:355, 2012

Sodium/Iodide Symporter (NIS)

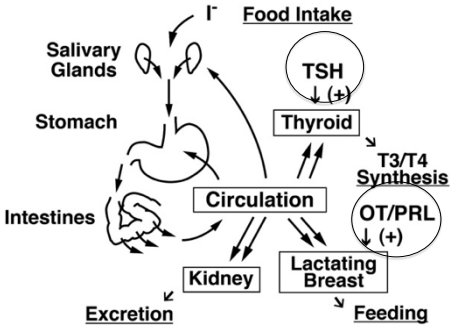


13 transmembrane domains

Iodide transport is passive, transported with sodium going down the gradient generated by Na+/K+ ATPase

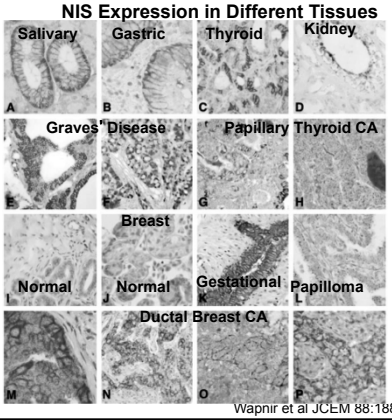
Dohan et al Endoc Rev 2003; 24:48

Free Iodide Cycle



OT-oxytocin PRL-prolactin Kogai and Brent Pharm & Therap 135:355, 2012

NIS Expression in Different Tissues



Wapnir et al JCEM 88:1880, 2003

Hierarchy of Tissue NIS Activity

- Graves' disease/toxic nodule (TSH Receptor driven)
- Normal Thyroid (TSH Receptor driven)
- Lactating Breast
- Salivary Gland/Gastric Mucosa
- Choroid Plexus and Placenta (?)
- Thyroid Cancer (some stimulated by high concentration TSH)
- Breast Cancer

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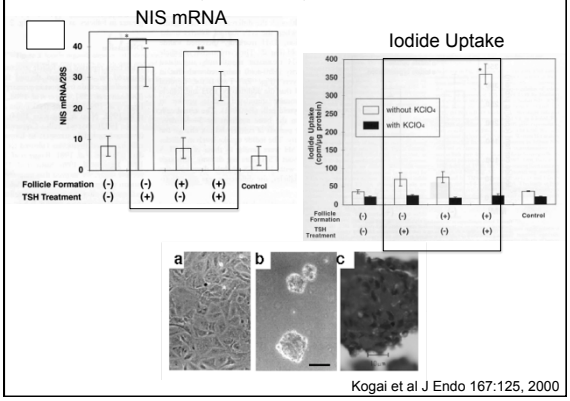
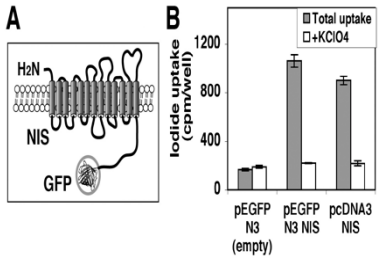
- Modifying NIS membrane insertion to enhance iodide uptake in thyroid and breast cancer.

Primary Human Thyroid Culture

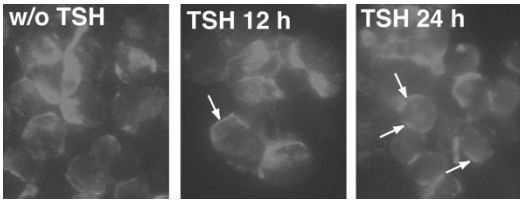


Kogai et al J Endo 167:125, 2000

Green Fluorescent Protein (GFP)-Labeled NIS

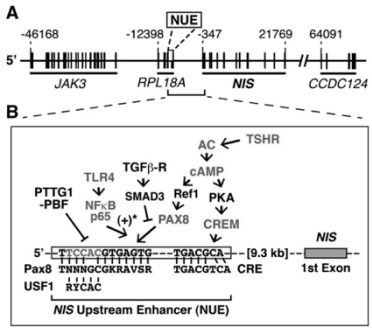


TSH Stimulates NIS Membrane Localization



Expression vector with NIS linked to
GFP (green fluorescent protein) in FRTL5 thyroid cells

NIS Gene 5'-Flanking Region

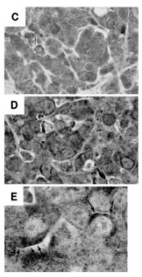


Kogai and Brent Pharm & Therap 135:355, 2012

NIS Induction in Thyroid Cancer

Agent	In Vitro	Clinical
TSH	++++	++++
Retinoids	+	+/- (~20% of tumors)
PPARγ Agonists	+	+/- (anecdotal)
Histone Deacetylase Inhibitors (depsipeptide, trichostatin A)	++ (also in rodent model)	-
Demethylation (5-azacytidine)	+/-	-

Retinoic acid induces sodium/iodide symporter gene expression and radioiodide uptake in the MCF-7 breast cancer cell line

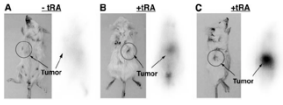


Control

+ RA

+ RA

Systemic RA treatment Induces Iodine Uptake in MCF7 Xenograft Tumors



Kogai et al Cancer Research 64:415, 2004

Kogai et al Proc Natl Acad Sci, USA. 97:8519, 2000

NEWS & VIEWS

Will radioiodine be useful in treatment of breast cancer?

Expression of a sodium iodide transporter by the thyroid gland causes iodide accumulation, and radioactive iodide is an effective treatment for thyroid cancer. The identification of a similar transporter in mammary tissue indicates that this approach might also be used to treat breast cancer (pages 871-878).

IODIDE IS AN essential component of thyroid hormones, which are required for normal development and metabolism. Iodide was first reported to accumulate in the thyroid in 1915 (ref. 1) and was

GILBERT H. DANIELS[1] & DANIEL A. HABER[2]

compared with 23% of surrounding

ovarian, melanoma, colon and liver cancer cell lines renders them susceptible to killing by ^{131}I (ref. 5). Although Tazebay *et al.* observed that NIS was expressed in some transgenic mouse mammary tu-

Nature Medicine 2000; 6:859

NIS Regulation and Iodide Uptake

- NIS gene upregulation is necessary, but not sufficient, to increase iodide uptake.
- NIS gene regulation differs significantly between thyroid, which is TSH-dependent and breast.
- Retinoic acid (RA) regulates NIS gene expression in breast cancer, but high concentrations are required to augment iodide uptake. RA analogs and dexamethasone stimulate NIS at lower concentrations than all trans RA, but don't increase the maximal response.
- Approaches that augment NIS membrane insertion and function are likely more generalizable, have the potential to translate into improved therapies for thyroid and breast cancer, as well as cancers treated with NIS gene therapy.

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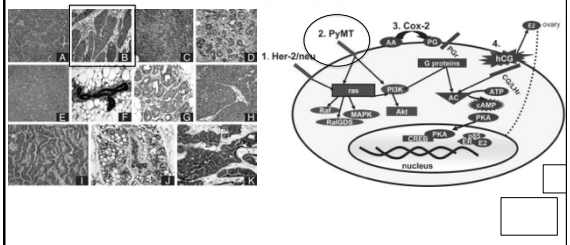
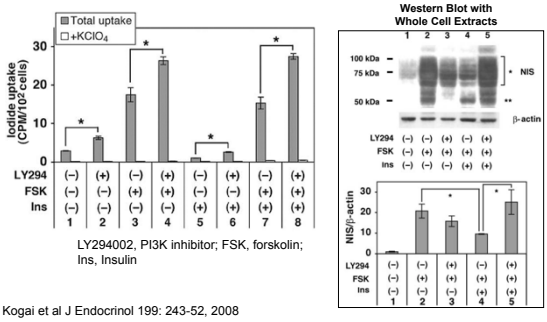
Signaling through 3′,5′-Cyclic Adenosine Monophosphate and Phosphoinositide-3 Kinase Induces Sodium/Iodide Symporter Expression in Breast Cancer

KATHERINE A. B. KNOSTMAN, JE-YOEL CHO, KWON-YUL RYU, XIAOQIN LIN, JAMES A. McCUBREY, TIMOTHY HLA, CATHERINE H. LIU, EMMA DI CARLO, RUTH KERI, MING ZHANG, DAE Y. HWANG, WILLIAM C. KISSEBERTH, CHARLES C. CAPEN, AND SISSY M. JHIANG



Knostman et al JCEM 89:5196, 2004

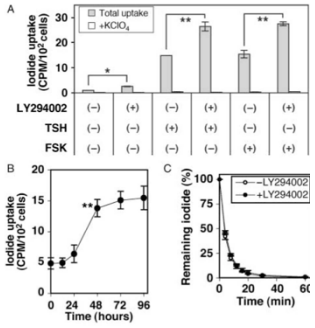
PI3K Inhibition Induces NIS Expression in FRTL5 Rat Thyroid Cells



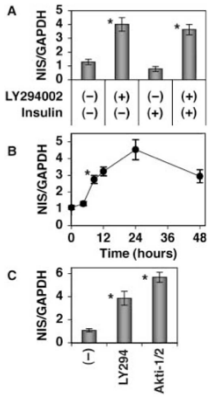
LY294002, PI3K inhibitor; FSK, forskolin; Ins, Insulin

Kogai et al J Endocrinol 199: 243-52, 2008

PI3 Kinase Inhibition and Iodide Uptake in FRTL5 Thyroid Cells

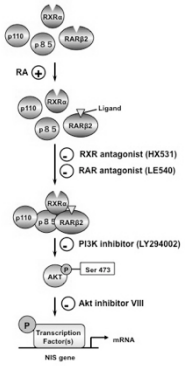


Kogai et al J Endocrinol 199:243, 2008



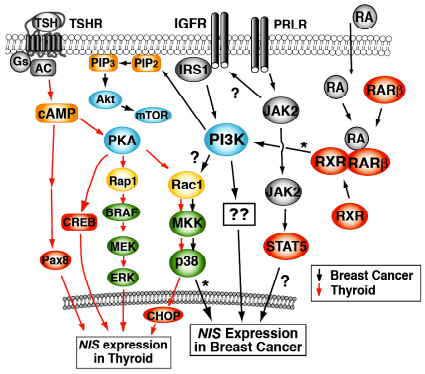
PI3 Kinase Inhibition
in BHP Papillary Thyroid Cells
Constituitively Expressing NIS

Kogai et al J Endocrinol 199:243, 2008

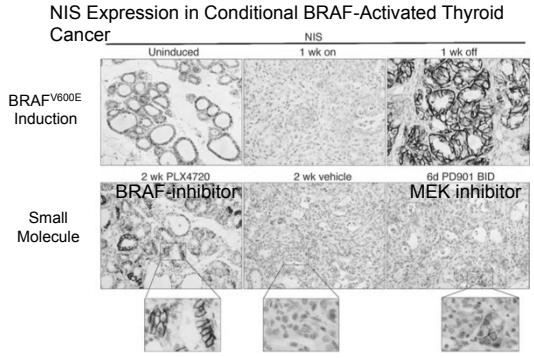


RA Receptor Directly
Interacts with PI3 Kinase

Ohashi et al Cancer Research 69:3443, 2009

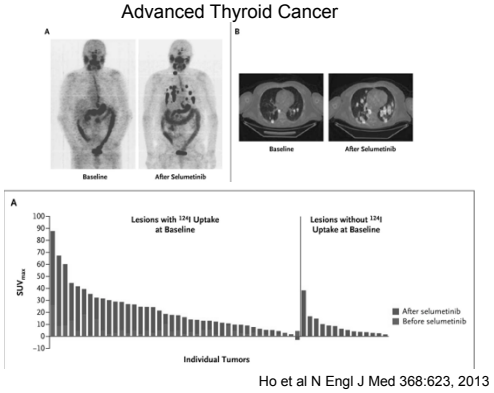


Kogai and Brent Endocr Rel Cancer 13:797, 2006



Chakravarty et al J Clin Invest 121:4700, 2011

Selumetinib (MEK1/2 inhibitor)-Enhanced Radioiodine Uptake in Advanced Thyroid Cancer



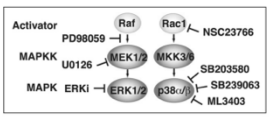
Ho et al N Engl J Med 368:623, 2013

Regulation of Sodium Iodide Symporter Gene Expression by Rac1/p38β Mitogen-activated Protein Kinase Signaling Pathway in MCF-7 Breast Cancer Cells*

Received for publication, October 20, 2011, and in revised form, December 1, 2011. Published, JBC Papers in Press, December 8, 2011, DOI 10.1074/jbc.M111.315523

Takahiko Kogai‡, Yan-Yun Liu, Kaixuan Mody, Deborah Y. Shamsian, and Gregory A. Brent‡
From the Molecular Endocrinology Laboratory, Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, California 90073 and the Departments of Medicine and Physiology, David Geffen School of Medicine at UCLA, Los Angeles, California 90073

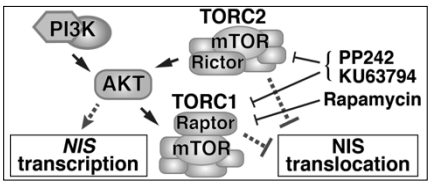
Distinct p38 pathways regulate NIS expression in FRTL-5 rat thyroid cells and MCF-7 breast cancer cells.



Thyroid cells	MCF-7 cells
TSHR	
↓	RAR/RXR
cAMP	↓
↓	Rac1
Rac1	↓
↓	MKK3B
MKK3/6	↓
↓	p38β
p38α	↓
↓	?
CHOP	↓
↓	*NIS*
NIS	

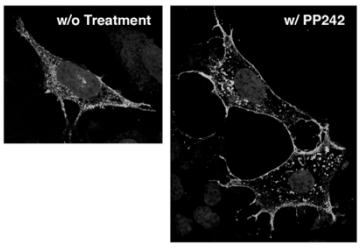
Kogai et al J Biol Chem 287:3292, 2012

PI3K/AKT/mTOR pathway and NIS regulation



Kogai and Brent Pharm & Therap 135:355, 2012

Expression of NIS-GFP fusion protein in BHP 2-7 cells



- Cells were transfected with pEGFP-NIS, treated with or without PP242 (2uM), and observed with confocal microscopy (objective lens, 63x).

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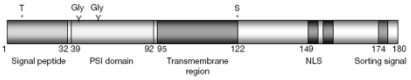
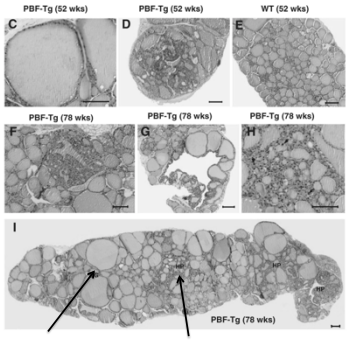
PBF-(Pituitary Tumor Transforming Gene) Binding Factor



- Professor Chris McCabe, Birmingham University
- Collaborator-Professor Jayne Franklin, Birmingham University
- PBF binds to PTTG
- Upregulated in thyroid and breast cancer
- Binds NIS and reduces membrane insertion
- *Src* phosphorylates PBF Tyrosine 174, required for NIS interaction.
- The *Src* inhibitor, PP1, reduced PBF-NIS interaction and increased iodide uptake in thyroid cancer cells.
- Smith *et al* JCEM 98:2876, 2013

Progressive Thyroid Growth and Appearance of Macrofollicular and Hyperplastic Lesions Associated with PBF Overexpression



Increased Expression
TSH-Receptor
Cyclin D1

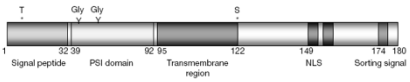
Reduced Expression
NIS

Iodide uptake reduced 70%

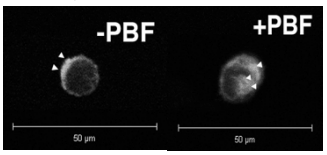
Diffuse Goiter Hyperplastic

Read et al Cancer Research 71:6153, 2011

PBF-(Pituitary Tumor Transforming Gene) Binding Fac

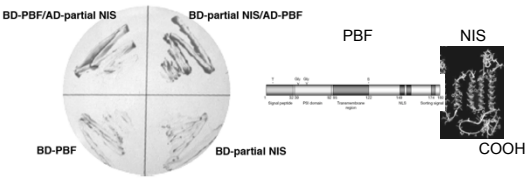


Thyroid Cancer Cells (BHP 2-7)



Expression vector with NIS linked to GFP (green fluorescent protein)

Interaction between NIS C-terminal portion and PBF reproduced in yeast two hybrid system



Combination of full length NIS and PBF did not result in yeast growth.

Yeast system utilized to screen small molecule compounds in library from the Developmental Therapeutics Program at National Cancer Institute, with 6 positive compounds from about 15,000 screened.

Summary and Future Directions

- Signal transduction pathway inhibition and stimulation shows significant promise for enhancing radioiodine treatment for thyroid, breast, and other cancers.
- Many of the agents tested act at multiple sites, influencing NIS gene expression, NIS mRNA stability, NIS protein transport and membrane insertion.
- Combination of agents that increase NIS gene expression and membrane insertion should be explored.
- The relatively short time period required to upregulate iodide uptake for adequate radioiodide treatment should permit the use of effective agents that might otherwise be rejected due to long-term toxicity.
- An improved understanding the role of postranslational modifications of NIS, important interacting proteins, cytoplasmic transport and membrane insertion will be critical to successful treatment strategies.