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.
Iodine Transport in the Thyroid Follicle

Dohan et al. Endoc Rev 2003; 24:48

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

Free Iodide Cycle

Wapnir et al. JCEM 88:1880, 2003

Graves` Disease Papillary Thyroid CA Gestational Breast

Normal Normal Ductal Breast CA

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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**Primary Human Thyroid Culture**

- **NIS mRNA**
- **Iodide Uptake**

*Kogai et al. J Endo 167:125, 2000*

**Green Fluorescent Protein (GFP)-Labeled NIS**

*A* Total uptake

*Kogai and Brent Pharm & Therap 135:355, 2012*

**TSH Stimulates NIS Membrane Localization**

- **w/o TSH**
- **TSH 12 h**
- **TSH 24 h**

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

**NIS Gene 5'-Flanking Region**

*Kogai et al. Cancer Research 64:415, 2004*

**NIS Induction in Thyroid Cancer**

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

**Systemic RA treatment Induces Iodine Uptake in MCF7 Xenograft Tumors**

*Kogai et al Cancer Research 84:415, 2004*

**Agent**

- Systemic RA treatment

*Kogai et al Proc Natl Acad Sci, USA. 97:8519, 2000*
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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PI3K Inhibition Induces NIS Expression in FRTL5 Rat Thyroid Cells


PI3 Kinase Inhibition and Iodide Uptake in FRTL5 Thyroid Cells

PI3 Kinase Inhibition in BHP Papillary Thyroid Cells Constitutively Expressing NIS


RA Receptor Directly Interacts with PI3 Kinase


NIS Expression in Conditional BRAF-Activated Thyroid Cancer

Kogai and Brent Endocr Rel Cancer 13:797, 2006

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


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


Regulation of Sodium Iodide Symporter Gene Expression by BRAF/RAF/MEK/ERK Pathway in MCF-7 Breast Cancer Cells

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

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 radiiodide treatment should permit the use of effective agents that might otherwise be rejected due to long-term toxicity.
- An improved understanding the role of posttranslational modifications of NIS, important interacting proteins, cytoplasmic transport and membrane insertion will be critical to successful treatment strategies.