Iodine and Breast Cancer: Is There a Link?

Endocrine Surgery & Laboratory
SGPGIMS
Clinical Background-
Thyroid and Breast

• Co-existence of Thyroid & Breast Diseases:
  – plenty of conflicting reports
  – hypothyroidism and BBD
  – hypothyroidism and ?? Ca Breast.
• Low s-T4: breast hypersensitive to prolactin: dysplasia and neoplasia.
  » Pavic Z, 1988
Clinical Background - Thyroid and Breast

Editorial, Ann Med 1997 Jun

- The Thyroid and Breast Cancer: a Significant Association?
  – Smyth PP
Clinical Background - Iodine and Breast

- Iodine essential for breast normality.
  - A natural anti-oestrogen.
    » Ghent, et al. 1993, CJS

- Iodine deficient state:
  - mammary duct epith. dysplasia & neoplasia via enhanced gonadotropin, subsequent hyperestrogenism.

- Fibrocystic rat model
  » Eskin BA, 1986, Frontiers Thyroidol.
Clinical Background - Iodine and Benign Breast Disease

• Correction of fibrocystic breast in rats
  » Eskin BA, 1986, Frontiers Thyroidol.

• Treatment of Human BBD with Iodine:
  – NaI, PBI, Molecular iodine
  – Mol. iodine most beneficial
  – symptomatic, radiological and pathological response
  » Ghent, et al. 1993, CJS.
Clinical Background-
Iodine and Breast Cancer

• Geographical variations in Ca-Br incidence attributed to iodine intake
• No conclusive information on causative relationship of iodine and Ca-Br
• Experimental studies showing suppressive effect of iodine on
  – DMBA induced breast tumors in rats
  – MCF-7 human breast cancer cells (in-vitro)
Iodine Transport Mechanism and Breast Cancer

- hNIS demonstrated in lactating breast tissue
  » Spitzweg, 1998
- hNIS demonstrated in Ca-Br and fibroad.; suggested Anti-hNIS Ab / Anti-TPO Ab in sera of CaBr pts., resulting in inhibition of $^{125}$I uptake.
  » Kilbane, 2000
Lacunae in Knowledge

• In experimental studies in rats:
  – it is known that the tumors which show suppression with iodine have higher tissue iodine content c.w. those which do not show as much suppression
  – It is not clear how iodine gets concentrated in some breast tumors, and not in the others.

• No evidence of similar role of iodine in human breast cancer tissues.
Research Questions

• Is there a molecular link between iodine and breast neoplasia
• Is there an active iodine transport mechanism in breast, akin to one in thyroid.
• If there is one, does it have a role in
  – iodine or deficiency induced changes in breast
  – response of BBD and Ca-Br to iodine?
Investigations Planned/ Initiated

• Thyroid hormones status and BBD and Ca-Br
  – hypothyroidism and BBD
    » presented at world cancer congress, 1994
• Urinary iodine excretion (reflection of dietary iodine intake) and BBD and Ca-Br
• Development of experimental models of
  – (BBD)
  – Ca-Br : DMBA induced in rats
  – (MCF-7 human breast cancer cell lines)
Investigations Planned/ Initiated

• In Experimental Ca-Br model:
  – Study of effect of iodine (molecular, elemental and protein bound) with or without MPA, in varying doses
  – study of iodine environment modulation on molecular markers of cell proliferation (BrdU), PCD (TUNEL), and apoptosis.

• Intra-mural project
• PhD topic
Investigations Planned/ Initiated

• In human Ca-Br Tissues:
  – Study of tissue iodine uptake (in-vivo & in-vitro) and content (ex-vivo)
  – Correlation with serum and urinary iodine
  – Correlation with established prognostic markers
  – Study of iodine transport mechanism: hNIS expression
    • offshoot: effect of CTx on iodine transport mechanisms
      – extra-mural project
      – publication of preliminary results
Investigations Initiated/ Planned

• Clinical Studies:
  – Study the effect of elemental iodine on
    • BBD
    • Ca-Br
  – in clinical trial setting.
  – Only after enough evidence and basis in experimental models
  – well planned clinical trial with approval of research and ethics committees
Introduction..2
Human sodium iodine symporter gene (hNIS)

- Responsible for trans-membrane iodine transport.
- Altered expression in thyroid cancer.
- Gene cloned & sequenced from lactating human mammary tissue.
- mapped to chromosome 19p12~13.2.
hNIS Map
hNIS Map
Introduction...4
Lacunae in Knowledge

- Whether hNIS is responsible for regulating iodine in breast tissue.
- Is hNIS expression is regulated by TSH and Prolactin.
- Is hNIS expression altered in breast carcinogenesis.
Introduction ..4
Aims and Objectives

• To study the hNIS expression in normal and cancerous breast tissues.
• To study the transcriptional pattern in cancerous breast tissue.
• To study the effect of serum TSH and Prolactin on expression of hNIS in breast tissue.
Material and Method

- Tumour and peri-tumoral (normal) tissue from 5 IDC patients
- Hyperplastic thyroid tissue from Grave’s dis. patient (control)
- All tissue specimen examined histologically.
- Cryo-preserved, stored at -80°C.
- Estimation of serum T3, T4, TSH and prolactin (RIA).
- Estimation of plasma inorg. iodine by ion exchange chromatography.
Material & Methods ..2
Preparation of hNIS Probe

• 600 base pair hNIS cDNA.
• hNIS gene was cloned in pCR II vector
• Plasmid was linearized with Hind III restriction enzyme.
• RNA probe was synthesized using T7 RNA Polymerase.
Material & Methods ..3
RNA isolation (Chommczynski & Sacchi)

- Tissue homogenized in 4M guanidine isothiocyanate
- Purified with equal vol. Water saturated phenol & chloroform (4:1)
- RNA in aqueous phase ppted. with 3 vol. Abs. Ethanol, stored at -80°C.
Material & Methods 4

RNAase protection assay (RPA).

• High specific activity RNA probe added to each RNA sample
• Hybridization at 42°C for 12 hr
• Hybridization mixture subjected to RNAase T & RNAase A, finally with Proteinase K Tt.
• Hybridized RNA analyzed on polyacrylamide / 7M urea gel electrophoresis
• Gel dried at 80°C & Autoradiograph developed
Material & Methods ..5
Transcription Analysis by Northern Blotting

- RNA separated on 1% agarose gel.
- Transferred from gel to nylon membrane by capillary transfer in 10x SSC (buffer).
- Hybridization with RNA probe in aq. soln. at 62°C for 12 hr.
- Autoradiograph developed.
## RESULTS

<table>
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<th>Sl.No.</th>
<th>T3</th>
<th>T4</th>
<th>TSH</th>
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<td>5</td>
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<td>0.48</td>
</tr>
</tbody>
</table>

T3 : 1.3- 2.6 nM/L  
T4 : 60- 160 nM/L  
TSH : 0.3- 5.0 mIU/ml  
Prolactin : <400 mIU/L  

Urinary Iodine excretion WNL in all
Results..2
Quality Control: Ethidium Bromide stained 1% agarose gel electrophoresis confirming intact RNA
Results..3

Quality Control: $\beta$-actin cDNA Probe confirming equal loading of RNA
hNIS expression by RNAase Protection Assay (RPA).
Results..5
Transcription Analysis

2.9 kb →
hNIS

4.4 kb

1.9 kb
Discussion

- hNIS is involved in iodine transport in human breast tissue.
- Serum parameters (T3, T4, TSH, Prolactin) were in normal range and therefore no effect on the expression of hNIS.
Discussion...

• Increased expression of hNIS in Ca breast as compared to normal breast tissue.

• Tissue specific transcriptional regulation of hNIS as confirmed by two transcripts of hNIS in Breast whereas only one transcript is present in thyroid gland.
Conclusions

- Serum parameters have no effect on hNIS expression.
- hNIS expression is altered in Ca. Breast.
- There is tissue specific regulation of hNIS.
- Splicing is defective in the Ca. breast tissue.
Preparation of hNIS Probe

- Linearization with Hind III digestion
- 0.6 kb hNIS insert
- T7 RNA polymerase
- rNTPs + $^{32}$P UTP
- Radiolabelled hNIS RNA Probe
- Remove DNA template with RQ1 DNAase
- Purified cRNA transcripts
Preparation of hNIS Probe

Linearization with Hind III digestion

+ T7 RNA polymerase
+ rNTPs + $^{32}$P UTP

Radiolabelled hNIS RNA Probe

Remove DNA template with RQ1 DNAase

Purified cRNA transcripts
Human sodium iodine symporter gene (hNIS)

- Responsible for active transport of iodine in thyroid gland.
- Altered expression in thyroid cancer.
- Gene cloned & sequenced from lactating human mammary tissue.
  » Spitzweg c 1998, JCEM.
- Mapped to chromosome 19p12~13.2.
RNAase Protection assay (RPA)

• Highly sensitive method to detect even one or two copies of mRNA present in a single cell.
• RPA was performed to study the hNIS expression in the breast tissue.
RPA...

- High specific activity RNA probe added to each RNA sample
- Hybridization at 42°C for 12 hr
- Hybridization mixture subjected to RNAase and Proteinase K Tt.
- Hybridized RNA analyzed on PAGE/UREA
- Gel dried at 80°C & Autoradiograph developed