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Report

Functional expression of sodium iodide symporter (NIS) in human breast cancer tissue*

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Summary

Sodium iodide symporter (NIS) is a molecule involved in active accumulation of iodine in thyroid gland for the biosynthesis of thyroid hormone. Its expression has also been demonstrated in extra-thyroidal tissues including lactating mice mammary gland and also in human breast cancers. Iodide transport in thyroid cells through NIS is the basis for using radioiodine for diagnosis and treatment of differentiated thyroid carcinoma. The similar approach may prove beneficial for the diagnosis and treatment of breast cancer if iodine uptake, its retention and NIS expression can be shown unequivocally in malignant tumors. The aim of the present study was to investigate NIS expression, *in vivo* iodine transport ability and fate of iodine in human breast tumors. Women (age 33–58 years) with infiltrating duct carcinoma confirmed by FNAC and subsequent histopathology were the subject of this study. Expression of NIS RNA and protein was confirmed by RNAase protection assay, western blot and immunohistochemistry respectively in surgically excised breast tumor tissue. Iodine transport ability and its nature was assessed both *in vivo* and *in vitro*. We report high NIS expression at both transcriptional and translational level and its ability to transport iodine in human breast tumors. The *in vivo* iodine transport ability was confirmed by scintigraphy. Unlike thyroid, perchlorate and thiocyanate do not inhibit iodine transport in breast tumors. The presence of iodinated proteins suggests the longer retention time. The unequivocal demonstration of NIS expression, its functionality and retention of iodine by organification further provides supportive evidence for use of radioiodine as an additional treatment modality of human breast carcinoma.

Introduction

Iodide accumulation is a fundamental property of thyroid cell and is the first step of thyroid hormonogenesis. In normal thyroid tissue, NIS is expressed on the plasma membrane at the basolateral surface of the follicular cells. This molecule is responsible for the ability of thyroid gland to concentrate iodide up to 40-fold with respect to plasma and depends upon the electrochemical gradient of Na^+, which is maintained by Na^+–K^+ ATPase. The transported iodine is organified as thyroglobulin by the action of thyroperoxidases. The active accumulation and retention of iodine into differentiated thyroid carcinoma forms the basis of radioiodine (^{131}I) therapy [1].

The similar approach may prove beneficial for the diagnosis and treatment of other cancer if iodine uptake, its retention and NIS expression can be shown unequivocally in malignant tumors. Thyroid gland shares its capacity to accumulate iodide actively with several other tissues like stomach, salivary gland and lactating mammary gland, where NIS expression has been documented [2–4]. The functional expression of endogenous NIS by scintigraphy in breast tumor of transgenic mice, one with activated *Ras* and other with overexpression of *Neu* oncogene, by scintigraphy, has also been reported [5]. The same study also reported NIS positivity in human breast carcinoma tissue [5]

*Part of this work was presented in the 12th International Thyroid Congress, Kyoto, Japan, October 2000 and also constitutes the PhD thesis of Geeta Upadhyay.

indicating that symporter if studied in detail, may be used as a breast cancer marker as well as target for therapy. Others [7, 8] have also reported beneficial effects of radioiodine therapy in experimental rat breast tumor models expressing NIS.

The functionality of endogenous NIS molecule expressed in human breast cancer tissue is still not understood. It has also been emphasized earlier that therapeutic use of radioiodine in carcinoma breast requires *in vivo* and *in vitro* quantitative measurements of tumor iodide concentration and its retention [6]. The detailed study of NIS molecule at RNA and protein level, its functionality and the fate of transported iodine are required before NIS may be targeted for therapeutic use.

Our current study was designed to evaluate, using RNAase protection assay, western blot and immunohistochemistry, the abundance of NIS in breast tumor tissue. Further, we investigated the iodide transport ability *in vivo* by scintigraphy and *in vitro* by ^{125}I uptake. We also studied the nature of iodine uptake and its subsequent organification.

Materials and methods

Subjects

This project was approved from our Institutional Ethical Committee. Twelve female patients (age 33–58 years) who underwent mastectomy for cytologically confirmed infiltrating duct carcinoma (IDC) (representing TNM stages I–III), were the subjects of study. Two of these patients had undergone anti-cancer chemotherapy with cyclophosphamide, methotrexate and 5-fluoro-uracil combination (CMF) regime before mastectomy. Other patients did not receive chemotherapy or radiotherapy before operation. Breast cancer tissue was obtained from the core of the tumor and ET tissue was obtained from mastectomy specimen atleast 5 cm away from the gross tumor margins. Total RNA and membrane fractions were extracted from tissues of five patients, including two patients who had undergone pre-operative chemotherapy. Tissue from thyroidectomy specimen of patients with Graves' disease was used as a positive control for NIS expression study [9]. Scintigraphic imaging was performed in subsequent four patients prior to mastectomy. *In vitro* iodine organification assay was performed in surgically excised breast tissues obtained from three patients.

RNAase protection assay (RPA)

Total RNA from tissue was isolated by the acid guanidinium/phenol/chloroform method from 500 mg tissue [10]. RNA (50 μg) was precipitated and dissolved in hybridization buffer (40 mM PIPES pH 6.4, 1 mM EDTA, 0.4 M NaCl and 80% formamide). NIS cDNA of 600 bp cloned in pCRII vector (Invitrogen Inc, USA) [A kind gift of Prof T. Onaya, Yamansahi Medical School, Japan], the 5′ end of the insert corresponds to 317 bp of human NIS (A in ATG is designated as +1), used for northern hybridization. The plasmid was linearized with *Hin*d III and ^{32}P-labeled antisense riboprobe was synthesized with T7 RNA polymerase and sense with SP6 RNA polymerase, using Riboprobe kit (Promega Inc, USA) according to the manufacture's protocol. The antisense and sense RNA probe was added in separate reaction mixtures. The mixture was denatured at 85°C for 5 min and hybridization was carried out at 42°C for 12 h. After incubation the mixture was first treated with RNAase digestion mixture (RNAase A + RNAase T) and then with proteinase K. After phenol/chloroform treatment, the mixture was precipitated with double volume of ethanol at −20°C for 30 min and dissolved in formamide loading buffer (80% formamide, 10 mM EDTA pH 8.0, 1 mg/ml xylene cyanol FF, 1 mg/ml bromophenol blue). Radiolabeled hybridized RNA was analyzed by electrophoresis through a polyacrylamide/7 M urea gel (UREA/PAGE). The gel was dried for 2 h on gel dryer (BioRad, USA) and exposed to Kodak OMAT, X-ray film (NEN Research Products, USA) for 72 h at −80°C [11]. Equal loading was confirmed by β-actin cDNA using northern hybridization.

Western blot analysis

The membrane protein was isolated by homogenizing the tissues in five volumes of the buffer (10 mM Tris–HCl [pH 7.4], 5 mM NaCl, 1 mM EDTA, 0.1 mM phenylmethylsulphonyl fluoride, 50 mg/ml leupeptin) containing 0.25 M sucrose. After gentle homogenization at 4°C, the lysates were centrifuged at 700 *g* for 10 min at 4°C. The supernatants were further centrifuged at 100,000 *g* for 90 min at 4°C to collect total postnuclear membrane fractions. The membrane fractions were resuspended in the homogenizing buffer at protein concentration of 1 mg/ml and stored at −80°C [9]. The membrane fractions (100 μg) were added to the sample buffer (10% glycerol, 2% SDS, 0.0625 M Tris–HCl [pH 6.8]) and heated with 2-mercaptoethanol at 70°C for 7 min. The samples were subjected to 0.1%

SDS-12% PAGE and electro-transfered to nitrocellulose membranes. The blots were stained by Ponceau-S to check the transfer of the proteins on the membranes.

Nonspecific binding sites were blocked with 3% BSA in TBST (20 mM Tris–HCl [pH 7.4], 137 mM NaCl, 0.1% Tween 20) for 1 h. The membranes were then incubated for 2 h at room temperature with a mouse monoclonal NIS antibody (NIS FP5a) (A kind gift from Dr John C. Morris, Mayo Clinic, Rochester, USA). It is a mouse monoclonal antibody against sodium/iodide symporter developed using a hNIS-MBP fusion protein containing the last extracellular loop and the carboxy terminus of human NIS (containing the amino acid sequence 625–643), capable of recognizing NIS protein with high affinity and specificity [12]. After washing three times with TBST, the membranes were incubated with ^{125}I labeled protein A (Pharmacia Biotech, Amersham, USA) (2×10^4 cpm per square centimeter of nitrocellulose filter) for 2 h. The filter was washed three times with TBST [10]. The filter was dried and exposed to Kodak OMAT, X-ray film (NEN Research Products, USA).

Immunohistochemistry

The tissue sections were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked by incubation of slides in 3% H_2O_2/methanol for 30 min. Subsequently, the slides were subjected to antigen retrieval with 10 mM citrate buffer at 100°C for 30 min. Nonspecific binding sites were blocked by 3% BSA diluted in PBS at room temperature for 30 min. The same primary antibody in 1% BSA-PBS-T was added and incubated for 1 h at room temperature [12] with biotinylated secondary antibody and HRP conjugated streptavidin (Universal LSAB kit, DAKO, Denmark). Finally color was developed with substrate 3, 3′ diaminobenzidine tetrahydrochloride (DAB) (Sigma Chemical, USA) and H_2O_2. In the negative control, instead of primary antibody, sections were incubated with 3% BSA. For positive control, thyroid tissue section obtained from Graves' patient was processed similarly.

Nuclear imaging

Imaging was conducted using a dual head gamma camera system (DST XL, Sopha Gamma Camera, France) with a LEAP collimator interfaced to an IBM computer. Imaging was done using Technetium-pertechnetate {TPT($^{99m}TcO_4^-$)}, a γ-emitting molecule, concentrated in thyroid by mechanism similar to iodine [13]. Appropriate energy peaks with 20% windows were used. Imaging was done after 10 min post-intravenous injection of 10 mCi (370 M bq) $^{99m}TcO_4^-$, obtained from BRIT, BARC, Mumbai, India. TPT images of breasts were acquired for 10 min each in lateral and anterior views after positioning the patient in prone position over a scintimammography cushion pad. On the first day the patient underwent imaging with only TPT as described above and on second day (after four half-lives of ^{99m}Tc), same set of images were acquired using same amount of TPT after 30 min of oral administration of 500 mg potassium perchlorate (Sigma, USA) dissolved in 100 ml of sterile water. Semi-quantitative assessment of uptake was done by drawing ROI over tumor and ET region in the same breast.

In vitro *iodine organification in human breast tissue*

Surgically excised tumor and ET tissues were collected on ice. Tissue slices of 0.3–0.4 mm in thickness were prepared quickly with sharp blade and washed again in fresh saline. Slices were briefly blotted, weighed on balance in 150 ± 15 mg [14, 15]. The tissues were transferred to vessels containing ^{125}I (0.25 μCi/ml; 0.3 ng/ml iodide) in 4 ml of Hanks' balanced salt solution (HBSS) buffer. Incubation was carried out in a rotary water bath at 37°C (120 cycles/min). The tissue was then weighed and homogenized in 2 ml 10% trichloroacetic acid (TCA) containing 0.1 mM NaI. Precipitated protein was collected by centrifugation at 2000 *g* for 10 min [16]. The pellet was washed twice with 10% TCA and radioactivity associated with TCA-insoluble fraction was determined. The experiment was performed in the presence of perchlorate and thiocyanate, well known iodine transport inhibitors and propylthiouracil (PTU), organification blocker [14]. Results of the iodide uptake studies were expressed as cpm/mg protein to account for differences in tissue cellularity.

Serum analysis

Sera from all the patients were subjected to Prolactin (PRL), TSH, total T3 (TT3) and total T4 (TT4) estimation by standard RIA kits (DPC kit, USA).

Statistical analysis

All the results were expressed as mean ± SEM. Data groups were compared using SPSS-10.0 (Chicago IL,

USA) software using Levene's independent sample *t* test.

Results

Increased expression of NIS RNA in cancerous breast tissue

Using cRNA probe of high specific activity we detected high expression of NIS in tumor compared to ET tissue. RPA analysis showed protected band of 600 bp corresponding to NIS on UREA/PAGE when exposed to X-ray film (Figure 1(A)). NIS expression at RNA level was consistently high in all the cancerous tissue studied (lanes 3–5, Figure 1). The tumor tissue from the patients who had undergone pre-operative chemotherapy (lanes 7 and 8, Figure 1) also showed high expression of NIS, inspite of low loading of RNA as confirmed by β-actin, as compared to expression in ET tissue.

The nonspecific binding was confirmed by using sense cRNA, and showed no bands (Figure 1(B)). The expression of NIS gene was high in Graves' thyroid tissue, taken as a positive control (lane 1, Figure 1) in consonance with earlier observations [8, 13, 15, 17].

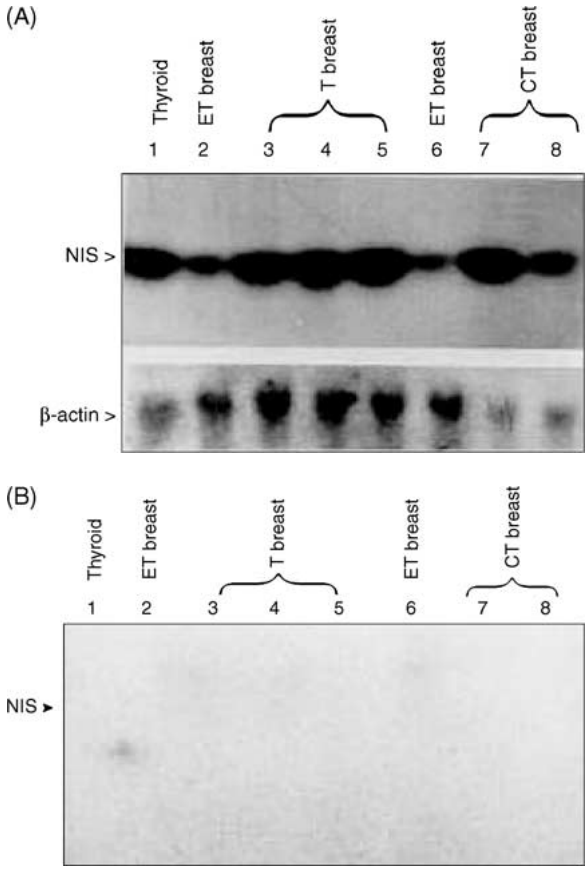

Figure 1. Increased levels of NIS RNA in cancerous breast tissue. RNAase protection assay of Graves' thyroid, ET, tumor and CT breast tissue. Total RNA from Graves' thyroid (lane 1), ET (lanes 2 and 6), tumor (lanes 3–5), and CT breast tissue (lanes 7 and 8) was incubated with NIS cRNA probe and protected band was analyzed on UREA-PAGE (A). Similar experiment using sense probe was also done and showed no signals confirming the specificity of probe (B). To compare gene expression equal RNA separated on denaturing agarose gel and transferred to nylon membrane and probed with β-actin cDNA probe (A).

Increased expression of NIS protein in cancerous breast tissue

The expression of NIS protein was confirmed by western blotting using NIS monoclonal antibody (NIS FP-5a). Western blotting revealed presence of 97 kDa band corresponding to NIS, both in tumor and ET breast tissue (Figure 2). The expression in breast tumor tissue was enhanced several fold in tumoral tissue as compared to ET tissue. The protein of 97 kDa was predominantly present in thyroid tissue as reported earlier [8, 14, 15, 17]. In tissues from two patients who had received pre-operative chemotherapy, band intensity was comparable to the ET breast (lanes 6 and 7, Figure 2).

Localization of NIS protein

Site-specific expression of NIS protein was observed by immunohistochemistry of the breast tissue by using the same monoclonal antibody against NIS. Most of the breast tumor cells were found positive for NIS immunoreactivity (Figure 3(C)). Higher magnification of showed intense cytoplasmic positivity for NIS (Figure 3(D)). Graves' thyroid tissue included as a positive control showed high immunoreactivity in thyroid follicles (Figure 3(A)) in consonance with earlier observation [8, 14, 15, 17]. The section of breast tumor tissue in the absence of primary antibody incubation showed absence of any immunoreactivity and served the purpose of negative control (Figure 3(B)). The results are in agreement with those reported in a recent study where in 87% of the breast tumors were found to be NIS positive [5].

In vivo *analysis of iodide transport in human breast tissue*

The *in vivo* iodide transport ability of breast tumor cells was assessed by TPT scintigraphy of breast

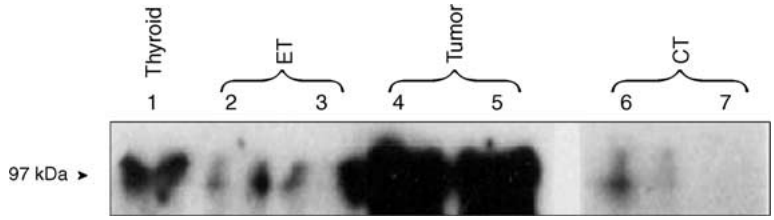

Figure 2. Increased presence of NIS protein in cancerous breast tissue. Western blot analysis of membrane fractions from Graves' thyroid, extra-tumoral, tumoral and CT breast tissue. Membrane fractions from Graves' thyroid (lane 1), extra-tumoral (lanes 2 and 3), tumor breast (lanes 4 and 5), and CT breast (lanes 6 and 7) was electrophoresed on 12% SDS-PAGE and was transferred on nitrocellulose membrane, subjected to western blot analysis with NIS monoclonal antibody. The bands were visualized with ^{125}I Protein A.

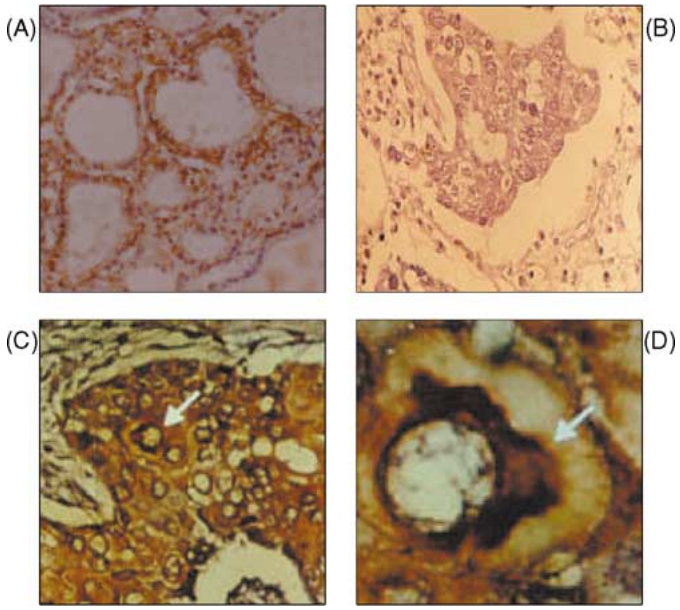

Figure 3. Breast tumor cells showed positivity for NIS. Immunohistochemical staining for NIS in infiltrating ductal carcinoma. Representative sections from breast carcinoma were subjected to immunohistochemical staining by mouse monoclonal antibody against NIS. (A) Photomicrograph showing immunoreactivity for NIS antibody in thyroid follicles of Graves' disease (400X) (Positive control). (B) Absent immunoreactivity in breast tumor section treated with 3% BSA instead of primary antibody (400X) (Negative control). (C) Photomicrograph of immunostained section from breast tumor showing diffuse cytoplasmic immunostaining for NIS antibody (400X). (D) Strong positivity with better cellular details could be appreciated at higher magnification (1000X) from (C).

cancer patients. TPT is used for iodine transport studies in thyroidal disorder and concentrated in thyroid by NIS. Scintigraphy showed the uptake of radioactive tracer in both the breasts with an increased uptake in malignant mass along with thyroid, salivary glands and stomach (Figure 4(A) and (C)). Administration of perchlorate, a known inhibitor of iodine transport in thyroid, resulted in blockade of TPT uptake in thyroid, salivary gland, stomach and extra-tumoral breast but not in the breast tumor (Figure 4(B) and (D)). This observation is in contrast to the release of radioiodine by the breast as reported in mice in response to perchlorate [5].

Analysis of iodine organification

Organic binding of the iodine to the proteins was assessed *in vitro* using radioactive iodine (^{125}I) in the tissue slices from breast tumor and ET tissue. Tissue from Graves' thyroid was included as positive control. We detected the radioactivity associated with TCA precipitates from tumor and ET breast tissue. The radioactivity associated with TCA precipitate from tumor tissue was significantly higher than ET tissue ($p < 0.05$) and lower than Graves' tissue ($p < 0.05$). Perchlorate and thiocyanate known inhibitors of iodine transport, at a concentration of 1 mM could block

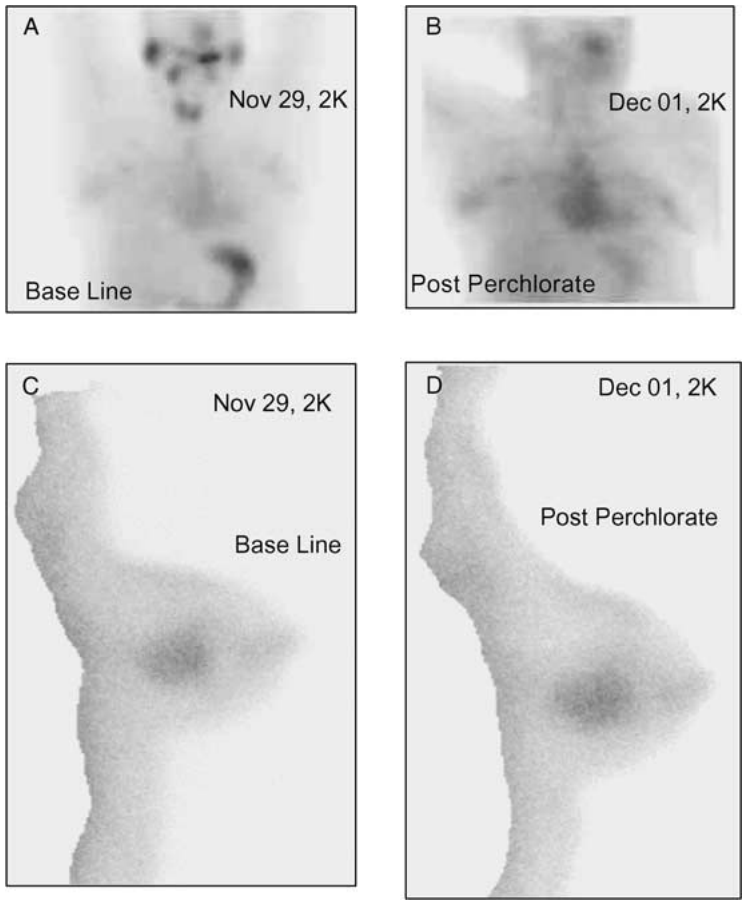

Figure 4. NIS protein is functional in tumorous breast. Scintigraphic imaging with TPT of breast cancer patients before and after administration of perchlorate. Anterior image of the neck and chest region of the patient showing (A) intense uptake in thyroid, salivary gland and stomach before perchlorate administration, after giving perchlorate uptake was blocked in thyroid (B). Lateral images of the breast showing (C) diffused tracer uptake in extra-tumoral breast with increased focal uptake in breast lesion (D) after giving perchlorate the uptake in the lesion was not blocked but the activity in ET area increased.

the iodine transport in ET and Graves' tissue, but not in the breast tumor tissue ($p < 0.005$). The protein bound iodine was significantly higher ($p < 0.005$) in tumor tissue in the presence of both iodine transport inhibitors. Propylthiouracil, a peroxidase inhibitor was employed to characterize the regulation of iodide incorporation into protein. The radioactivity associated with TCA precipitable fraction decreased significantly with PTU treatment both in tumor ($p < 0.005$) and ET tissue ($p < 0.005$) and also in Graves' thyroid tissue (Figure 5). The results indicate longer retention of transported iodine in breast tumors.

Serum analysis

Serum total T3 and T4, TSH and PRL levels were within normal range in all the patients studied (Table 1).

Discussion

In this study we report unequivocal presence of endogenous NIS, it's functional nature and organification of iodine in human breast carcinoma. Interestingly, in both *in vivo* and *in vitro*, we observed that iodine, which is transported in human breast tumors, is handled differently (in terms of iodine uptake in the presence of perchlorate and thiocyanate) compared to normal thyroid gland, extra-tumoral breast tissue in humans, and by mice breast tumors respectively [5, 14, 17].

We analyzed tumor and ET tissue from five-breast cancer patient for NIS mRNA expression by RPA, which is highly sensitive technique and can detect even one or two copies of mRNA. NIS mRNA was expressed in both extra-tumoral and tumor tissue, its

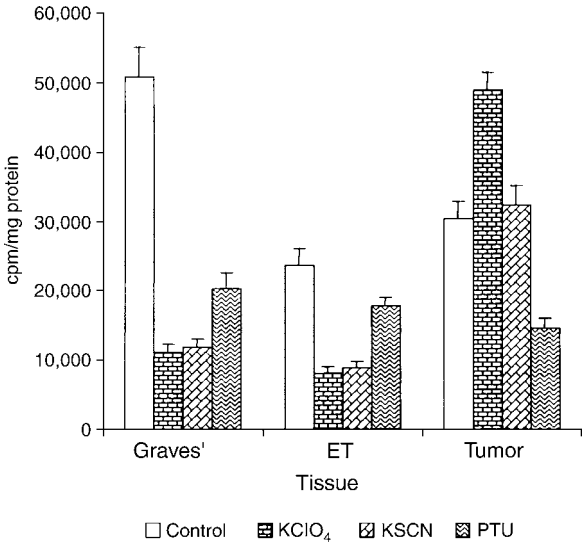

Figure 5. Iodine is organified in breast tissue. Iodine organification in ET and tumor tissue in the presence of perchlorate, thiocyanate and PTU. Surgically excised tissue was sliced and was incubated with ^{125}I (0.1 mCi) in presence and absence of perchlorate (1 mM), thiocyanate (1 mM) and PTU (100 µg/ml) for 4 h at 37°C. After incubation, the tissue was homogenized in 10% TCA, protein was precipitated and counts were taken. The results were repeated three times independently and expressed in cpm/mg protein as mean ± SEM.

Table 1. Serum analysis of breast cancer patients: the representative serum analysis of patients who had undergone total breast mastectomy and tissue was collected for RNA and protein analysis. The total serum T3, T4, TSH and PRL were determined in each patients by RIA (DPC kit, NY, USA)

T3 (nM/L)	T4 (nM/L)	TSH (mIU/ml)	PRL (mIU/L)
1.40	100	2.20	93.28
1.65	150	3.20	89.00
1.39	115	0.52	127.20
1.39	140	1.90	94.28
1.54	145	0.48	90.09
1.45	130	0.70	84.80

Reference range
T3: 1.3–2.6 nM/L; T4: 60–160 nM/L; TSH: 0.3–5.0 mIU/ml; PRL: < 400 mIU/L.

expression was enhanced many fold in tumor tissue. The western blot analysis further confirmed the enhanced expression of NIS protein in tumorous tissue as compared to ET. It was interesting to observe the reduced NIS protein expression in tumor tissues of two patients who had undergone pre-operative chemotherapy, inspite of increased NIS mRNA levels in the same tissues is in accordance with the known protein synthesis inhibitory action of cyclophosphamide. Although NIS has been shown to be located in plasma membrane in lactating breast tissue of rat, but the strong cytoplasmic staining of NIS in breast tumors observed by us indicate differential processing of NIS in breast malignancy and is in agreement with earlier report [5].

The question whether highly expressed NIS molecule is functionally active in breast tumors has not been adequately answered. *In vivo* iodine transport ability of NIS molecule expressed in breast tumors was assessed with TPT ($^{99m}TcO_4^-$) is a γ-emitting molecule actively concentrated in the thyroid by the mechanism similar to iodine through NIS. It also offers practical advantage of a much shorter half-life (6 h) than ^{131}I (half-life of 8 days) and ^{125}I (half life of 60 days) [13]. Imaging analysis showed the tracer concentration in thyroid, salivary gland and stomach with an enhanced localized uptake in malignant breast mass. Administration of perchlorate blocked tracer uptake in thyroid, salivary gland, stomach and extra-tumoral breast, but not in breast tumor tissue. Our observation is in agreement to earlier report of strong TPT uptake in human breast lesion after perchlorate administration by Cancroft and Goldsmith in 1973. It was suggested that scintigraphic evaluation with TPT after perchlorate administration could help in obtaining better assessment of malignant breast masses [19]. However, this suggestion was ignored, possibly because molecular basis of iodine uptake was unknown at that time. The unresponsive of TPT uptake in human breast lesion to perchlorate administration observed by us is in contrast with recently reported TPT uptake inhibition in mice mammary tumor [5]. This discrepancy, though may be species specific, needs a detailed study of iodine transport mechanism in different pathophysiological conditions of breast. These observations emphasize on future experiments to explore the use of NIS in management of breast cancer.

The retention of radioiodine within the tumor cells for a longer time decides the efficacy of therapy, as it increases the radiation exposure within the cell. The prerequisite is fulfilled in differentiated thyroid carcinoma as transported iodine is bound with thyroglobulin by organification [16–18]. The organification of iodine in resting mammary gland and breast tumors is poorly understood. Therefore, to understand whether iodine transported in breast tissue is retained, *in vitro* experiments were done with ^{125}I and protein bound iodine was analyzed by TCA precipitation. We observed that iodine is organified after its uptake both

in tumor and extra-tumoral tissue. The iodine transport inhibitors namely perchlorate and thiocyanate blocked iodine uptake in ET, as low counts were observed in TCA precipitate from ET, whereas no blockade was observed in breast tumor tissue. This indicates that iodine transporter present in breast tissue is akin to that present in thyroid whereas the fate of transported iodine is fundamentally different in tumor tissue. These *in vitro* experiments further support the observation of TPT uptake *in vivo* and its response to perchlorate.

Our experiments also suggest that iodination is peroxidase mediated as the protein bound iodine decreased significantly in the presence of peroxidase inhibitor PTU. The peroxidase activity is known to be present in human mammary tumors but its role in iodination of the proteins is not known [2, 20, 21]. In lactating mammary gland, iodine is organified to casein and other milk proteins with help of lactoperoxidase, expressed in the alveolar cells, to provide iodine to new born [2, 3]. The evidence provided by others [2] and us indicate that iodoprotein formation may occur in inactive mammary tissue as well. Thus it is not unreasonable to assume that more extensive iodide organification may occur in other tissues through the presence of tissue specific peroxidases as reported earlier [22]. The significance of iodination in non-lactating, extra-tumoral and breast tumors is not understood and needs to be explored further.

The above evidences suggest that NIS is expressed at high levels and is functional in breast tumors. The regulation of NIS gene in human mammary gland has not been investigated in detailed. Hormones are considered to be an important factor in the etiology of the breast cancer and also known to regulate NIS expression. TSH regulates the NIS expression in thyroid gland and PRL has been shown to enhance iodine uptake in mouse mammary gland explants [9, 14, 17]. We did not observe any change in circulating levels of total T3, T4, TSH and PRL in the study subjects, indicating that regulation of NIS expression in breast tumor may involve factors other than these hormones. The functional expression of NIS in estrogen receptor negative breast tumor in transgenic mice overexpressing *Ras* or *Neu* oncogene reported previously, also supports the hypothesis of NIS induction by non-hormonal factors involved in malignant transformation of breast epithelial cells [5].

We demonstrated high level of NIS expression in breast tumor and its ability to concentrate iodine. The active accumulation of iodide into differentiated thyroid carcinoma forms the basis of radioiodine therapy (^{131}I). In this treatment, ^{131}I is given orally, absorbed into the blood stream and transported by NIS into the susceptible tissues. Similar approach may be useful for the breast carcinoma but thyroid gland should be protected to avoid radiation exposure. Our results suggest that perchlorate effectively block radioiodine uptake in thyroid and other extra-thyroidal tissue, thus allowing specific uptake in breast tumor tissue. However, perchlorate is known to be toxic to thyroid follicles, therefore cannot be used for long term protection of thyroid. The administration of exogenous T3 or T4 may also help, leading to inhibition of TSH release and concomitant down regulation of thyroidal NIS [16].

Localization of NIS and organification of transported iodine in breast tumors observed by others and us raises exciting possibilities for both diagnosis and treatment of breast cancers. The necessary *in vitro* and *in vivo* quantitative measurements provided in the present study, albeit in small number of cases needs to be followed by comprehensive clinical analysis in large number of patients before this approach can be considered as a breast cancer therapy.

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