Human sodium iodide symporter (hNIS) in fibroadenoma breast—A immunohistochemical study

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Human sodium iodide symporter (hNIS), responsible for the active transport of iodine is an integral plasma membrane glycoprotein present in the thyroid cells and extrathyroid tissues like breast and salivary glands. If its functional form is unequivocally shown in benign or malignant breast tissues, then it may serve as a basis for diagnosis and treatment using radioactive iodine. With an aim to analyze the hNIS expression in a distinct benign breast condition of fibroadenoma, biopsy proven fibroadenoma tissues, normal non-lactating breast tissue and biopsy proven infiltrating duct carcinoma tissues were examined for hNIS expression using immunohistochemistry. Out of 20 biopsy proven fibroadenoma tissues, 19 (95%) showed positivity for hNIS protein and only one was negative. Of these 10% were mildly positive, 50% cases were moderately positive and 35% showed intense positivity. None of the control tissue obtained from reduction mammoplasty specimens or normal breast tissues samples (5 cms away from the tumor) were positive. hNIS was also intensely positive in 9 out of 10 (90%) infiltrating duct carcinoma tissues and moderately positive in one case. These preliminary results show that hNIS was present in high frequency as demonstrated by immunohistochemistry in fibroadenoma breast.

Keywords: Fibroadenoma breast, Human sodium iodide symporter (hNIS), Immunohistochemistry

The sodium iodide symporter (NIS) is a key protein responsible for the active transport of iodide from the blood into the thyroid gland. NIS is expressed on the basolateral surface of the thyroid follicular cells as an integral plasma membrane glycoprotein and enables the thyroid to actively concentrate iodide into the thyroid follicular cells against a concentration gradient of 20-40 fold with respect to plasma. The iodide is then translocated across the apical membrane into the follicular lumen, where it is organified into thyroglobulin. This is the first crucial step in the thyroid hormone (TH) biosynthesis. Thyroid function and its systemic implications depend on an adequate supply of iodide into the gland and this, in turn, depends on the dietary intake of iodine and proper hNIS function. Iodine accumulation via hNIS into the thyroid glands is the basis for diagnosis i.e. scintigraphic imaging of the thyroid with radiiodine and the destruction of the hyper-functioning tissues for its treatment. Besides the thyroid gland, hNIS is also known to mediate active iodide transport in other tissues, viz. salivary glands, gastric mucosa, lactating mammary gland and ciliary body of the eye. The tissue specific regulation of hNIS expression and the functional significance of extra-thyroidal iodide concentration remain elusive. The hNIS protein is expressed under physiological conditions in the mammalian breast during late pregnancy and lactation and is reported to be under the hormonal influence of estrogen, prolactin and oxytocin. In the lactating mammary gland, hNIS mediates the transport of iodine from maternal circulation into the milk thus making this essential trace element available for the newborn for its own TH production during oxytocin mediated stimulation of lactation increased expression of hNIS has been reported. In ovariectomised rats, a combination of estrogen, oxytocin and prolactin treatment leads to maximal hNIS expression in mammary cells. Its role in end
organ cancers was examined recently by using anti hNIS antibody immune-staining on 93 thyroid and breast cancer tissue samples and Western blot experiments were carried out to determine the amount of hNIS protein in 20 samples. It was suggested however, that the staining was due to non-specific binding of antibodies and the protein levels were low in thyroid and breast cancers.\(^8\) Conversely, another study reported 80% of human breast cancer samples positively staining for hNIS protein.\(^7\) Equally, its functionality and retention of iodine by organification was provided as supportive evidence for use of radioiodine as an additional treatment modality of human breast cancer. These authors also reported high hNIS expression by RNAaase protection assay and Western blot studies. The in vivo iodine transport ability was confirmed by scintigraphy.\(^7\) Iodide accumulation mechanisms in extra-thyroidal tissue are similar to that in the thyroid in the sense that they are also blocked by thiocyanate and perchlorate ions. However thyroid stimulating hormone (TSH) exerts no regulatory influence on extra thyroidal tissue. hNIS was functionally expressed in transgenic mouse mammary tumor.\(^6\) Another study investigated the correlation between the expression of hNIS mRNA and the uptake of 99mTc–pertechnetate in 25 breast tumors. On scintigraphy, positive uptake was seen in 4 patients. The normalized mRNA expression of hNIS was higher in tumors with positive uptake on the scintigraphy than that in negative uptake tumors. Hence, hNIS expression in the breast tumor was correlated with the 99mTc-pertechnetate uptake.\(^10\)

Till now only a few studies are available on benign conditions of the breast like the fibroadenoma. In a study carried out to demonstrate hNIS expression by reverse transcriptase polymerase chain reaction (RT-PCR), NIS was demonstrated in both the fibroadenoma examined and in mRNA isolates from 6 out of 7 breast carcinoma, and in addition, mean total tissue iodine levels in benign tumors (fibroadenomata) were significantly higher than those in breast cancer.\(^12\)

The present study has been designed to see the expression of hNIS in breast tissue viz. fibroadenoma and juxtapositioned normal breast tissues using immunohistochemistry and compare it with breast carcinoma. This may provide a better understanding how hNIS is differentially expressed in benign and malignant proliferation of breast i.e. fibroadenoma and infiltrating duct carcinoma.

Materials and Methods

Tissues—Ethical clearance for this work was obtained from the institutional ethical committee of CSM Medical University, Lucknow. Informed consent was obtained from the patient prior to surgery and operating surgeon requested to provide a part of excised tissue in all cases. The study was carried out from May 2007 to April 2008. A purposive sample of 20 women attending the breast clinic of CSM Medical University with fibroadenoma breast belonging to the age group 18-50 years was included in this study. No formal sample size calculation was done at this stage as this was considered pilot data for further hypothesis testing. Proforma based demographic and clinical details were recorded and physical examination was performed in all subjects. Excision biopsy was done in each patient and was histologically proven as fibroadenoma (intracanalicular and pericanalicular). Normal breast tissue was obtained from reduction mammoplasty or 5 cm away from the tumor margin (confirmed to be normal by histological examination). Normal non-lactating tissue was obtained from the excised mastectomy breast tissue. The tissue was submitted for both routine histopathology as well as immunohistochemical analysis with hNIS. Ten consecutive cases of breast carcinoma were selected from the archival material available in the Department of Pathology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow. These subjects were pre-menopausal women between 18 to 50 years of age with histologically proven infiltrating duct carcinomas. Grave’s thyroid known to over express hNIS was used as a positive control for NIS staining.\(^13\)

Immunohistochemistry—Sections (4-6 µm) thick made from the formalin fixed tissue were charged on glass slides. All sections were incubated at 60°C for 2 h. Tissue sections were de-paraffinized by serial passages in xylene and graded ethanol. Endogenous peroxidase activity was blocked by incubating tissue sections in 3% H\(_2\)O\(_2\) in methanol for 15 min. Antigen retrieval was performed using 10% citrate buffer in a water bath for 15 min. All washes were performed with TSBT (0.3 M NaCl, 0.1% Tween-20, and 0.05 M Tris-HCl, pH 7.6) three times for 5 min each. Immunostaining was performed essentially according to manufacturer’s protocol (Vectastatin Universal Quick Kit, Vector Laboratories, Burlingame, CA). Briefly, sections were blocked using blocking serum.
Slides were incubated for 2 h with primary anti-hNIS antibody (a polyclonal antibody against cytosolic domain of hNIS, raised in rabbits) diluted in buffer containing 1.5% blocking serum. After TBST washes, sections were incubated in biotinylated universal secondary antibody for 10 min. Subsequently, sections were incubated in streptavidin/peroxidase complex for 5 min. Sections were incubated in DAB chromagen (Dako cytomation, Carpinteria, CA) solution until desired intensity developed (observed as a brown product). Slides not incubated with hNIS antibody were used as primary antibody control. All slides were counterstained with haematoxylin (30-60 sec). All slides were DPX mounted. Sections were visualized and photographed on a light microscope (Nikon Microphot 4).

**Interpretation and grading**—The tissues were scored as follows: negative (0), mild focal positivity (+), patchy or diffuse positivity of moderate intensity (++) and diffuse positivity of high intensity (+++). The subjective scoring system was done by 2 observers who were blinded to each other. Inter observer agreement for the scoring was good (kappa was over 0.8). Normal breast tissue was also examined in 8 cases. Two normal specimens were taken from reduction mammoplasty and 2 from excised axillary breasts.

### Results

Expression of hNIS protein was observed in the fibroadenoma tissues using the technique of immunohistochemistry. A total of 19 samples of fibroadenoma, out of 20 (95%) biopsy proven cases of fibroadenomas examined showed strong positivity for the protein. Pattern of staining was diffuse cytoplasmic in epithelial cells and it was also seen in scattered stromal cells. The results were as follows: 2 (10%) slides were mildly positive (+), 10 (50%) cases were moderately positive and 7 (35%) slides showed intense positivity (Table 1). None of the position normal breast sample tissue were positive. Thyroid tissue obtained from a patient of Grave’s disease showed intense positivity in each run, likewise sections from rat brain showed no positivity (Fig. 1). In addition, intracellular staining of hNIS was also observed in 9 out of 20 fibroadenoma samples analyzed (Table 1). hNIS was intense positive (+++)

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<th>Type</th>
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Fig. 1—Immunohistochemical staining for hNIS in fibroadenoma breast. Representative sections from fibroadenoma were subjected to immunohistochemical staining using a rabbit polyclonal antibody against hNIS. (A): Photomicrograph showing immunoreactivity for NIS antibody in thyroid follicles of Grave’s disease (400X) (positive control). (B): No immunostaining in normal breast tissue section for hNIS antibody (400X). (C): Infiltrating duct carcinoma strong immunoreactivity in cytoplasm of neoplastic epithelial cells (400X). (D): Fibroadenoma breast cells showed positivity for hNIS. Diffuse cytoplasmic immunostaining is also observed in epithelial cells and in scattered stromal cells also (100X). (E): Stromal cells show variable staining (400X). (F): Non-neoplastic glands in higher magnification show hNIS positivity (400X).
in 9/10 (90%) infiltrating duct carcinoma cases and it was moderately positive in one case.

**Discussion**

Since 19 out of 20 (95%) specimens of fibroadenomas showed positivity for the hNIS expression, further studies are required to find out the functionality of hNIS in fibroadenomas in relation to potential radioiodine therapeutics. This is a small study and further studies are required to corroborate the present findings. One can perhaps find a correlation between the rate of growth and variants of fibroadenomas (multiple/giant) and the abundance of hNIS. Most of the patients in this study had the history of accidentally discovering the lump, hence, at present one cannot comment upon aggressiveness of fibroadenoma but future studies can be carried out taking the rate of growth into account.

Recently, functional hNIS expression has been reported in fibroadenoma also, besides other breast cancer and lactating mammary gland. In a case reported by Berger et al., radioactive iodine given to a patient with multifocal papillary thyroid carcinoma also showed focal solid lesion in her right breast which was proven by various investigations to be fibroadenoma.

Absence of hNIS in the normal tissues seen in the present study may be false negative on account of scanty epithelial tissue and large amount of fat present in the normal breast tissue. It may be imprudent to surgically excise a large amount of normal tissue and, hence, normal breast tissues were only taken from the specimens where the surgeon on his own discretion had done a wide excision, or from the axillary breast excision.

The observation of active iodine transport in a given tissue constitutes the proof of both hNIS protein expression and its proper targeting to the plasma membrane in that tissue. The presence of hNIS properly targeted to the plasma membrane is required for active iodide transport. In the present study, a total of 9 out of 20 samples showed intracellular staining. Demonstration of hNIS in a given tissue solely by immunohistochemistry does not necessarily mean that hNIS is fully functional in that tissue or at least in part, because the expressed protein may remain in intracellular membrane compartment and is not targeted to the plasma membrane.

Only a few studies till now have been carried out on fibroadenoma breast (a common benign condition). In one study carried out to demonstrate hNIS expression by RT-PCR, NIS was demonstrated in the mRNA isolates from 2/2 fibroadenoma tissues and 6/7 breast carcinoma tissues. In addition, the mean total tissue iodine in 23 benign tumors (fibroadenoma) was significantly higher than those in 19 breast cancers or normal tissue.

In another study, using immunohistochemical methods, 1278 samples were probed with anti hNIS antibodies which included 253 thyroid and 169 breast conventional whole tissue sections (CWTS). Four high density tissue microarrays containing a wide variety of breast lesions, normal tissues and carcinoma cores were tested. The results of the normal tissue arrays were corroborated in 50 CWTS. This study examined a total of 10 fibroadenoma specimens by immunohistochemistry. Out of these 2 were negative, 3 weakly positive, and 5 strongly positive showing a total 80% positivity. Simultaneously, 40 normal breast tissues were also examined out of which 28 were negative, 8 weakly positive, and 4 strongly positive i.e. a total 30% positivity was observed. All normal tissues were taken from the vicinity of the carcinomas (35 invasive and 5 DCIS). Therefore, according to the authors, these may correspond to tissues that have undergone biochemical changes that are not morphologically apparent. In another study, it was found that 80% of the human breast carcinoma samples expressed hNIS whereas none of the normal samples did the same and, hence, they hypothesized the possibility of hNIS as a valuable diagnostic and prognostic marker in cancer of the breast.

In conclusion, intense staining of hNIS in fibroadenoma tissue in comparison to controls has been demonstrated which may signify an increased iodine metabolism in this pathological breast condition that need to be further characterized.

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