Relationship of sodium/iodide symporter expression with I\textsuperscript{131} whole body scan uptake between primary and metastatic lymph node papillary thyroid carcinomas

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ABSTRACT. The aim of the present study was to evaluate total and membranous Na\textsuperscript{+}/I\textsuperscript{-} symporter (NIS) expressions in papillary thyroid carcinoma (PTC) tissue, correlation of NIS expression between primary and metastatic lymph node (LN) PTC tissues, and relationship of NIS expression with I\textsuperscript{131} whole body scan (WBS) uptake between primary and metastatic LN PTC tissues by analyzing 17 pairs of primary and metastatic LN PTC tissues. Staining positivity was calculated, and staining intensity was graded as negative (0), weak (1+), moderate (2+) and strong (3+). In primary PTC tissues, positivities and intensities of normal cells were higher than those of carcinoma cells but had no correlation with those in matched metastatic LN PTC tissues. In classic type, positivities, intensities and membranous intensities (mIS) were correlated between primary and matched metastatic LN PTC tissues. In patients aged younger than 45 yr, positivities and intensities in primary PTC tissues had correlation with those in matched metastatic LN PTC tissues. Positivities, intensities, mIS and pathological subtype of carcinoma cells in primary PTC tissues were not correlated with age, tumor size, TNM stage, MACIS score and thyroglobulin (Tg) levels at the time of I\textsuperscript{131} WBS. Sensitivity, specificity, as well as positive and negative predicted values of mIS in patients with I\textsuperscript{131} WBS uptake were 69.2, 75, 90 and 42.9% in primary PTC tissues, and 92.3, 100, 100 and 80% in metastatic LN PTC tissues. The results of mIS taken either as positive or negative were correlated with those of I\textsuperscript{131} WBS after controlling for age. Our results demonstrate that PTC tissues have altered total and membranous NIS expressions, suggesting that NIS expression in primary PTC tissues may predict NIS expression and I\textsuperscript{131} WBS uptake in matched metastatic LN PTC tissues.

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INTRODUCTION

In thyroid gland, iodide is trapped and transported against an electrochemical gradient across basolateral plasma membrane of thyrocyte by Na\textsuperscript{+}/I\textsuperscript{-} symporter (NIS), a membrane glycoprotein with 13 putative transmembrane domains which acts through Na\textsuperscript{+} ion gradient generated by Na\textsuperscript{+}-K\textsuperscript{+} ATPase (1-4). Because iodide uptake is usually decreased in thyroid cancer relative to surrounding normal thyroid tissue (5-7), total thyroidectomy with remnant thyroid gland ablation should be performed before radioactive iodine (RAI) therapy in treatment of metastatic thyroid cancer (8). These findings suggest that NIS expression may be lower in thyroid cancer than normal thyroid tissue, although molecular mechanisms related to decrease of iodide concentrating ability in thyroid cancer are not completely understood. However, there are divergent data in the literature regarding NIS expression in thyroid cancer. While several studies reported loss or decrease of NIS mRNA and protein levels in thyroid cancer (3, 9-14), Saito et al. (15) showed the opposite results, that NIS mRNA and protein levels in thyroid cancer tissue were more increased than those in normal thyroid tissue by using immunoblotting and immunohistochemistry (IHC). Moreover, Doohan et al. (16) demonstrated predominant intracellular overexpression of NIS protein in thyroid cancer by using IHC, due to advantages of this technique over immunoblotting. Considering that NIS is functional only at plasma

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membrane, it is also possible that membranous NIS expression would be decreased in thyroid cancer despite increased total NIS expression in thyroid cancer relative to normal thyroid tissue. Regarding NIS expression, it has been investigated whether NIS expression in primary thyroid cancer may predict NIS expression and thereby RAI uptake in matched metastatic lesion because of its clinical significance in treatment of metastatic thyroid cancer (7, 10-12, 17-21). Castro et al. (19) reported that NIS staining of primary thyroid cancer predicted RAI uptake in metastatic lesion, whereas Park et al. (17) showed that NIS mRNA levels were not correlated between primary and matched metastatic lymph node (LN) papillary thyroid carcinoma (PTC) tissues. However, the relationship of membranous NIS expression with RAI uptake between primary and metastatic LN PTC tissues has not been studied as yet. Functional studies about transcription, translation, targeting and distribution to subcellular structure may possess discrepant results according to methodology. For example, the results by Northern blotting may be different from those by Western blotting or IHC, because mRNA levels may not always reflect protein levels. IHC can be performed on archival tissue, and requires only a small amount of sample tissue, allowing for analysis of cancer tissues and surrounding normal tissues from same specimen; it reflects protein expression levels, and above all provides information on subcellular localization (16). The present study was designed to investigate total and membranous NIS expressions in PTC tissue, correlation of NIS expression between primary and metastatic LN PTC tissues, and relationship of NIS expression with RAI uptake between primary and metastatic LN PTC tissues by analyzing 17 pairs of primary and metastatic LN PTC tissues.

MATERIALS AND METHODS

Preparation of thyroid tissue samples

Study protocol was designed according to the Declaration of Helsinki and approved by the Ethical Committee of the ChunCheon Sacred Heart Hospital, Hallym University. PTC tissues were obtained during thyroid surgery from ChunCheon Sacred Heart Hospital between January 2003 and March 2005, and fixed in formaldehyde and embedded in paraffin. PTC tissues were classified into classic and follicular variant types according to the World Health Organization recommendations (22). TNM stage was graded by tumor size, regional LN metastasis and distant metastasis (23), and MACIS score was calculated in accordance with presence of metastasis, age, completeness of resection, invasiveness and tumor size (24). Metastatic LN PTC tissues were obtained by surgical excision after scanning $^{131}$ whole body image and measurement of serum thyroglobulin (Tg) levels. At the time of surgical excision, all patients had no history of RAI therapy.

$I^{131}$ whole body scan (WBS)

All patients discontinued thyroid hormone replacement for 6 weeks, and whole body image including head and neck area was obtained after 48 h of $^{131}$ 3 mCi administration. We confirmed serum TSH levels >30 mIU/l just before $^{131}$ WBS.

Measurement of total $T_{4}$, TSH, Tg and anti-Tg antibody levels

Total $T_{4}$ and TSH levels were measured at the times of initial surgery and $^{131}$ WBS, respectively. Tg and anti-Tg antibody levels were measured at the time of $^{131}$ WBS. Total $T_{4}$ was measured with AxSYM Total $T_{4}$ Kit (Abbott, USA) by fluorescence polarization immunoassay, TSH using AxSYM TSH Kit (Abbott, USA) by multiparticle enzyme immunoassay, Tg using Tg-S IRMA CT Kit (Radim, Italy) by IRMA and anti-Tg antibody using Serodia-ATG Kit (Fujirebio, Japan) by microtiter particle agglutination test. The normal ranges are as follows: total $T_{4}$, 4.5–12.0 ug/dl; TSH, 0.4–4.7 mIU/l; Tg, 0–30 ng/ml; anti-Tg antibody, 1:27.

Immunostaining (IS) for NIS protein

Paraffin-embedded sections (5 μm) of thyroid and LN tissues were used for the detection of NIS protein. Anti-NIS monoclonal antibody (Cat. No. MS-1653-P0, Labvision, Fremont, CA, USA) was applied, and IS was performed modifying the method described by Gerard et al. (25). In brief, slides were deparaffinized, rehydrated and pre-treated in TRIS-citrate buffer (pH 9.5). Slides were washed with phosphate buffered saline (PBS) supplemented with 1% bovine serum albumin (BSA) and incubated in PBS-BSA containing normal goat serum (1:50 dilution) for 60 min at room temperature. Primary antibody was applied and incubated for 48 h at room temperature. After two washes in PBS-BSA, binding of antibody was detected using secondary antibody conjugated to peroxidase-labeled polymer (DAKO, Glostrup, Denmark) for 60 min at room temperature. After two washes in PBS-BSA, peroxidase activity was revealed with 3-amino-9-ethylcarbazole substrate (DAKO, Glostrup, Denmark). Slides were counterstained with Mayer’s hematoxylin, rinsed and mounted in Paramount (DAKO, Glostrup, Denmark). Negative control, absence or replacement of primary antibody were tested, and IS before and after inhibition of endogenous peroxidase activity with $H_{2}O_{2}$ was performed.

Evaluation of IS

In samples with NIS IS, the number of positive cells was counted in 10 microscopic fields at a magnification of x250, and then positivity percentage of positive cells was calculated. Intensity was graded as negative (0), weak (1+), moderate (2+) and strong (3+). All slides were independently reviewed by two expert pathologists (Choi K-C, Park Y-E) who were blinded as to the clinical data.

Statistical analysis

Between normal and carcinoma cells in primary PTC tissues, staining positivities were compared using paired t-test, and staining intensities were compared using Wilcoxon signed ranks test. Between carcinoma cells of primary and matched metastatic LN PTC tissues, correlation of staining positivities was analyzed by Pearson’s analysis, and correlation of staining intensities was analyzed by Spearman rank order analysis, and partial correlation analysis after controlling for age was performed. Correlations of staining positivities and intensities with clinical variables, respec-