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Immunohistochemical Expressions of Sodium/Iodide Symporter (NIS) and Thyroid Transcription Factor-1 (TTF-1) and Their Relationship in Primary Pulmonary Adenocarcinoma

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Sodium iodide symporter (NIS) plays a key role in thyroid hormone production by efficiently accumulating iodide from the circulating blood into the thyocytes, and this is done against an electrochemical gradient. Thyroid transcription factor-1 (TTF-1) is a homeodomain-containing protein expressed in embryonic diencephalons, thyroid, and lung and has been found to bind to thyroid specific promoters and to activate their transcriptional activity. TTF-1 may be one of the factors capable of activating NIS gene expression in the thyroid gland, thus it accounts for the lower levels of NIS gene expression that are seen in the extrathyroidal tissues. However, a high frequency of TTF-1 expression has been observed, especially in primary lung adenocarcinoma. The present study was undertaken in order to elucidate the relationship between the expression of NIS and TTF-1 in primary lung adenocarcinoma. Immunohistochemical studies for NIS and TTF-1 were performed in 64 primary lung adenocarcinomas. Immunoreactivities for NIS and TTF-1 were found in 49 (76.6%) and 45 (70.3%) out of 64 cases, respectively. Forty-one (83.7%) of the 49 cases with positive NIS immunoreactivity showed positive TTF-1 expression, whereas 11 (73.3%) of the 15 cases with negative NIS immunoreactivity showed negative TTF-1 expression ($P<0.05$). So the NIS expression was significantly associated with the TTF-1 expression. These findings suggest that TTF-1 may be one of the factors capable of activating NIS gene expression in primary lung adenocarcinoma. Further studies are needed to define the relation between NIS and TTF-1 for examining the mechanisms of tissue-specific NIS expression.

Key Words: Lung adenocarcinoma, Sodium iodide symporter, Thyroid transcription factor-1

INTRODUCTION

The sodium iodide symporter (NIS) is a transmembrane glycoprotein most commonly studied in connection with the thyroid gland that functions by co-transports two sodium ions along with one iodide ion into cells (Dadachova and Carrasco, 2004). NIS plays a key role in thyroid hormone production by efficiently accumulating iodide from the circulation into the thyrocytes against an electrochemical gradient (Zhang et al., 2003). As thyroidal NIS has been cloned, NIS mRNA and protein expressions have been reported in a variety of extrathyroidal tissues, including salivary and lacrimal glands, gastric mucosa, kidney, and mammary gland, suggesting that iodide transport in these tissues is mediated by the expression of functional NIS protein (Spitzweg et al., 1998; Tazebay et al., 2000; Spitzweg et al., 2001). The ability of thyroid cancers to concentrate radioiodine is dependent, at least in part, upon the expression and functional integrity of the NIS. Compared with the thyroid gland, extrathyroidal tissues have a much lower ability to transport and concentrate iodide. Because identical NIS proteins are encoded in thyroid and extrathyroidal tissues, the diminished iodide transport seen in the extrathyroidal

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tissues may result from altered NIS gene transcriptional activity (Spitzweg et al., 1998).

Thyroid transcription factor-1 (TTF-1) is a 38-kDa homeodomain-containing, DNA-binding protein, which is expressed in the thyroid, lungs, and some restricted areas of diencephalon. TTF-1 plays a decisive role in the determination and maintenance of cellular phenotype by activating thyroperoxidase and thyroglobulin gene transcription (Guazzi et al., 1990). Spitzweg et al. (1998) have suggested that TTF-1 may be one of the factors capable of activating NIS gene expression in the thyroid gland, its absence thus accounting for the lower levels of NIS gene expression seen in extrathyroidal tissues. Endo et al. (1997) have reported that TTF-1 activated the rat NIS promoter by a direct interaction with its proximal enhancer region in the rat NIS gene. In lung cancer, a high frequency of TTF-1 expression has been observed in adenocarcinoma (75~80%), whereas squamous cell carcinomas and large cell carcinomas showed no, or low, TTF-1 expression (Zamecnik and Kodet et al., 2002). Recently, we have reported that NIS was immunohistochemically detected in 54.0% of human primary lung cancers and NIS expression was significantly higher in tissues of lung adenocarcinoma than in the other histologic types (Lee et al., 2006). These findings suggest that TTF-1 may be one of the factors capable of activating NIS gene expression in lung adenocarcinomas, and that its absence accounts for the lower levels of NIS expression in the other histologic types of lung cancer.

To the best of our knowledge, the relationship between NIS and TTF-1 expression in tissues of primary lung adenocarcinoma has not yet been studied in Korea. Herein we investigated the expression patterns of NIS and TTF-1 using immunohistochemistry to elucidate the tissue-specific relationship between NIS and TTF-1 expressions in primary lung adenocarcinoma.

MATERIALS AND METHODS

1. Materials

We retrospectively examined 64 cases of primary lung adenocarcinoma from the surgical pathology archival of the Department of Pathology at Dong-A University Medical Center. The records were complied 2000 to 2004. No preoperative chemotherapy or radiotherapy had been administered in any of theses cases. Standard lobectomy or pneumonectomy and lymph node dissections were performed in every case. Tissues, clinical records and pathological reports were obtained for all 64 cases. This study was approved by the institutional review board, and written informed consent was obtained from all of the subjects at the time of surgery. The hematoxylin and eosin-stained slides were reviewed in each case to confirm the original diagnosis, based on the World Health Organization criteria (Travis et al., 2004).

2. Immunohistochemical evaluation

Immunohistochemical studies for NIS and TTF-1 were performed on formalin-fixed, paraffin-embedded, 4 μm-thick tissue sections, using the avidin-biotin-peroxidase complex method. The primary antibodies were mouse monoclonal antibodies directed against NIS (Neomarkers, Fremont, CA, USA) used in a 1:50 dilution and TTF-1 (Neomarkers, Fremont, CA, USA) at a 1:50 dilution. Deparaffinization of all the sections was performed through a series of xylene baths, and rehydration was performed with a series of graded alcohol solutions. To enhance immunoreactivity, microwave antigen retrieval was performed at 750 W for 30 min in citrate buffer (pH 6.0). After blocking endogenous peroxidase activity with 5% hydrogen peroxidase for 10 min, primary antibodies incubation were performed for 1 hours at room temperature. Cap-Plus™ Biotinylated secondary antibody (Zymed, USA) was applied for 30 min at room temperature, and then Cap-Plus™ Streptavidin-HRP (Zymed, USA) was applied for 30 min at room temperature. After washing the tissue samples in Tris-buffered saline for 10 min, 3, 3'-diaminobenzidine was used as a chromogen, and Gill's hematoxylin counterstain was applied. Tissue from a Graves' disease patient was used as a positive control for NIS and normal thyroid tissue was used as positive control for TTF-1.

3. Interpretation of immunohistochemical staining

Immunoreactivity for NIS expression was defined by presence of granular cytoplasmic or membranous staining and that for TTF-1 expression was defined by presence of nuclear staining. The NIS and TTF-1 staining intensities were classified as negative, weak, moderate, or strong. A tumor was defined as NIS or TTF-1 positive if more than 10% of the cells showed moderate or strong staining. A negative staining result was defined as weak or absent staining in any number of cells, or moderate/strong staining in

Table 1. Relationship between the pathological characteristics and the sodium iodide symporter and thyroid transcription factor-1 expressions in 64 primary lung adenocarcinomas

Pathological characteristics	No. of cases	NIS[a] expression		TTF-1[b] expression		*P* value
		Negative (%)	Positive (%)	Negative (%)	Positive (%)	
Tumor size						NS*
≤3 cm	31	8 (25.8)	23 (74.2)	11 (35.5)	20 (64.5)	
> 3 cm	33	7 (21.2)	26 (78.8)	8 (24.2)	25 (75.8)	
Lymph node metastasis						NS*
Negative	28	6 (21.4)	22 (78.6)	8 (28.6)	20 (71.4)	
Positive	36	9 (25.0)	27 (75.0)	11 (30.6)	25 (69.4)	
Lymphovascular invasion						NS*
Negative	50	10 (20.0)	40 (80.0)	15 (30.0)	35 (70.0)	
Positive	14	5 (35.7)	9 (64.3)	4 (28.6)	10 (71.4)	

[a] NIS, sodium iodide symporter, [b] TTF-1, thyroid transcription factor-1, * NS: not significant

less than 10% of the cells.

4. Statistical analysis

Statistical analysis was performed with the Statistical Package Service Solution software (SPSS for Windows, Standard version 12.0, Chicago, USA). Fisher exact test was performed to assess the relationship between the expression patterns of NIS and TTF-1. A *P* value less than 0.05 was considered to be statistically significant.

Table 2. Relationship between sodium iodide symporter and thyroid transcription factor-1 expressions in 64 primary lung adenocarcinomas

TTF-1[b] expression	NIS[a] expression		*P* value
	Negative (%) n=15	Positive (%) n=49	
Negative (n=19)	11 (73.3)	8 (16.3)	<0.05
Positive (n=45)	4 (26.7)	41 (83.7)	

[a] NIS, sodium iodide symporter
[b] TTF-1, thyroid transcription factor-1

RESULTS

1. Clinicopathological characteristics

The ages of the 64 patients ranged from 28 to 74 years (median age: 59 years), and there were 35 men and 29 women. Tumor sizes ranged from 1 cm to 9.5 cm (median size: 3.7 cm). Histologically, they were all adenocarcinomas, showing a mixture of the various histological subtypes, including acinar, papillary, solid and bronchioloalveolar carcinomas. Lymph node metastases were present in 36 of the 64 cases, and lymphovascular tumor invasion was seen in 14 of the 64 cases.

2. NIS and TTF-1 expressions and their relationship with the clinicopathological characteristics

Immunoreactivities for NIS and TTF-1 were found in 49 (76.6%) and 45 (70.3%) out of the 64 cases, respectively. There was no NIS expression in normal alveolar tissue or bronchial mucous glands, and only weak and focal immunoreactivity was seen in the apical portion of the ciliated columnar cells in normal bronchial mucosa. TTF-1 immunoreactivity was limited in alveolar and bronchiolar epithelial cells and interstitial cells, lymphocytes and alveolar macrophages did not stain for TTF-1 in non-neoplastic lung. There were no significant associations between NIS or TTF-1 expression and clinicopathological characteristics, including age, sex, tumor size, lymphovascular invasion or lymph node metastasis (Table 1).

3. Relationship between the expressions of NIS and TTF-1

Forty-one (83.7%) of the 49 cases with positive NIS immunoreactivity showed positive TTF-1 expression (Fig. 1), whereas 11 (73.3%) of the 15 cases with negative NIS immunoreactivity showed negative TTF-1 expression (Fig 2) (Table 2) ($P<0.05$). So the NIS expression was significantly associated with the TTF-1 expression.

DISCUSSION

The NIS is an intrinsic plasma membrane protein that mediates the active transport of iodide in the thyroid gland

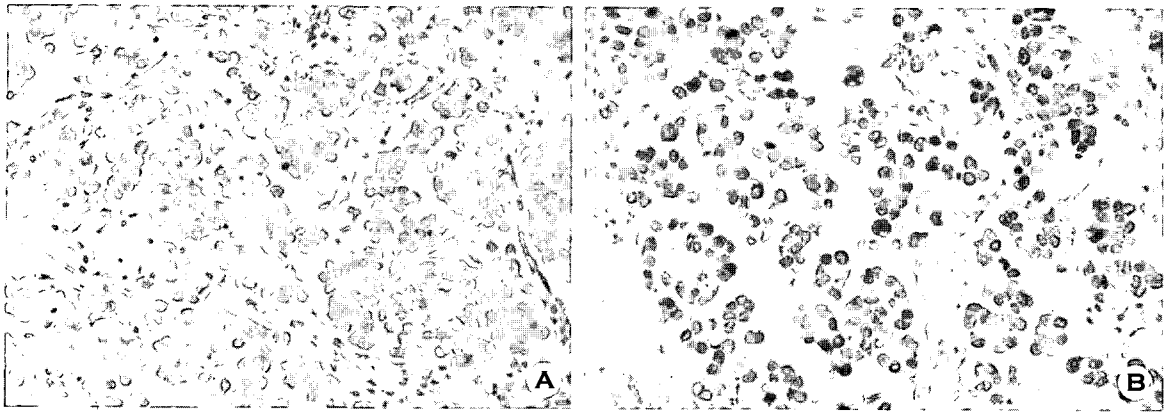

Fig. 1. An adenocarcinoma shows positive cytoplasmic reactions for sodium iodide symporter **(A)** and positive nuclear reactions for thyroid transcription factor-1 **(B)**.

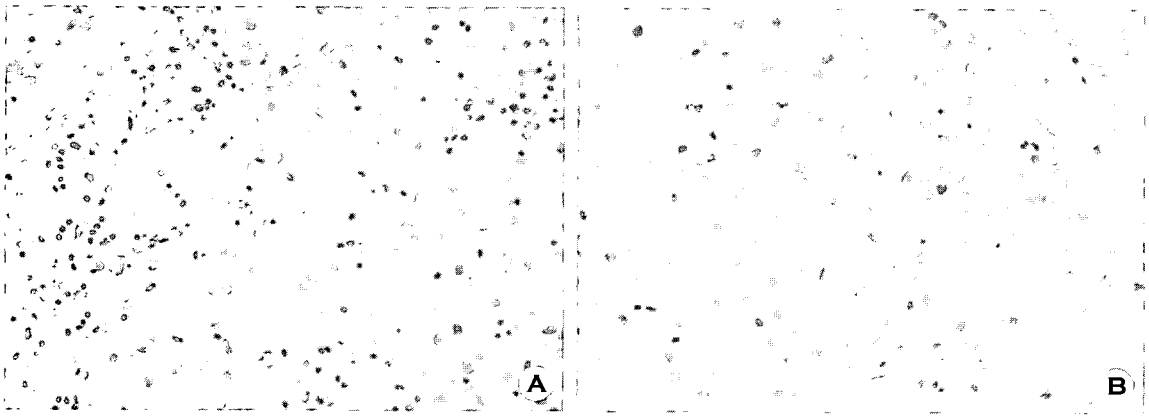

Fig. 2. The other adenocarcinoma shows negative immunoreactivities for sodium iodide symporter **(A)** and thyroid transcription factor-1 **(B)**.

and plays a key role thyroid hormone production. The iodide-concentrating activity of the thyroid gland allows for the use of radioiodide in thyroid scintigraphy for the diagnosis of thyroid nodules, as well as for ablation of post-surgical remnants and treatment of recurrent or metastatic thyroid cancer. The expression of NIS in normal and cancerous tissues is of interest because of the potential of NIS to serve as a specific conduit for the targeted therapeutic destruction of NIS expressing malignant cells with radioiodide (Ajjan et al., 1998). It was generally thought until recently that the lack of I^- oragnification in the extrathyroidal tissues that functionally express NIS would preclude the use of radioiodide to treat cancer in these tissues. For this reason, many strategies have been used and are currently in progress in order to re-establish iodide uptake function by means of re-expressing the NIS in tumor cells. Experimentally, NIS has been either transfected and/or transferred with an adenoviral or retroviral vector to test the concept of targeted ablative therapy (Mandell et al., 1999; Spitzweg et al., 2000; Spitzweg et al., 2001). Transfection of the NIS gene into a variety of malignancies, including melanoma, colon carcinoma, ovarian adenoma, and lung and prostate cancers, confers radioiodide uptake (Mandell et al., 1999). Novel evidence in prostate cancer cells expressing exogenous NIS after adenoviral gene transfer has convincingly proved that prolonged retention time and therapeutic efficacy of ^{131}I are achievable in NIS expressing extrathyroidal cells even in the absence of I^- organification (Spitzweg et al., 2000).

Compared with the thyroid gland, iodide transport and concentrating activity is much lower in extrathyroidal tissues. Because identical NIS proteins are encoded in thyroid tissue and extrathyroidal tissues, diminished iodide transport in extrathyroidal tissues may result from altered NIS gene

transcriptional activity, or from altered NIS mRNA or protein turnover. Variable and lower NIS transcriptional activity in extrathyroidal tissues may be accounted for, in part, by thyroid-specific transcription factors that act on the NIS promoter to control NIS gene expression in the thyroid gland (Spitzweg et al., 1998). TTF-1, a homeodomain-containing protein, has been found to bind to all three thyroid-specific promoters and to activate their transcriptional activity (Saiardi et al., 1995). In lung cancers, several reports described the differences in TTF-1 expression between histological types. Yatabe et al. (2002) have reported that TTF-1 was expressed in 62.5~90% of adenocarcinomas and 88~100% of small cell carcinomas, whereas it was not found or found at a very low frequency in squamous carcinoma (0~27%) and large cell carcinomas (0~25%). Recently, we have reported that NIS was immunohistochemically detected in human primary lung cancer (Lee et al., 2006). In our study, NIS expression was significantly higher in tissues of lung adenocarcinoma than in the other histologic types. In this study, forty-one (83.7%) of the 49 cases with positive NIS immunoreactivity showed positive TTF-1 expression, whereas 11 (73.3%) of the 15 cases with negative NIS immunoreactivity showed negative TTF-1 expression, which suggests that the NIS expression was significantly associated with the TTF-1 expression. Recently, cloning and functional analysis of rat NIS gene has identified a binding sequence for TTF-1 within the rat NIS promoter region (Endo et al., 1997). These findings suggest that TTF-1 may be one of the factors capable of activating NIS gene expression in the lung, thus accounting for especially higher level of NIS expression in tissues of adenocarcinoma of the lung showing a high frequency of TTF-1 expression.

However, the regulatory mechanisms defining a relationship between NIS and TTF-1 has not yet been well defined. Endo et al. (1997) have reported a TTF-1 binding site between -245 and -230 bp in the NIS 5' flanking region conferring thyroid-specific transcription of NIS. Furuya et al. (2004) have reported that adenovirus vector containing TTF-1-infected cancer cells that stably expressed NIS increased their radioiodide uptake and organification in vitro and in vivo. The other report showed that the TTF-1 binding site was in the human NIS upstream enhancer region, but TTF-1 did not act as a trans-activating factor on the human NIS upstream element (Taki et al., 2002).

In conclusion, we have shown that TTF-1 may be one of the factors capable of activating NIS gene expression in primary lung adenocarcinoma. Further studies are needed to define the relationship between NIS and TTF-1 for examining the regulatory mechanisms of tissues-specific NIS expression, so to propose the use of radioiodide as a valuable tool in diagnosis and treatment of adenocarcinoma of the lung.

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