

Expressions of Id-1 and Id-2 in Hyperplastic Thyroid Tissue and Thyroid Carcinoma

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Received : December 5, 2005

Accepted : January 24, 2006

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* This work was supported by 2004 research grant from Seoul National University Boramae Hospital (03-2004-1).

Background : Id proteins are a family of helix-loop-helix proteins and are regarded to be negative regulators of cell differentiation. In general, Id-1 and Id-2 expressions are upregulated during tumor development and progression in a variety of neoplasms, and these expressions may be associated with aggressive tumor behavior. However, little is known about the roles of Id-1 and Id-2 in thyroid neoplasms. **Methods :** The expressions of Id-1 and Id-2 were assessed immunohistochemically in 310 normal, hyperplastic, and neoplastic thyroid tissues using tissue microarrays. **Results :** Normal thyroid tissues rarely expressed Id-1 or Id-2. Moreover, whilst Id-1 expression was more elevated in malignant thyroid tissue than in hyperplastic thyroid tissue, Id-2 expression was more variable. No significant differences were observed between histologic subtypes of thyroid carcinomas with respect to Id-1 or Id-2 expression. Follicular adenomas showed higher expressions of Id-1 and Id-2 than thyroid carcinomas. No significant association was found between clinicopathological parameters and Id-1 expression, though Id-2 expression was significantly reduced in metastatic, stage IV tumors. **Conclusion :** The expressions of Id-1 and Id-2 were elevated in hyperplastic and neoplastic thyroid tissues. However, neither appears suitable as a marker of malignancy or an aggressive phenotype, although Id-2 expression in advanced thyroid carcinomas may reflect a favorable prognosis.

Key Words : Inhibitor of differentiation protein 1; Inhibitor of differentiation protein 2; Thyroid gland; Carcinoma, papillary; Adenocarcinoma, follicular; Adenoma

Thyroid carcinoma represents 1% of all cancers and is the most common type of endocrine cancer.^{1,2} Nonmedullary thyroid carcinoma, which arises within the follicle cells of the thyroid, can be classified into the following three types; papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), and anaplastic or undifferentiated thyroid carcinoma (ATC). Recent advances in imaging modalities have increased detection rates for microcarcinoma, which has been credited with up to 20% of recurrences; moreover, several cases of distant metastases with a fatal outcome have also been described.³ Therefore, it is important to differentiate highly invasive rapidly growing thyroid carcinoma with a propensity for metastasis from minimally invasive disease.

Id (inhibitor of DNA binding) proteins are a family of four helix-loop-helix (HLH) proteins (Id-1, Id-2, Id3, and Id4) that lack a basic DNA binding domain.⁴ They act as dominant inhibitors of basic HLH transcription factors by heterodimerization,

and thus inhibit gene expression.⁵ Moreover, Id gene expression is down-regulated on differentiation in many cell types in vitro and in vivo, and although Id proteins are viewed as negative regulators of cell differentiation, they participate in cell cycle progression, and tumor biology.^{4,6} In general, the expressions of Id-1 and Id-2 are up-regulated during tumor development and progression in a variety of benign and malignant neoplasms,^{4,7} and some studies have revealed that the overexpression of Id-1 in malignant tumors is associated with an aggressive behavior, a poor prognosis, and increased tumor angiogenesis.^{6,8-12} To date, only data regarding Id-1 expression in thyroid carcinoma is available,^{13,14} and these investigations were performed on relatively small numbers of cases. Moreover, nothing is known about Id-2 expression in thyroid carcinoma.

The aim of this study was to investigate the expressions of Id-1 and Id-2 proteins in hyperplastic and neoplastic thyroid tissues, and their associations with aggressive tumor behavior.

MATERIALS AND METHODS

Patients and Tissues

Three hundreds and ten tissue samples were examined during this study, and were obtained from the surgical pathology files of the Department of Pathology, Seoul National University Boramae Hospital and the Department of Pathology, Seoul National University Hospital from January 1993 to December 2003. Clinical information was obtained from patients' medical records. These tissue samples comprised, samples from patients with a normal thyroid (n=10), multinodular goiter (n=57), Graves' disease (n=17), follicular adenoma (FA, n=57), FTC (n=58), PTC (n=94), ATC (n=17). Hematoxylin and eosin stained slides were reviewed and one appropriate paraffin block was selected for each case.

Construction of the tissue microarrays

Core tissue biopsies (2 mm in diameter) were taken from individual paraffin embedded thyroid tissues (donor blocks) and arranged in new recipient paraffin blocks (tissue array blocks) using a trephine apparatus (Super Biochips Laboratories, Seoul). Each tissue array block contained up to 60 cores, and total 6 tissue

Table 1. Histological scoring of Id-1 and Id-2 protein expression in thyroid tissue by immunohistochemistry

	Id-1	Id-2
Normal (n=10)	0.50±0.93	2.78±0.67
Multinodular goiter (n=57)	2.45±1.59	4.29±0.94
Graves' disease (n=17)	3.88±0.86	3.56±1.37
Follicular adenoma (n=57)	4.73±1.14	5.57±0.80
Follicular carcinoma (n=58)	2.95±1.65	3.66±1.00
Papillary carcinoma (n=94)	3.53±1.92	4.67±1.24
Anaplastic thyroid carcinoma (n=17)	3.88±1.41	3.87±1.25

microarray blocks were made. Four micrometer-thick sections were cut from the completed array blocks and transferred to silanized glass slides.

Immunohistochemical staining

Immunohistochemistry for Id-1 and Id-2 (rabbit polyclonal antibodies, 1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was performed on formalin-fixed, paraffin embedded tissue sections using microwave-induced epitope retrieval and the Dako EnVision+ detection system (Dakocytomation, Carpinteria, CA, USA), according to the manufactures' instructions.

Cytoplasmic expressions of Id-1 and Id-2 were semiquantitatively analyzed as described previously.¹⁰ Percentages of positive cancer cells were classified as; 0, negative; 1, <33%; 2, 33 to 67%; 3, ≥67% of cancer cells exhibiting immunoreactivity. Immunohistochemical signal intensities were also stratified into four groups: 0, no immunoreactivity; 1, weak; 2, moderate; 3, strong. Finally, cell and intensity scores were summed. Statistical analysis was performed using the Statistical Package for the Social Sciences Ver. 10.1 for Windows (SPSS Inc., Chicago, IL). Data were compared using the non-parametric Kruskal-Wallis test for ANOVA, and the Mann-Whitney test was used to assess differences between individual data sets. p values of <0.05 were considered significant.

RESULTS

The expressions of Id-1 and Id-2 were variable in normal, hyperplastic, and neoplastic thyroid tissues. Immunohistochemical expression data for Id-1 and Id-2 in various thyroid tissues are summarized in Table 1, Fig. 1. Id-1 and Id-2 immunoreactivities were significantly lower in normal thyroid tissues than in

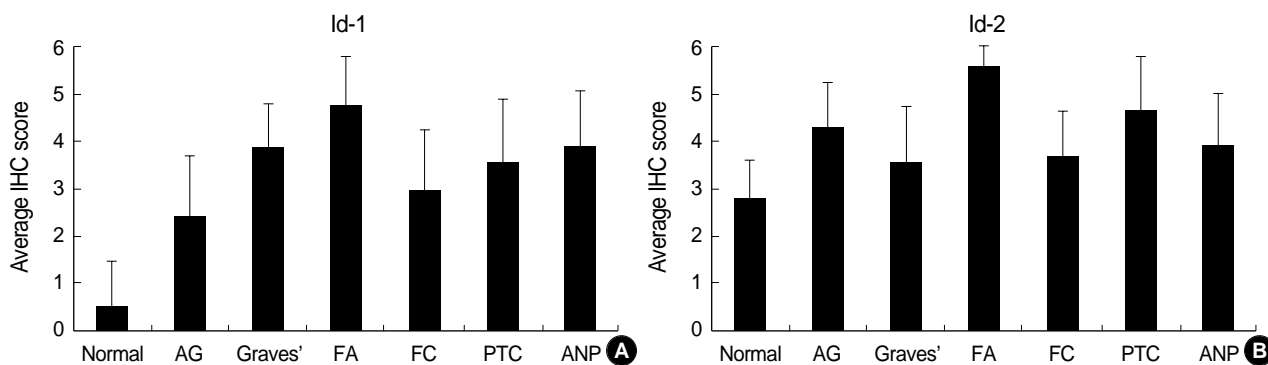


Fig. 1. Expression level of Id-1 (A) and Id-2 (B) in various thyroid tissues. Values are mean immunohistochemical scoring.

hyperplastic and neoplastic thyroid tissues ($p < 0.01$). In multinodular goiter and Graves' disease tissues, Id-1 was expressed in hyperplastic regions, whereas Id-2 expression in these cells was more variable and ranged from moderate to occasionally strong (Fig. 2).

Id-1 expression was significantly higher in PTC and ATC than in multinodular goiter tissues ($p < 0.01$). ATC showed slightly higher Id-1 expression than PTC, but this was not statistically significant. In contrast, Id-2 expression in ATC was significantly lower than in PTC ($p < 0.05$). FA showed higher Id-1 and Id-2 expressions than malignant thyroid carcinomas (PTC, FTC and ATC, Fig. 3), and this was statistically significant ($p < 0.01$). Due to the marked variability of Id-1 and Id-2 immunostaining in Graves' disease tissues, differences between Graves' disease and neoplastic thyroid tissues did not achieve statistical significance.

Clinicopathological parameters and Id-1 and Id-2 protein expression in thyroid carcinomas (FTC, PTC, and ATC) are summarized in Table 2. No significant association was observed between age, sex, tumor size, number of tumors, LN metastasis, and the expression levels of Id-1 or Id-2. However, Id-2 expres-

sion was significantly lower in stage IV than stage III ($p < 0.05$). Id-2 expression was also lower in metastatic carcinomas than nonmetastatic carcinomas ($p < 0.05$). But, Id-1 expression was not found to be correlated with stage or metastasis status.

DISCUSSION

Due to the ability of the inhibitor of DNA binding (Id) proteins to inhibit differentiation of certain lineages and their potential to stimulate, they are expected to have important roles in the cancer progress.¹⁵ Id-1 and Id-2 expressions have been reported to be up-regulated during tumor development and progression in the epidermis,^{16,17} colon,^{10,18} pancreas,^{19,20} prostate,¹² uterine cervix¹¹ and ovary,²¹ and levels of Id-1 and Id-2 expression have been correlated with poor differentiation and more aggressive clinical behavior.^{6,8-12} However, little data is available on Id-1 expression in thyroid cancer cells,^{13,14} and the data available were derived from a relatively small number of cases. Moreover, nothing is known about the expression and function of Id-2 in

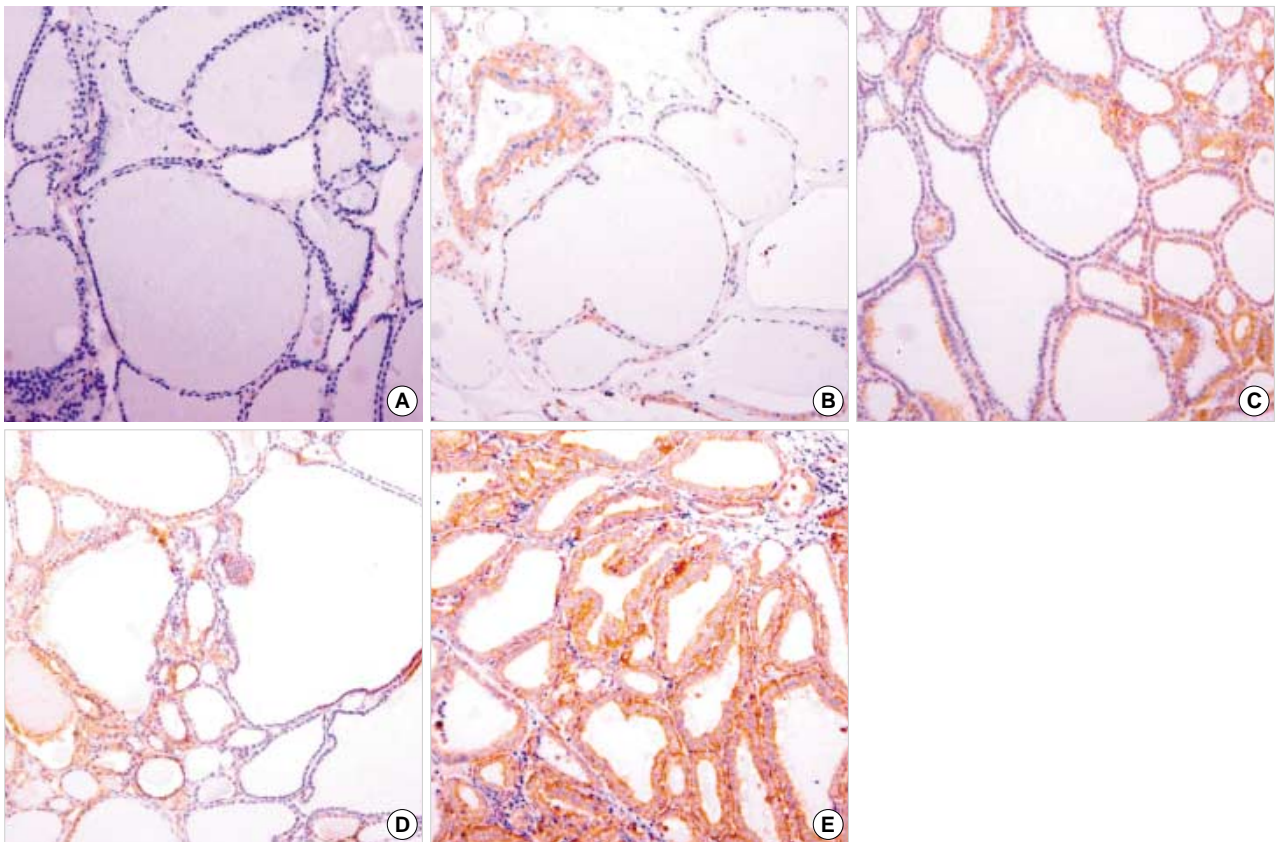


Fig. 2. Id-1 (A-C) and Id-2 (D-E) protein expression in normal and hyperplastic thyroid tissues. Id-1 is not expressed in normal thyroid glands (A). In multinodular goiter, Id-1 (B) and Id-2 (D) are expressed in hyperplastic foci of the tissues. In Graves' disease, Id-1 (C) and Id-2 (E) are more strongly expressed in most of the hyperplastic follicular cells.

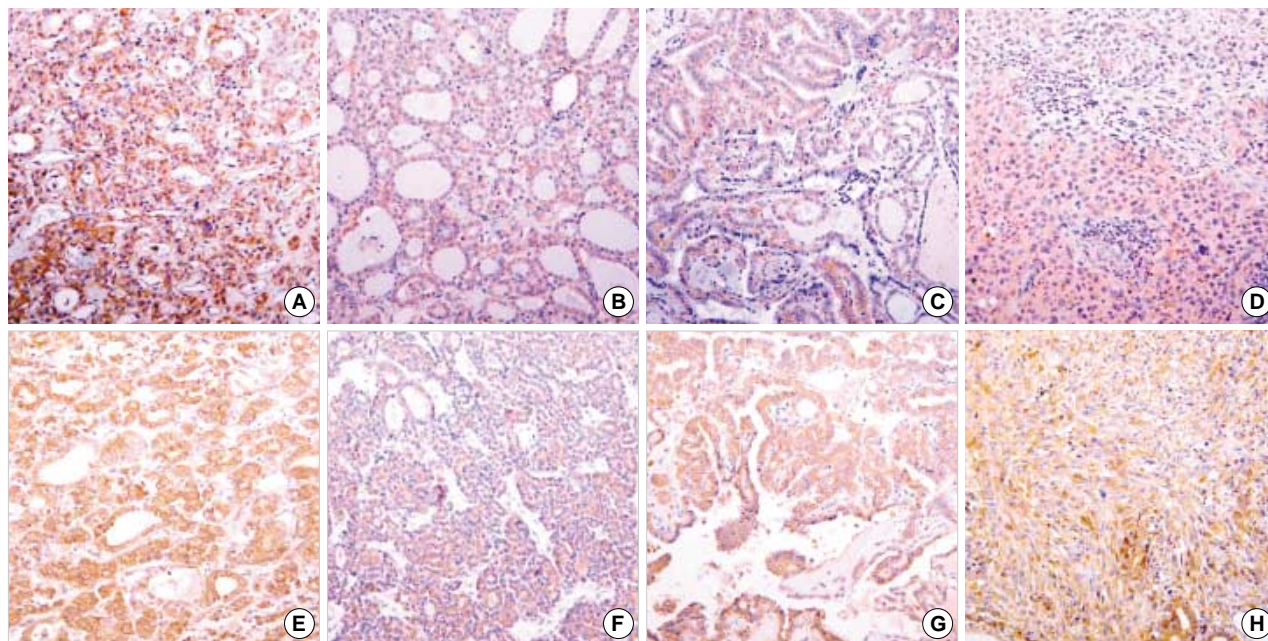


Fig. 3. Representative immunohistochemical staining of Id-1 (A-D) and Id-2 (E-H) in neoplastic thyroid tissues. In follicular adenoma, Id-1 (A) and Id-2 (E) are strongly expressed in tumor cells compared to follicular carcinomas (B, Id-1; C, Id-2). Id-1 and Id-2 are also expressed in papillary carcinomas (C and G) and anaplastic carcinomas (D and H).

Table 2. Level of Id-1 and Id-2 protein expression and clinicopathological parameters in patients with thyroid cancer

Clinicopathologic parameters ^a	Id-1 score	Id-2 score
Age		
<45 year (n=101)	3.05 ± 1.86	4.26 ± 1.32
≥ 45 year (n=63)	3.54 ± 1.76	4.24 ± 1.21
Sex		
Male (n= 29)	3.55 ± 1.92	4.52 ± 1.24
Female (n=136)	3.32 ± 1.78	4.17 ± 1.25
Tumor size		
<2 cm (n=38)	3.24 ± 1.82	4.24 ± 1.20
2-4 cm (n=81)	3.47 ± 1.90	4.23 ± 1.32
≥ 4 cm (n=37)	3.24 ± 1.57	4.37 ± 1.19
Number of tumor		
Single (n=119)	3.24 ± 1.77	4.16 ± 1.24
Multiple (n=44)	3.73 ± 1.82	4.49 ± 1.31
TNM stage		
I (n=50)	2.92 ± 1.99	4.15 ± 1.30
II (n=34)	3.47 ± 1.54	4.09 ± 1.19
III (n=52)	3.67 ± 1.75	4.63 ± 1.14
IV (n=29)	3.41 ± 1.82	3.81 ± 1.33 ^b
Lymph node metastasis ^b		
Positive (n=59)	3.71 ± 1.76	4.55 ± 1.27
Negative (n=33)	3.42 ± 1.79	4.36 ± 1.41
Distant metastasis		
Positive (n=35)	3.03 ± 1.81	3.67 ± 1.29
Negative (n=130)	3.45 ± 1.80	4.38 ± 1.21 ^c

^aNot equal to total number of thyroid carcinoma samples because not all primary tumors are available for clinical or immunohistochemical analysis; ^bStage IV showed significantly lower Id-2 expression relative to stage III (p<0.01); ^cp<0.01.

hyperplastic and neoplastic thyroid cells, or on the roles of Id-2 in thyroid cancer invasiveness.

In this study, we found that Id-1 and -2 are upregulated in hyperplastic and neoplastic thyroid cells, but that these expressions are not associated with an aggressive malignant thyroid carcinoma phenotype. Moreover, only Id-2 expression was significantly reduced in stage IV metastatic carcinomas than non-metastatic thyroid carcinomas. And, although increased Id-1 protein expression in some human cancers has been associated with an aggressive cancer phenotype, others found that high Id-2 protein expression is correlated with a favorable prognosis and reduced cellular invasiveness in breast cancer cells.^{22,23} These results concur with our data, as Id-2 appears to be more implicated in differentiation rather than in proliferation. However, more functional studies of Id proteins are required to determine the roles of Id-2 in the differentiation and invasiveness of thyroid cancer cells.

The thyroid gland is a well known and interesting model for the investigation of many signal transduction pathways, such as, the TSH-cyclic AMP pathways, the epidermal growth factor-protein tyrosine kinase receptor/ras/MAPK pathways, and the phorbol esters-protein kinase C pathways, and all of these pathways require the presence of insulin or insulin-like growth factor-1 or hepatocyte growth factor.^{24,25} Deleu *et al.* demonstrated that Id proteins are upregulated after TSH and insulin stim-

ulation in thyrocytes,²⁶ and previous data from Kebebew *et al.* also showed Id-1 gene up-regulation in hyperplastic and benign follicular adenoma, though this was not significant,¹³ which may have been due to a limited number of samples. In most cell types, the gene expressions of some Id isoforms are strongly induced by mitogen/growth factor stimulation.²⁷⁻³⁰ Therefore, increased expressions of Id-1 and Id-2 proteins in AG, Graves' disease, and FA suggest that Id protein expressions reflects deregulated growth, and that they could play roles during early stage tumorigenesis.

In conclusion, this study demonstrates that the expressions of Id-1 and Id-2 proteins are increased in hyperplastic and neoplastic thyroid tissue cells. Moreover, Id-1 and Id-2 expressions were higher in benign follicular adenoma than in malignant thyroid carcinoma, and thus, these expressions cannot be viewed as markers of malignancy or of an aggressive phenotype. However, metastatic carcinomas were found to show reduced Id-2 expression, which raises the possibility that Id-2 could be of use as a prognostic marker in malignant thyroid carcinomas.

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