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RAPID COMMUNICATION

c-Kit Proto-oncogene Is More Likely to Lose Expression in Differentiated Thyroid Carcinoma Than Three Thyroid-specific Genes: Thyroid Peroxidase, Thyroglobulin, and Thyroid Stimulating Hormone Receptor

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Abstract. Although c-*kit* proto-oncogene product is known to be weakly expressed on normal thyrocytes, its function is unclear. In order to investigate the significance of thyroid c-*kit*, c-*kit* gene expression in 37 various thyroid tissues was analyzed by comparing c-*kit* gene expression with the mRNA expression of three thyroid-specific genes: thyroid peroxidase, thyroglobulin, and thyroid stimulating hormone receptor. c-*kit* mRNA was hardly detected by the usual northern blot method in 2 of 7 follicular carcinomas, 11 of 12 papillary carcinomas, and a medullary carcinoma. On the other hand, a high level of c-*kit* mRNA expression was found in all 17 benign thyroid tissues (4 normal thyroid tissues, 4 Graves' disease, 2 adenomatous goiters, and 7 follicular adenomas). This study found that c-*kit* proto-oncogene is more likely to lose expression in differentiated thyroid carcinoma than any thyroid-specific gene. Decreased c-*kit* gene expression may serve as an indicator for the de-differentiation of thyrocytes.

Key words: c-*kit*, Differentiated thyroid carcinoma, Papillary carcinoma, Follicular carcinoma, Thyroid differentiation

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C-*KIT* PROTO-oncogene is a member of receptor tyrosine kinase subclass III which also includes receptors for platelet-derived growth factor and colony stimulating factor-1 [1]. Genetic studies have found that the c-*kit* receptor in mice is mapped to the dominant white spotting (*W*) locus [2, 3], and that the c-*kit* ligand, which is stem cell factor (SCF), is a growth factor encoded by the mouse *steel (Sl)* locus [4–6]. The phenotypes of *W* and *Sl* mutant mouse strains that bear germ line loss of function mutations in c-*kit* and its ligand, respectively, demonstrate roles for c-*kit* in melanogenesis, hematopoiesis, and germ cell development. The development of anti-c-*kit* antibodies has enabled systematic immunohistochemical analyses of the distribution of c-*kit* receptor protein to be carried out. Results of these analyses have demonstrated c-*kit* expression in hippocampus, cerebellum, thyrocytes, cortical stroma of the ovary, breast epithelial cells, and salivary gland. However, the functions of the c-*kit* receptors in these tissues remain unknown [7–9].

There have been reports that c-*kit* is highly ex-

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pressed or mutated in small cell lung cancer [10], leukemia cells [11], testicular tumor [12], colon cancer [13], neuroblastoma [14] and other tumors. This indicates that c-*kit* activity may play an important role in their oncogenesis. On the other hand, c-*kit* expression is lost in breast cancer [15] and melanoma [16, 17]. This seems to indicate that c-*kit* may have functions in normal breast epithelia and melanocytes.

The expression of c-*kit* product in thyrocytes is weak [8, 9]. In a few thyroid carcinoma tissues used in preliminary studies, no c-*kit* product was detected immunohistochemically [7, 8]. Levels of c-*kit* protein expression in thyrocytes are too low to be measured, and probably because of this difficulty, there are no reports on changes in c-*kit* expression during thyroid carcinogenesis.

In order to investigate whether c-*kit* is positively associated with thyroid carcinogenesis, we analyzed c-*kit* gene expression in neoplastic thyroid tissues. We also compared the c-*kit* gene expression in thyroid carcinomas with the mRNA expression of three thyroid-specific genes known to be suppressed in carcinoma.

Materials and Methods

Thyroid tissues

All thyroid tissues were obtained surgically from Japanese patients with various thyroid diseases. The normal thyroid tissues analyzed in this study were derived from normal tissue adjacent to excised thyroids with papillary carcinoma. Histopathological diagnosis was made according to the WHO criteria for thyroid tumors [18].

Northern blot analysis

Total cellular RNA was prepared from excised thyroid according to the guanidinium-CsCl method. Ten μg of RNA was electrophoresed through formaldehyde-agarose gels, transferred to a Zeta-probe membrane (Bio-Rad, NY) and baked at 80 °C for 2 h. Filters were prehybridized for 3 h, hybridized overnight, and washed according to the manufacturer's instructions. Autoradiographic films were exposed to the filters with intensifying screens for 2 h to 7 days at −70 °C. The cDNA probes were labeled with α-^{32}P-dCTP using a Takara Random Primer DNA Labeling Kit (Takara Co., Ltd., Otsu, Japan). The human TPO cDNA probe was a 2.4 kb Eco RI-Sac I fragment isolated by our group [19]. The human thyrogrobulin cDNA probe was a 0.96 kb Bam HI-Pst I fragment obtained from JCRB (Japanese Cancer Research Resources Bank) [20]. The human TSH-R cDNA probe was a 2.3 kb Xho I-Bam HI fragment (courtesy of Prof. G. Vassart, Universite Libre de Bruxelles, Belgium) [21]. Both a 3.8 kb Hind III-Hind III human c-*kit* cDNA fragment [1] and a 0.4 kb EcoR I-EcoR I human β-actin cDNA fragment [22] were kindly given by Dr. S. Tohda (Tokyo Medical & Dental University, Japan).

Results

c-*kit* gene expression was analyzed in 37 various thyroid tissues by the northern blot method. Figure 1 shows one of the northern blot analyses. All results are summarized in Table 1. A high level of c-*kit* mRNA expression was found in all benign thyroid tissues (4 normal thyroids, 4 Graves' disease, 2 adenomatous goiters, and 7 follicular adenomas), while strong underexpression of c-*kit* mRNA was found in differentiated thyroid carcinoma and medullary carcinoma. c-*kit* mRNA was not detected by the usual northern blot method in 2 of 7 follicular carcinomas (FTCs), 11 of 12 papillary carcinomas (PTCs), and a medullary carcinoma (MTC).

In order to study the correlation between c-*kit* expression and thyroid-specific gene expression (Fig. 1, Table 1), the same northern blot filters used for the study on c-*kit* expression were re-hybridized with cDNA probes of 3 thyroid-specific differentiation antigens, thyroid peroxidase (TPO), thyroglobulin (Tg), and thyroid stimulating hormone receptor (TSH-R). TPO mRNA expression was strongly suppressed in all 12 PTCs and in one of 7 FTCs (case 24). In case 23, a high level of TPO mRNA expression was found, but c-*kit* mRNA expression was strongly suppressed. Tg mRNA was strongly underexpressed in 6 of 12 PTCs, and TSH-R mRNA expression was slightly suppressed in 6 of 12 PTCs.

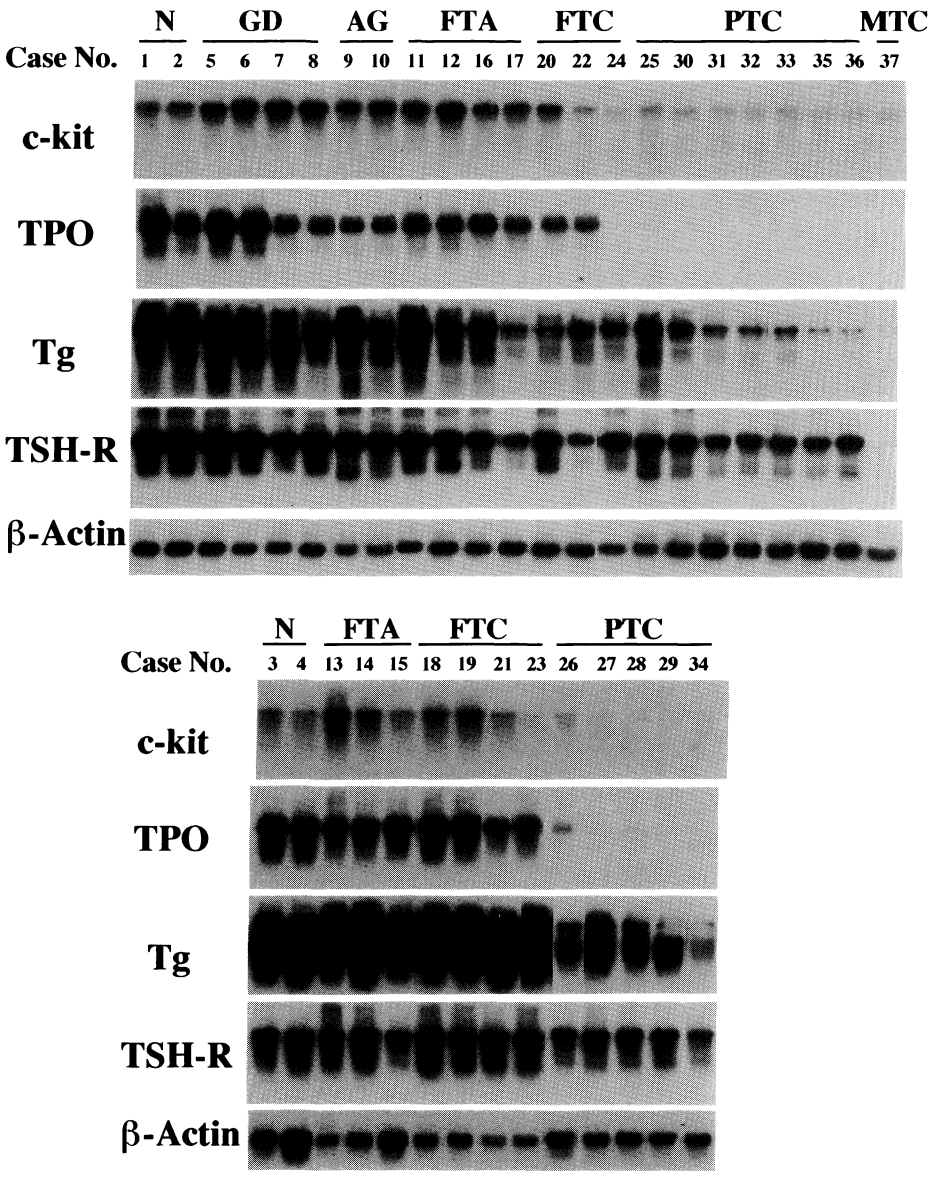

Fig. 1. Northern blot analyses of neoplastic thyroid tissues. N, normal thyroid tissue; GD, Graves' disease; AG, adenomatous goiter; FTA, follicular adenoma; FTC, follicular carcinoma; PTC, papillary carcinoma; MTC, medullary carcinoma.

Discussion

This comparative study of c-*kit* and the thyroid-specific genes in thyroid carcinomas found that the c-*kit* gene is more likely to lose expression in thyroid carcinoma than the three thyroid-specific genes. The hierarchy of expression loss was as follows: c-*kit* was most likely to lose expression, TPO next most likely, Tg third, and TSH-R least likely among the four (Table 1). This suggests that c-*kit* may be a good indicator of underexpression of the three thyroid-specific genes, in other words, of the de-differentiation of thyrocytes.

Our results do not agree with others' studies on c-*kit*. First, a difference can be found in terms of the significance of thyroid c-*kit* in differentiation. Based on our result that underexpression of c-*kit* mRNA was not found in benign thyroid tumors, we hypothesized that c-*kit* functions in the differ-

Table 1. Summary of northern blot studies

Case No.	Histopathological Diagnosis	mRNA expression			
		c-*kit*	TPO	Tg	TSH-R
1	N	+++	++++	++++	++++
2	N	+++	++++	++++	++++
3	N	+++	++++	++++	++++
4	N	++	++++	++++	++++
5	GD	+++	++++	++++	++++
6	GD	++++	++++	++++	++++
7	GD	++++	+++	++++	++++
8	GD	++++	+++	+++	++++
9	AG	+++	+++	++++	++++
10	AG	+++	+++	+++	++++
11	FTA	+++	++++	++++	++++
12	FTA	++++	++++	+++	++++
13	FTA	++++	++++	++++	++++
14	FTA	+++	++++	++++	++++
15	FTA	++	++++	++++	+++
16	FTA	+++	++++	+++	++++
17	FTA	+++	++++	++	++
18	FTC	+++	++++	++++	++++
19	FTC	+++	++++	++++	++++
20	FTC	+++	+++	++	++++
21	FTC	++	+++	++++	++++
22	FTC	+	+++	+++	++
23	FTC	−/+	+++	++++	++++
24	FTC	−/+	−/+	++	++++
25	PTC	+	−/+	+++	++++
26	PTC	−/+	+	++	+++
27	PTC	−/+	−/+	+++	+++
28	PTC	−/+	−	++	+++
29	PTC	−/+	−	++	+++
30	PTC	−/+	−/+	++	+++
31	PTC	−/+	−/+	+	++
32	PTC	−/+	−	+	++
33	PTC	−/+	−/+	+	++
34	PTC	−/+	−/+	+	++
35	PTC	−/+	−/+	+	++
36	PTC	−/+	−	+	++
37	MTC	−/+	−	−	−

N, normal thyroid tissue; GD, Graves' disease; AG, adenomatous goiter; FTA, follicular adenoma; FTC, follicular carcinoma; PTC, papillary carcinoma, MTC, medullary carcinoma. ++, +++, ++++, positive; +, weakly positive; −/+, faint expression detected on long-exposed films; −, not detected by usual northern blot method.

entiation rather than the proliferation of thyrocytes, and accordingly that loss of thyroid c-*kit* plays a role in the thyroid de-differentiation of thyrocytes associated with malignant transformation. On the other hand, Natali *et al.* reported while we were preparing this paper that transformation of thyroid epithelium is associated with loss of c-*kit* receptor, and hypothesized that thyroid c-*kit* plays a role in the normal proliferation of thyrocytes [23]. We are now studying the effects of SCF on thyrocytes, and the results of the study should help us clarify the function of c-*kit*.

Second, a difference can be found in whether or not c-*kit* product was detected. In our study, we

did not detect any c-*kit* product in thyroid tissues by immunohistochemical analysis or by western blot analysis using four anti-human c-*kit* antibodies: a monoclonal anti-human c-*kit* antibody L6 [24] and three antibodies which were available commercially (data not shown). Others have reported that weak expression of c-*kit* product was immunohistochemically detected in thyroid tissue using different anti-c-*kit* antibodies from ours [8, 9, 23]. It is possible that this difference in detection is due to differences in antibody specificity to thyroid c-*kit* product.

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