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A specific Translocation of Chromosome 3 Generating Pax8-PPARg Fusion mRNA is Rare in Japanese Patients with Follicular Carcinoma

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Abstract: Thyroid follicular neoplasm is commonly encountered in clinical medicine. However, it is difficult to pathologically differentiate benign from malignant tumors. Recently t (2,3)(q13; p25), a translocation resulting in the generation of Pax8-PPARγ fusion gene has been reported in American patients with follicular carcinoma with high incidence suggesting that Pax8-PPARγ fusion mRNA could be a molecular marker for the diagnosis of follicular carcinoma. In this study we examined the expression of this fusion gene in follicular carcinomas in Japanese patients. Various thyroid nodules (follicular carcinoma, follicular adenoma, papillary carcinoma, adenomatous goiter and normal tissue) were obtained at surgery in our institute and total RNAs were used to detect the Pax8-PPARγ fusion mRNA by RT-PCR. In all the follicular carcinoma samples, Pax8-PPARγ fusion mRNA was not detected. In all the other samples the expression was also negative. On the other hand, wild type Pax8 mRNA was detected in all the samples and wild type PPARγ mRNA was detected in 83.3% of the follicular adenoma and the adenomatous goiter samples, in 100% of the follicular carcinoma, the papillary carcinoma and the normal tissue samples respectively. In contrast to the previous report, no expression of Pax8-PPARγ fusion gene was detected in follicular carcinomas. Ethnic background may affect the translocation on the pathogenesis.

Key words: Pax8, PPARγ, thyroid, follicular carcinoma

Thyroid follicular neoplasm is commonly encountered in clinical medicine. However, it is difficult to differentiate benign from malignant tumors if a distant metastasis is not present simultaneously. Recently, Kroll et al. reported t (2,3)(q13; p25), a translocation resulting in the generation of Pax8-PPARγ fusion mRNA in follicular thyroid carcinomas with high incidence (63%).[1)] However, Pax8-PPARγ fusion was not recognized in papillary carcinomas and follicular adenomas.[1)] Pax8 is one of the thyroid transcription factors for thyroid specific genes such as thyroglobulin (TG), thyroperoxidase (TPO).[2)] PPARγ is a ligand-dependent nuclear receptor that plays an important role in adipocyte differentiation and glucose metabolism.[3)] PPARγ also regulates the expression of genes involved in differentiation and cell cycle.[4)] Several studies reported that thiazolidinediones, one of the synthetic PPARγ ligands, inhibits proliferation of breast, prostate, colon cancer and liposarcoma in vivo or in vitro.[5-8)] In this study, we evaluated the expression of mRNA for Pax8, PPARγ and Pax8-PPARγ fusion in various kinds of nodules and normal thyroid glands obtained at surgery in our department.

Materials and Methods

Surgical specimens from 3 thyroid follicular carcinomas, 6 thyroid follicular adenomas, 6 thyroid papillary carcinomas, 6 adenomatous goiters and 6 normal thyroid tissues were obtained at Nagoya University Hospital between 1990 and 2000. The diagnosis of each tumor was made by pathologists in our hospital.

Total RNAs were prepared using acid-guanidium thiocyanate phenol chloroform.[9)] RNA (1μg) was reverse transcribed with Moloney Murine Leukemia Virus reverse transcriptase (Invitrogen Corp., Carlsbad, CA, USA) at 37°C for 60 min in a 10μl mixture. Two microliters of a reverse-transcribed mixture was amplified by PCR. Sense and antisense primer were designed in exons separated by at least 1 intron to avoid the artifact due to contamination of DNA. The primers for Pax8 were 5'-CATTTGAGCGGCAGCACTACCCAGAGG-3' (876-902, exon 7; sense) and 5'-CCGTGGTGGGGATGTGGGGTGGGTAT-3' (1279-1305, exon10; antisense) The primers for PPARγ were 5'-TTCTCCAGCATTTCTACTCCACATTAC-3' (296-322, exon 1; sense) and 5'-ATGGTGATTTGTCTGTTGTCTTTCCTG-3' (888-914, exon 2; antisense). The primers for Pax8-PPARγ were 5'-

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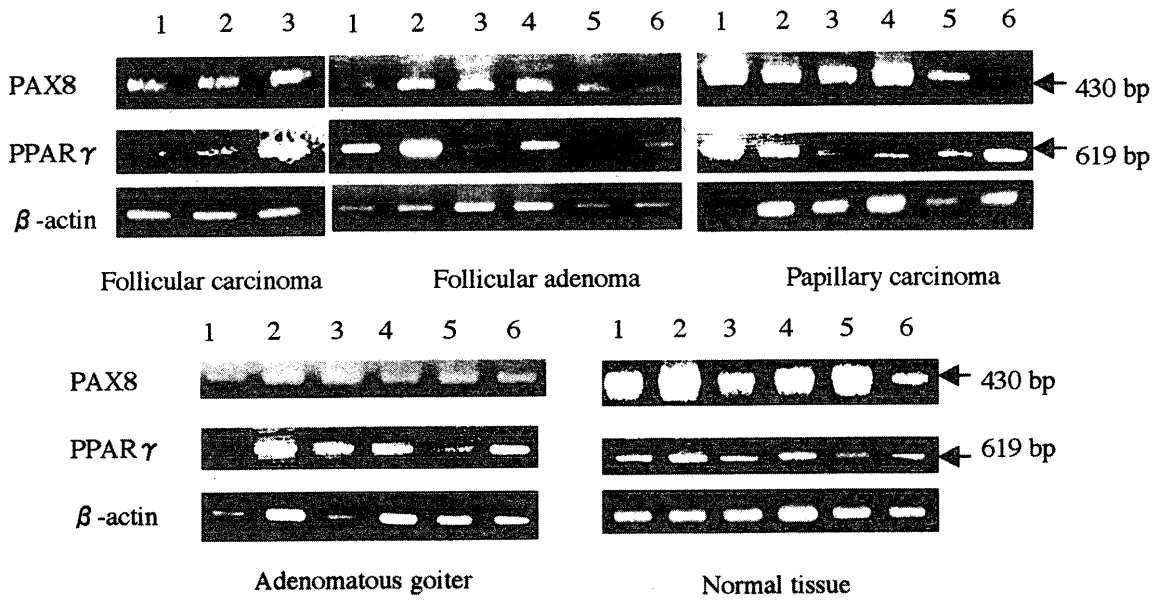

Fig. 1 RT-PCR to detect Pax8, PPAR γ and Pax8-PPARγ fusion.
After reverse transcription from human thyroid follicular carcinomas (3), papillary carcinomas (6), follicular adenoma, adenomatous goiters (6) and normal thyroid tissues (6), Pax8 and PPARγ cDNAs were amplified by PCR. PCR products were resolved mRNA by electrophoresis and stained with ethidium bromide. Pax8-PPARγ fusion mRNA was not detected in any of the follicular carcinomas, the adenomatous goiters, the follicular adenomas, the papillary carcinomas, and the normal thyroid tissues (data was not shown).

ATTTGAGCGGCAGCACTACCCAGAGGC-3' (297-323 of Pax8, exon 4; sense) and 5'-ATGGTGATTTGTCTGTTGTC-TTTCCTG-3' (292-318 of PPARγ, exon 1; antisense). Fidelity of RT-PCR was monitored by amplifying human beta-actin using the primers of 5'-ACCTTCAACACCCCAATG-3' (sense) and 5'-GGCCATCTCTTGCTCGAAGTC-3'. PCR products were subcloned into pGEM-T Easy vector (Promega, Madison, WI).

Result

Pax8-PPARγ fusion mRNA was detected by RT-PCR in none of the 3 follicular carcinoma samples. The expression was neither detected in adenomatous goiters, follicular adenomas, papillary carcinomas, nor normal thyroid tissues (data was not shown). Wild type Pax8 mRNA was detected in all the samples whereas wild type PPARγ mRNA was detected in 5 of 6 the adenomatous goiters (83.3%), in 5 of 6 the thyroid follicular adenomas (83.3%), in all the papillary carcinomas, the follicular carcinomas and the normal thyroid tissues (Fig.1).

Discussion

Genetic alterations have been described in thyroid follicular carcinomas.[10-16] Knoll et al. showed t (2,3)(q13; p25), a translocation resulting in production of fusion Pax8-PPARγ mRNA. They attributed the dominant negative fusion gene product on PPARγ to carcinogenesis.[1] Of note that they demonstrated the specific gene alteration detected in thyroid follicular carcinoma with high frequency (63%). However, in the present study, we could not detect the Pax8-PPARγ fusion in 3 cases with follicular carcinoma. Ethnic background may affect the translocation.

The expression of PPARγ is distributed mainly in adipose tissues and intermediately or less in the large intestine, retina and some immune cells. The present study revealed that PPARγ could also be expressed in normal thyroid tissue. Although the physiological role of PPARγ is still unknown, it is tempting to speculate that it may be important for differentiation of thyroid follicular cell.

Pax8 is a member of the murine Pax family of paired domain-containing genes, expressed in the developing kidney, the nueral tube, and the developing and adult thyroid.[17] Pax8 contributes to the thyroid organogenesis and the maintenance of differentiated function.[3] Mansouri et al. showed that Pax8 knockout mouse revealed a total absence of the thyroid follicular cells.[18] In the present study Pax8 mRNA was detected by RT-PCR in all the thyroid tumor. The fact that Pax8 is known to maintain differentiated function of follicular cells is compatible with its expression in all the thyroid nodules and in normal tissues.

In conclusion, we did not detect the Pax8-PPARγ fusion gene in our series. Further studies with larger numbers of cases should provide insight into of this fusion gene in thyroid follicular carcinoma to confirm the possibility to be the marker of follicular carcinoma.

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