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Nuclear Localization of Immunoreactive β-Catenin Is Specific to Familial Adenomatous Polyposis in Papillary Thyroid Carcinoma

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Thyroid carcinoma is the first symptom in some patients with familial adenomatous polyposis (FAP). We evaluated the cellular localization of β-catenin in thyroid carcinomas associated (n=4) or not associated (n=173) with FAP, since loss of functional protein of the adenomatous polyposis coli (*APC*) gene leads to nuclear accumulation of β-catenin in adenomas and carcinomas of the FAP colon. Immunoreactive β-catenin was demonstrated at the cell membrane of glandular cells of the non-neoplastic thyroid and non-FAP carcinomas. On the other hand, cytoplasmic and nuclear accumulation of β-catenin is specific to FAP-associated papillary carcinomas. The abnormality in the APC/β-catenin pathway is thus also important in FAP-associated thyroid carcinoma, and β-catenin immunohistochemistry is a feasible screening method to identify occult FAP in young patients with thyroid tumors.

Key words: Familial adenomatous polyposis — β-Catenin — Thyroid carcinoma — Papillary carcinoma — Immunohistochemistry

Thyroid carcinoma occurs much more frequently in young female patients with familial adenomatous polyposis (FAP) than in matched controls.[1,2] The relative risk for thyroid carcinoma in FAP women under 35 years of age is 160 times normal, and more importantly, 30% of thyroid carcinomas are diagnosed 4–12 years before the development of polyposis.[2] Germline mutation of the adenomatous polyposis coli gene (*APC*) is responsible for the FAP. APC protein controls the nuclear accumulation of β-catenin, a transcriptional activator regulated by the Wnt signaling pathway, by a combination of nuclear export and cytoplasmic degradation.[3,4] The latter function of APC is lost by the mutation and/or deletion of *APC* in both alleles in the colon carcinomas of FAP patients, and this results in the nuclear accumulation of β-catenin.[5] Then, it interacts with Tcf-4, the Lef/TCf transcription factor, leading to activation of several developmentally related genes, such as *c-myc*, and subsequent cell proliferation. To test the possibility that the abnormality of the APC/β-catenin pathway is also involved in FAP-associated thyroid carcinoma, we immunohistochemically evaluated β-catenin in FAP-associated thyroid carcinoma as well as sporadic thyroid carcinomas. If β-catenin-accumulation is specific to FAP in thyroid carcinomas, it can be a hallmark to identify occult FAP in young patients with thyroid carcinoma.

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Four FAP patients with thyroid carcinoma were enrolled in this study. Three were sisters and the other was a daughter of one of them. The thyroid carcinoma was the first symptom of the proband at age 16, five years before the diagnosis of FAP with a rectal carcinoma. Thyroid carcinomas were detected at the age of 12 in the case of her sister, and at 13 in her daughter. The clinical course of the sisters has been previously reported, and germline *APC* mutation was found at codon 848 (AAA to TAA, stop).[6] In the case of the fourth patient, who had no relation to the three patients described above, multiple colon carcinomas and multiple thyroid carcinomas were diagnosed simultaneously (Fig. 1A). The clinical course of this 26-year-old woman has also been reported previously.[7] Germline *APC* mutation was detected at codon 175 (ACT to AT, C deletion), resulting in truncation at codon 184. A somatic mutation was also demonstrated at codon 886 (CAG to TAG, stop) in one of the thyroid carcinomas.

As for the sporadic thyroid carcinomas, two series were evaluated: the first series was papillary thyroid carcinomas which had been consecutively resected at the Department of Surgery, Jichi Medical School (n=140, age 17–83 years old), including 11 cases younger than 30 years of age. The second series consisted of thyroid carcinomas of patients aged less than 30, treated at Tokyo Metropolitan Komagome Hospital. Twenty-seven cases were retrieved from the file of the Department of Pathology, and all of them were papillary carcinomas. Thus, the sporadic thyroid car-

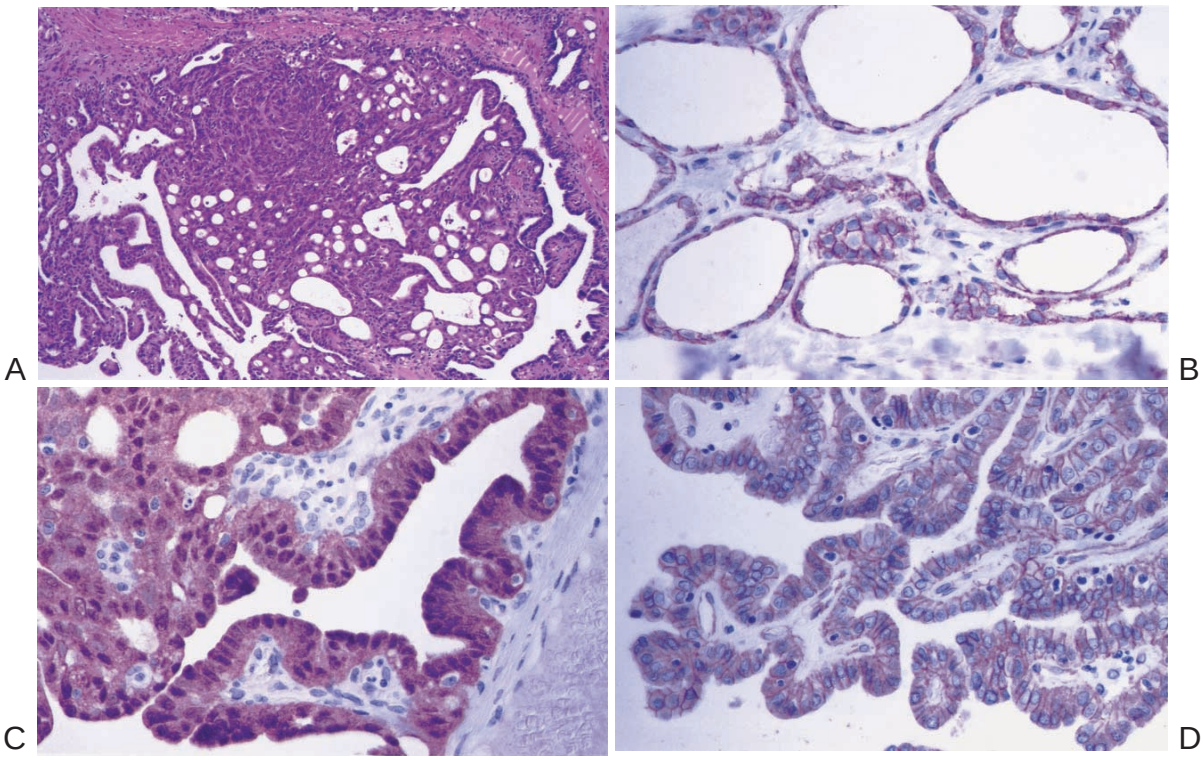

Fig. 1. (A) Thyroid carcinoma of a FAP patient, showing papillary and follicular architecture with whorl formation of solid spindle cells. (B) Membranous staining for β-catenin in non-neoplastic thyroid gland of a FAP patient (26-year-old woman). (C) Cytoplasmic and nuclear staining for β-catenin in a FAP-associated thyroid carcinoma. Note that membranous staining is hardly to be recognized. (D) Membranous staining for β-catenin is also characteristic for sporadic thyroid carcinoma. (B)–(D): β-catenin immunostaining.

cinomas consisted of 38 cases younger than 30, and 129 cases older than 30. Six medullary carcinomas (age 22–56 years old) were also evaluated.

Formalin-fixed and paraffin-embedded tissues of the thyroid carcinomas and adjacent non-neoplastic glands were used in the study. Four-micrometer-thick slides were subjected to immunostaining for β-catenin. For antigen retrieval, the sections were immersed in 0.01 *M* citrate buffer (pH 6.0) and heated in a microwave oven at 750 W for 20 min. Then, the avidin-biotin peroxidase complex (ABC) method was applied to the sections, using a mouse anti-human β-catenin monoclonal antibody (Transduction Laboratories, Lexington, KY). Each stained section was always accompanied by another section, which had been similarly processed except that the monoclonal antibody was omitted.

β-Catenin immunohistochemistry produced uniform membranous staining without nuclear staining in normal thyroid glands, whether FAP or non-FAP (Fig. 1B). It is apparent that inactivation of only one allele of *APC* by germline mutation did not affect the subcellular localization of β-catenin in the thyroid gland. As for the thyroid carcinomas, membranous staining was similarly demonstrated in all of 167 papillary and 6 medullary thyroid carcinomas of non-FAP patients (Fig. 1D). On the other hand, localization of immunoreactive β-catenin was strikingly shifted from the cell membrane to the cytoplasm and the nucleus in all of four FAP-associated thyroid carcinomas (Fig. 1C): nearly all of the carcinoma cells exhibited cytoplasmic and nuclear accumulation of β-catenin in each case. Thus, the abnormality in the APC/β-catenin pathway is specific to FAP-associated thyroid carcinoma, at least in the specimens in this study. Additional inactivation of the *APC* gene on another allele by somatic mutation and/or loss of heterozygosity is most likely to be responsible for this abnormality, and this has been confirmed in one of the patients in this study.[6]

Recently, involvement of the APC/β-catenin pathway has been shown in anaplastic thyroid carcinomas.[8, 9] Garcia-Rostan *et al.*[8] demonstrated β-catenin mutation in 19 of 31 patients and cytoplasmic or nuclear localization in 15 of 36 anaplastic thyroid carcinomas. However, abnormality of β-catenin in anaplastic carcinoma appears to be different from that in FAP-associated thyroid carcinoma:

nuclear β-catenin staining was only focally observed in all of the carcinomas, and several different somatic mutations were detected in the same tumor.

Several features of FAP-associated thyroid carcinomas have been described, such as young age of the patient and multiplicity of the carcinoma. Certain histologic and cytologic features can be recognized to be relatively specific to FAP-associated thyroid carcinoma, but the differences are not remarkable.[10] On the other hand, the localization of immunoreactive β-catenin was definite and striking, being of 'all-or-none' type. Furthermore, since immunostaining is a standard technique in a surgical pathology laboratory, β-catenin immunostaining can be easily applied to surgical materials and needle aspiration cytology samples to identify occult FAP in young patients with thyroid tumors.

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