Somatic Mutation of the \textit{APC} Gene in Thyroid Carcinoma Associated with Familial Adenomatous Polyposis

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We report the existence of both germline and somatic mutations of the \textit{APC} gene in thyroid carcinomas from familial adenomatous polyposis (FAP) patients. One papillary thyroid carcinoma from a 20-year-old woman, with germline mutation of the \textit{APC} gene (TCA to TGA at codon 1110), showed a somatic mutation of AAAAC deletion between codons 1060 and 1063. Another somatic mutation of CAG to TAG at codon 886 was also found in one of multiple thyroid carcinomas from a 26-year-old woman with attenuated FAP and germline mutation at codon 175 (C deletion). This is the first evidence that total absence of the normal function of the \textit{APC} gene is involved in development of thyroid carcinomas in FAP.

Key words: Familial adenomatous polyposis — Thyroid carcinoma — Somatic mutation of \textit{APC} gene — Histology of thyroid carcinoma

Familial adenomatous polyposis (FAP; MIM 175100) is an inherited condition caused by germline mutation of the \textit{APC} gene.\textsuperscript{1,2} It is an accepted theory that complete inactivation of the \textit{APC} gene by 2 mutations is involved in the development of colonic adenoma of the general population and FAP.\textsuperscript{3-5} We have previously demonstrated that gastric and duodenal tumors from FAP patients have somatic mutations of the \textit{APC} gene,\textsuperscript{6} as do other FAP extracolonic tumors, such as desmoids.\textsuperscript{7} Adrenal tumors from FAP patients have also been demonstrated to have somatic alterations of this gene.\textsuperscript{8,9} Although thyroid carcinoma is a common tumor in young women with FAP, its genetic changes have yet to be clarified.

In the present study, we examined two cases of thyroid carcinoma associated with FAP. With informed consent, DNA was extracted from the tumor and corresponding peripheral blood, and analyzed for the \textit{APC} mutation using polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP) and direct sequencing methods, as previously described.\textsuperscript{5}

One FAP patient (PLK294), a 20-year-old woman, had multiple thyroid tumors, of which the largest was 3.5×2.5 cm. Examination of the colon revealed scattered small adenomas without carcinoma. Congenital hypertropic retinal pigment epithelium was detected in the left eye. Subtotal thyroidectomy followed by histological examination revealed multiple papillary carcinoma of columnar cell type with cribriform and solid areas (Fig. 1A). A germline mutation of the \textit{APC} gene was detected at codon 1110 (TCA to TGA, stop). The second largest tumor (PLK294-TCa2) exhibited mutant bands in PCR-SSCP, and direct sequencing of these mutant bands revealed a 5-bp deletion (AAAAC deletion) between codons 1060 and 1063, resulting in a stop signal at codon 1063 (Fig. 2, Table I).

The second case (PLK29), a 26-year-old woman, underwent resection of the rectum, and excision of a metastatic tumor of the liver. The number of colonic polyps was approximately 25, and there were multiple thyroid carcinomas, the largest of which was 4 cm in diameter. Histopathological diagnosis of these thyroid carcinomas was papillary carcinoma with solid areas (Fig. 1B). This patient was recognized as FAP after a germline mutation of the \textit{APC} gene was detected at codon 175 (ACT to AT, C deletion), resulting in a truncation at codon 184. In one of the thyroid carcinomas (PLK29-TCa1), a somatic mutation was found at codon 886 (CAG to TAG, stop) (Table I).

In the case of PLK294-TCa2, to make detection of mutation easier and clearer by means of PCR-SSCP and direct sequencing methods, we used primer sets, which separately amplified two short regions that include codons 1060–1063 and 1110, as shown in Fig. 2. To clarify whether germline and somatic mutations of PLK294-
TCa2 existed on different alleles of the APC gene, we also performed PCR-SSCP analysis using primers which amplify the region including both germline (at codon 1110) and somatic (at codons 1060–1063) mutations. The SSCP pattern of PLK294-TCa2 showed multiple mutant bands, and direct sequencing of DNA fragments from
These authors have concluded that APC abnormality does not play a pathogenic role in thyroid tumorigenesis in patients not affected by FAP. There may be other factors that contribute to the difference between FAP and non-FAP thyroid carcinomas. By using immunochemical methods, enhanced expression of the RET-PTC oncogene has been detected in thyroid carcinoma in 2 of 3 patients with FAP in a kindred, and it was suggested that such activation is unique to sporadic papillary thyroid tumors. It is possible that inactivation of the APC gene through germline and somatic mutations brings about increased expression of cancer-related genes, such as RET, in cells of thyroid origin, although the previous study did not analyze somatic APC mutation in thyroid tumors which showed no loss of heterozygosity of the APC gene.

With respect to genotype-phenotype relationship, several studies have reported a correlation between the location of the germline mutation in the APC gene and the number of colorectal polyps in FAP patients. Examples of phenotypic heterogeneity caused by the mutation site or type have also been described, but it seems to be difficult to predict the severity of disease from the position of germline mutation. Previously, germline mutations in FAP patients with thyroid carcinoma have been reported at codon 1061, 1309, or 848, and our recent cases with thyroid carcinoma had germline mutation at codon 1105 or 1061. However, germline mutations of the present 2 cases were not at these spots, suggesting that the development of thyroid carcinoma does not depend on whether the germline mutation is at the 5' attenuated FAP phenotype region, or in the severe phenotype area (codon 1309).

Regarding the site of somatic mutation, approximately 94% of somatic mutations in gastrointestinal tumors were clustered between codons 1280 and 1500 (MCR). Although in our 2 cases, mutation occurred outside of the MCR, analyses of many thyroid tumors will be needed to establish whether the mutation pattern in thyroid tumor is consistently different from that of gastrointestinal tumor.

In FAP patients, as in the general population, thyroid carcinoma predominantly occurs in women, which suggests that tumor formation in FAP patients requires not only total loss of function of the APC gene, but also some other factors, such as hormonal environment, ileal-pouch formation, inflammatory factor, or surgical injury, for specific tumors. Further study of genetic changes is also needed to clarify the cause of the histological differences between FAP and non-FAP thyroid carcinomas.

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| Tumor | Somatic mutation | Germline mutation |
|-------|------------------|------------------|
|       | Codon  | Mutation   | Codon | Mutation   |
| PLK294-TCa2 | 1060–1063 | AAAAC deletion | 1110 | TCA→TGA |
| PLK29-TCa1    | 886    | CAG→TAG    | 175   | C deletion |

Table I. Somatic and Germline Mutations of the APC Gene in Thyroid Tumors from FAP Patients
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