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Short Communication

Molecular Evidence for Multicentric Development of Thyroid Carcinomas in Patients with Familial Adenomatous Polyposis

Michiko Miyaki,*† Takeru Iijima,*† Reiko Ishii,* Tsunekazu Hishima,‡ Takeo Mori,§ Keigo Yoshinaga,¶ Hiroshi Takami,‖ Toshio Kuroki,† and Takeo Iwama**

From the Hereditary Tumor Research Project, Department of Pathology,‡ Department of Surgery,§ Tokyo Metropolitan Komagome Hospital; Tokyo Medical and Dental University,¶ Teikyo University;‖ Institute of Molecular Oncology;† Showa University; and Kyoundo Hospital,** Sasaki Institute, Tokyo, Japan*

Familial adenomatous polyposis is characterized by multiple colorectal adenomas and an increased incidence of colorectal carcinomas. Patients also develop various extracolonic tumors, of which, thyroid carcinoma is common in young females. The occurrence of multiple carcinomas in one thyroid is frequently observed, although some carcinomas are solitary. To clarify whether each carcinoma develops independently or metastatically spreads from the first one formed, we analyzed the adenomatous polyposis coli (APC) gene mutation in each carcinoma. We found that each carcinoma had a different somatic mutation of the APC gene. This is molecular confirmation for the multicentric development of thyroid carcinomas in familial adenomatous polyposis through biallelic inactivation of the APC gene. ***(Am J Pathol 2000, 157:1825–1827)***

Familial adenomatous polyposis (FAP) is an autosomal dominant disease characterized by multiple colorectal adenomas and an increased incidence of colorectal carcinomas. It is also accompanied by various benign and malignant extracolonic manifestations, including gastric and duodenal tumors, osteomas, desmoid tumors, retinal pigmentation, and thyroid and adrenocortical tumors.[1–3] We have previously demonstrated that gastric, duodenal, and desmoid tumors, and an adrenocortical carcinoma, in FAP patients develop by inactivation of both alleles of the adenomatous polyposis coli (APC) gene through germline mutation and somatic mutation occurring in the normal allele,[4–6] in the same manner as in colorectal tumors.[7–9] We also demonstrated that thyroid carcinomas from FAP patients had both germline and somatic mutations of the APC gene.[10] Thyroid cancer, being common in young FAP females, develops either as a single carcinoma or as multiple carcinomas in one thyroid.[11–14] However, whether each carcinoma develops independently or metastatically spreads from the first one formed is still unclear. To clarify the mechanism of such multicentric development of thyroid carcinomas, we analyzed somatic mutation of the APC gene in each carcinoma in thyroids from FAP patients.

Materials and Methods

Patients and Samples

Thyroid carcinomas were obtained from two FAP patients who gave informed consent. Patient PLK29 (a 26-year-old female) had one large carcinoma in the left lobe and multiple carcinomas in the right lobe, as shown in Figure 1. Patient PLK294 (a 21-year-old female) had two carcinomas in the left lobe. All carcinomas were histopathologically diagnosed as papillary carcinoma. Genomic DNA was extracted from each carcinoma and corresponding normal tissue, using proteinase K, sodium dodecyl sulfate, and phenol-chloroform.

Mutation Analysis

DNA samples were amplified using polymerase chain reaction (PCR) and analyzed by the single-strand conformation polymorphism (SSCP) method. Primers for mutation analysis for the APC gene were the same as those

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Address reprint requests to Michiko Miyaki, Hereditary Tumor Research Project, Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku Tokyo 113-8677, Japan. E-mail: mmiyaki@opal.famille.ne.jp.

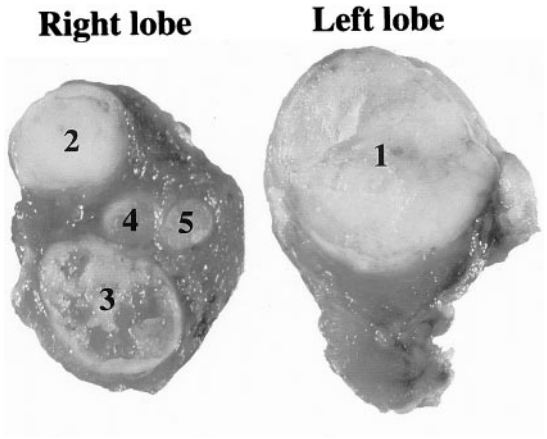

Figure 1. Multicentric thyroid carcinomas in FAP patient PLK29. Numbers of carcinomas correspond to those in Table 1.

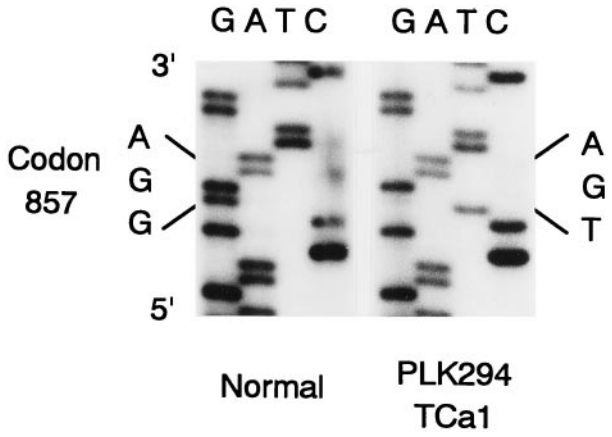

Figure 2. Example of sequencing of DNA fragments in SSCP corresponding to somatic mutation of the APC gene in thyroid carcinoma.

previously reported.[15] Conditions for PCR were the same as those previously described.[9] When abnormal bands were detected in the SSCP analysis, single-strand DNA fragments were extracted, amplified by asymmetrical PCR, and then subjected to direct sequencing by dideoxy chain-termination reaction.[9]

Loss of Heterozygosity Analysis

Loss of heterozygosity at chromosome 5q near the APC locus was analyzed using D5S346 and (AC)10. Loss of the normal allele was estimated by comparison of intensities between abnormal bands (corresponding to germline mutation) and normal bands in PCR-SSCP analysis.

Results

PCR-SSCP analysis and direct sequencing revealed both germline and somatic mutations of the APC gene. Data are shown in Table 1 and Figure 2. Patient PLK29, with a germline mutation of C deletion at codon 175, developed multiple papillary carcinomas in the thyroid (Figure 1). One large carcinoma in the left lobe had a somatic APC mutation of CAG to TAG (stop) at codon 886. Two of the multiple carcinomas in the right lobe of the same patient exhibited different somatic mutations of the APC gene, the mutation in one carcinoma (TCa3) being GAA to TAA (stop) at codon 1536, and that in the other (TCa5) being an A insertion at codons 1554 to 1556. Loss of the normal allele was detected in two carcinomas, TCa2 and TCa4. FAP patient PLK294, with a germline mutation of TCA to TGA (stop) at codon 1110, developed two papillary carcinomas in the left lobe. In these carcinomas, different somatic mutations were detected. Mutation in TCa1 was GGA to TGA (stop) at codon 857, and that in TCa2 was AAAAC deletion at codons 1060 to 1063. All of these somatic mutations occurred in exon 15, and formed stop codons resulting in truncated APC protein.

Discussion

The histopathological characteristic of FAP-associated thyroid carcinomas has been reported to be papillary carcinoma with a cribriform pattern and solid areas with a spindle-cell component.[11,13,14] With respect to the pattern of development of these carcinomas, both solitary and multicentric types have been reported.[11] In some cases, more than 10 separate tumors of various sizes have been detected in one thyroid. However, it is difficult to assess, by morphological features, whether these tumors are independent primary tumors or metastatically spread tumors from an originally developed one. We have recently demonstrated that FAP-associated thyroid carcinomas develop by biallelic inactivation of the APC gene through germline and somatic mutations.[10] Accordingly, to clarify the origin of multiple cancer in one thyroid, it is important to examine whether all tumors have the same or different somatic APC mutations. The present study revealed that three of the five carcinomas from a FAP patient with an identified germline mutation had different somatic mutations, and the other two carcinomas exhibited loss of the normal allele of the APC gene. In

Table 1. Somatic and Germline Mutations of the APC Gene in Multicentric Thyroid Carcinomas from FAP Patients

Patient	Thyroid carcinoma	Somatic mutation	Germline mutation
PLK29	TCa1	Codon 886 CAG → TAG	Codon 175 C deletion
PLK29	TCa2	Loss of normal allele	Codon 175 C deletion
PLK29	TCa3	Codon 1536 GAA → TAA	Codon 175 C deletion
PLK29	TCa4	Loss of normal allele	Codon 175 C deletion
PLK29	TCa5	Codon 1554–1556 A insertion	Codon 175 C deletion
PLK294	TCa1	Codon 857 GGA → TGA	Codon 1110 TCA → TGA
PLK294	TCa2	Codon 1060–1063 AAAAC deletion	Codon 1110 TCA → TGA

another patient with a known germline mutation, two carcinomas had different somatic mutations as well. The identification of such different somatic alterations of the APC gene confirms independent development of multicentric thyroid carcinomas in FAP patients. Moreover, all carcinomas were revealed to be formed by biallelic inactivation of the APC gene, because all somatic mutations resulted in truncated APC protein.

The position of somatic mutation within the APC sequence in thyroid carcinoma was not restricted to the region (codons 1281 to 1556) where more than 90% of somatic mutations of gastrointestinal tumors are clustered.[4,7,9] Three of five somatic mutations of thyroid carcinomas in the present study occurred outside of that region (codons 857, 886, and 1061). Somatic mutation of a solitary thyroid carcinoma in an additional patient was at codon 456 (data not shown). Germline mutations in our cases with thyroid carcinomas were at codons 175 and 1110. These patients exhibited sparse-type development of colorectal tumors. Germline mutations in our other cases with thyroid carcinomas were at codons 278, 1061, and 1106 (data not shown), and 848.[16] The position of the germline mutation in our cases is consistent with a recent report that a higher incidence of thyroid cancer has been observed in the patients with germline mutation before codon 1220,[17] different from the position with respect to colorectal tumors.[18,19]

Although the range of position of germline and somatic mutations in the APC gene in thyroid carcinoma is somewhat different from that of colorectal tumors, the present results suggest that the phenomenon of multicentricity of thyroid carcinoma formation is analogous to the multiplicity of colorectal tumor formation, because tumors in both cases have different somatic mutations. This molecular evidence for multifocal development of thyroid carcinoma may have value in elucidating the mechanism of thyroid tumorigenesis, and in the diagnosis and treatment of FAP patients. Because FAP-associated thyroid carcinomas occasionally occur before diagnosis of colonic adenomatosis, detection of multiple somatic APC mutations in thyroid carcinomas predicts that the patient is affected by FAP. Moreover, multicentricity of thyroid carcinoma in FAP patients implies the necessity of careful observation after partial thyroidectomy is selected.

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