Rearrangement, Amplification and Overexpression of c-Ha Ras Gene in Premalignant Lesion of Turcot's Syndrome

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Ras gene family (c-Ha, Ki, N ras) and c-myc proto-oncogenes were analyzed in seven colonic and three gastric adenomatous polyps obtained from a patient with Turcot's syndrome. The rearrangement and amplification as well as overexpression of c-Ha ras gene in one colonic adenomatous polyp were determined. The amplified c-Ha ras gene in this polyp revealed larger fragments of BamHI, PstI and SacI than those in the normal colonic mucosa from the same patient. But, such abnormalities were not observed in other polyps. No abnormalities of c-Ki ras, c-N ras or c-myc gene were observed in any polyps. These results suggest that the alternations of c-Ha ras gene in this patient may not be responsible for the adenomatous change, but may be related to the transition from adenoma to carcinoma of the colon.

Key words: c-Ha ras proto-oncogene, Colonic polyp, Malignant potential

Through the years there has been considerable interest in colonic carcinogenesis. Various investigations have been performed on the development of carcinoma in the colon. Molecular biological analysis has revealed chromosomal (1, 2) and proto-oncogene (3-10) abnormalities in some colonic tumors.

Many adenomatous polyps in the colon and rectum are present from childhood in familial polyposis coli (FPC). If left untreated, colon carcinomas will arise from those polyps in all affected patients. Therefore, FPC seems to be a good model to understand the process of carcinogenesis. Chromosomal (1, 11) and proto-oncogene (12-15) abnormalities have also been reported in adenomas and carcinomas of the colon in FPC patients, and the gene for FPC was recently mapped on chromosome 5 (16, 17). However, the relationship of these abnormalities and carcinogenesis is still largely obscure. FPC is often associated with extra-colonic lesions (18, 19). An increased risk of upper gastro-intestinal carcinomas in polyposis patients (20, 21) has been reported. Polyposis coli with brain tumor is called Turcot's syndrome, but several authors have described that this term should remain restricted to those cases with polyposis coli in association with gliomas (22). Colonic polyps of patients with Turcot's syndrome are thought to develop carcinomas as those of FPC. Although FPC is inherited as an autosomal dominant trait, the mode of inheritance of Turcot's syndrome has not been conclusively established (22-25). In addition, the molecular biological analysis of Turcot's syndrome has not yet been reported.

In the present study, the proto-oncogenes in colonic and gastric polyps obtained from a patient with Turcot's syndrome were analyzed in order to reveal a relationship of proto-oncogenes and tumorigenesis in the gastro-intestinal tract of this syndrome.
METHODS

The subject of the present study

A 40-year-old man was admitted because of consciousness disturbance. A brain tumor, polyposis coli and four gastric adenomas were demonstrated by cranial CT, barium enema and endoscopic examination. No polyps were found in the small intestine. The brain tumor was surgically resected. The histologic diagnosis was astrocytoma (grade II). Then, he was diagnosed as having Turcot's syndrome. No abnormalities were observed on the skin. Analysis of lymphocyte prometaphase chromosomes showed no abnormalities. Radiographs of the jaw revealed no abnormalities. His parents were first cousins, and his paternal grandparents were consanguineous, too. He had three sisters. His younger sister died at the age of 20 of unknown cause. No brain tumors by cranial CT were found in his two older sisters. No colonic polyps by colonoscopy were found in one of these two older sisters, but the other with no visceral symptoms had no examination on the colon. There was no history of colonic or brain tumors in the other family members. In this patient colectomy and ileo-rectal anastomosis were performed after this study. No colon carcinomas were demonstrated in the surgical specimen.

Samples

Samples, endoscopically obtained from the patient with Turcot's syndrome, were as follows: seven colonic polyps (sample Nos. 1–7) by polypectomy, several biopsy specimens from each of three gastric polyps (sample Nos. 8–10), normal colonic mucosa and normal gastric mucosa. A peripheral blood sample was obtained from this patient. These samples were quickly frozen and maintained at −70°C until analysis. Histologic analysis was performed on each gastrointestinal sample.

Probes

Nucleotide probes used were as follows: c-Ha ras (SacI-Sacl), v-Ki ras (PvuII-PvuII), c-N ras (PvuII-PvuII, exon1), c-myc (Clal-EcoRI, exon3), gastrin (HindIII-HindIII, exon2) and β-actin (HindIII-EcoRI). These probes were labeled by a mixed random primer system using α-32P-deoxy CTP (26).

DNA extraction and Southern blot analysis

Genomic DNA was extracted from each sample by a previously described method (27), digested with the appropriate restriction enzymes, electrophoresed in a 0.7% agarose gel, transferred to a GeneScreen Plus membrane, and hybridized with radioactive probes as described by Southern (28). Gastrin probe (29) was used as the internal control.

RNA extraction and Northern blot analysis

Total RNA was extracted from seven colonic polyps (sample Nos. 1–7), one gastric polyp (sample No. 8), normal colonic mucosa and normal gastric mucosa by the acid guanidium-phenol-chloroform method (30). Ten micrograms of total RNA from each sample was electrophoresed, transferred and hybridized with radioactive c-Ha ras, v-Ki ras, c-N ras and c-myc probes, respectively (27), and rehybridized with radioactive β-actin probe.

RESULTS

Southern blot analysis

Digested with BamHI, c-Ha ras gene revealed a remarkably amplified 8.6 kb band, a moderately amplified 7.4 kb band and a normal 6.6 kb band in sample No. 1, but revealed only a normal 6.6 kb band in sample No. 2, normal colonic mucosa and sample No. 8, compared with the internal control, a 3.8 kb gastrin band (Fig. 1). It was demonstrated that the amplified bands were not the gastrin gene, hybridized with only gastrin probe. C-Ha ras gene revealed a 6.6 kb BamHI fragment in other polyps (sample Nos. 3–7, 9, 10), normal gastric mucosa and peripheral blood obtained from the patient with Turcot's syndrome. C-Ki ras and c-N ras genes revealed 3.0 kb and 9.0 kb EcoRI fragments in normal tissues, peripheral blood and all polyps including sample No. 1. C-myc gene revealed a 12.2 kb EcoRI fragment in all samples. No amplification or rearrangement of c-Ki ras, c-N ras or c-myc genes were observed in any colonic or gastric samples.

In an attempt to characterize the rearranged c-Ha ras locus of the polyp (sample No. 1), genomic DNA of sample No. 1 and normal colonic mucosa obtained from this patient with Turcot's syndrome was digested with restriction enzymes BamHI, SacI and PstI, respectively. Southern blots were probed with c-Ha ras gene (Fig. 2). C-Ha ras gene showed 8.9
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Fig. 1. Rearrangement and amplification of c-Ha ras gene. Genomic DNA of two colonic polyps (sample Nos. 1, 2), normal colonic mucosa and one gastric polyp (sample No. 8) obtained from the Turcot's syndrome patient was digested with BamHI. Southern blots were probed with c-Ha ras and the internal control gene, gastrin. A 8.6 kb remarkably amplified band, a 7.4 kb moderately amplified band and a 6.6 kb normal band of c-Ha ras gene in lane 2 and normal 6.6 kb c-Haras bands in lanes 1, 3, 4 are observed compared with control, a 3.8 kb gastrin band in each lane. Lane 1, sample No. 2; lane 2, sample No. 1; lane 3, normal colonic mucosa; lane 4, sample No. 8.

Fig. 2. Rearrangement of c-Ha ras gene. Genomic DNA of the colonic polyp (sample No. 1) and normal colonic mucosa from the Turcot's syndrome patient was digested with BamHI, PstI and Sacl. Southern blots were probed with c-Ha ras gene. C-Ha ras gene revealed amplified 8.6 kb, 7.4 kb and normal 6.6 kb BamHI fragments in lane 1, and a 6.6 kb BamHI fragment in lane 4. C-Ha ras gene showed 9.0 kb and 7.8 kb PstI fragments in lane 2. But, digestion with PstI c-Ha ras gene was observed as only faint bands of 3.9 kb, 1.0 kb and 0.8 kb in lane 5, likely because of the small size of the hybridizing DNA sequence. C-Ha ras gene revealed 8.9 kb and 6.2 kb Sacl fragments in lane 3, and a 2.9 kb Sacl fragment in lane 6. Lanes 1–3, sample No. 1 were digested with BamHI, PstI and Sacl, respectively. Lanes 4–6, normal colonic mucosa from the Turcot's syndrome patient were digested with BamHI, PstI and Sacl, respectively. An internal control was not used in this experiment.

Northern blot analysis

In the colonic polyp (sample No. 1) with rearrangement and amplification of c-Ha ras gene, overexpression of this gene was observed (Fig. 3). The size of c-Ha ras mRNA of sample No. 1 was 1.4 kb, which was the same as previous reports. C-Ki ras and c-N ras mRNAs in sample No. 1 revealed the same intensity bands as in other colonic polyps (sample Nos. 2–7) and in normal colonic mucosa.

C-myc was observed as a 2.5 kb band, and overexpression of this gene, frequently reported in colonic polyps, was not detected in seven colonic polyps (sample No. 1–7). Overexpression of ras gene family and c-myc was not detected in sample No. 8, compared with normal gastric mucosa.

Histologic analysis

Histologic examination of seven colonic polyps (sample Nos. 1–7) revealed vilious adenomas with severe atypia (Figs. 4a and 4b). No distinctive difference on histologic examination was observed among these seven colonic polyps. Histologic examination of three gastric polyps (sample Nos. 8–10) revealed adenomas with mild atypia (Fig. 4c).
Fig. 3. A: Overexpression of c-Ha ras gene. Total RNA (10 µg) of four colonic polyps (sample Nos. 1–4), one gastric polyp (sample No. 8), normal colonic mucosa and normal gastric mucosa from the Turcot’s syndrome patient was electrophoresed and hybridized with c-Ha ras probe. B: The same filter was rehybridized with radioactive β-actin probe. Over-expression of c-Ha ras gene is observed in lane 6. Lane 1, normal gastric mucosa; lane 2, sample No. 8; lanes 3–5, sample Nos. 2–4 respectively; lane 6, sample No. 1; lane 7, normal colonic mucosa.

DISCUSSION

C-Ha ras has been found as a normal cellular counterpart of Harvey murine sarcoma viral transforming gene (31), and its abnormalities have been reported in some human malignancies (3–6). In the present study, rearrangement and amplification as well as overexpression of c-Ha ras gene were detected in one colonic adenomatous polyp obtained from a patient with Turcot’s syndrome. This is the first report of rearrangement and amplification as well as overexpression of c-Ha ras gene in colonic adenomatous polyps. The amplified c-Ha ras gene in sample No. 1 revealed larger fragments of BamHI, PstI and ScaI than those in normal colonic mucosa from the same patient. These results suggest that the rearranged c-Ha ras locus included the sites of these three enzymes, and that the rearranged locus may be associated with gene amplification rather than rearrangement occurring during the process of amplification of c-Ha ras gene.

On Northern blot analysis, c-Ha ras gene overexpression was observed only in the colonic polyp with rearrangement and amplification of c-Ha ras gene. These results offer two possible explanations. First, the gene amplification may lead to overexpression. Second, the rearranged locus may play a role in increasing gene transcription.

In the present study, abnormalities of c-Ha ras gene were observed in only one colonic adenomatous
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polyp, but not in the other six colonic adenomatous polyps of this patient with Turcot's syndrome. No distinctive difference on histologic examination was found among those seven colonic polyps. These results suggest that the alternations of c-Ha ras gene observed here are not likely responsible for the adenomatous change. Of the many polyps present in the colon of polyposis patients, only a few will become carcinomas during a normal lifespan. C-Ha ras gene overexpression has been reported to be more frequent and more remarkable in colon carcinomas than in colonic adenomatous polyps (3-5, 12). Ha-ras amplification was observed during mouse skin carcinogenesis (32), and human progressive colon carcinomas (33). Taking these findings into account, the adenoma with rearrangement and amplification as well as overexpression of c-Ha ras gene might have higher malignant potential, even if there is no histologic difference, than others with no alternations of c-Ha ras gene. Such an adenoma, accompanied by other factors, might develop malignant transformation in this patient. If so, our results that abnormalities of c-Ha ras gene were not detected in gastric adenomas of this patient would not be inconsistent with previous reports (20, 21) that upper gastrointestinal carcinomas in general arise after the mean age of development of colon carcinomas in polyposis patients.

The genetics of Turcot's syndrome has caused much controversy, and many authors (22-25) have argued whether the mode of inheritance is autosomal dominant or autosomal recessive. There were no histories of colonic tumors or brain tumors in other family members of this patient. It is, therefore, unlikely that it is an autosomal dominant mode of inheritance in this case.

Since several rectal polyps and four gastric adenomas, which seem to have high malignant potential, were residual after surgical operation in this patient, a regular endoscopic follow-up is necessary in this patient. Also, molecular biological analysis of those polyps would be useful to give foresight into their malignant transformation and lead to early treatment. Moreover, these studies would provide a clue to the disclosure of the mechanisms of the transition from premalignant to malignant lesion of the gastro-intestine.

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REFERENCES


