Turcot’s Syndrome of Glioma and Polyposis Occurs in the Absence of Germ Line Mutations of Exons 5 to 9 of the p53 Gene

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Abstract

The term “Turcot’s syndrome” has been used to describe approximately 55 patients with an association of colonic polyposis and primary neuroepithelial tumors of the central nervous system. The p53 tumor suppressor gene is a possible candidate underlying the syndrome because (a) mutations in the p53 gene are ubiquitous in human cancer, including colon carcinoma and gliomas, and (b) somatic or germ line mutations of the p53 tumor suppressor gene cause the Li-Fraumeni syndrome, which is characterized by the association of breast and soft tissue tumors. We determined the DNA sequence of the conserved regions of the p53 gene (exons 5 to 9) in the tumor tissues and lymphocytes of two patients with polyposis-lymphoma and found that mutations did occur as independent tumor-specific alterations but did not involve the germ line of these patients, suggesting that p53 may play a role in progression but not initiation of the disease.

Introduction

The rare association of colonic polyposis with primary neuroepithelial tumors of the central nervous system was described as early as 1949 by Crail (1) and a genetic basis for this association was first proposed by Turcot in 1959 (2). Since then the term “Turcot’s syndrome” has been used to describe this association but controversy has continued as to the mode of inheritance of such a trait and whether the syndrome is a genetic disorder distinct from familial polyposis, whether it represents a random association between two separate diseases (colonic polyposis and gliomas), and whether the sporadic simultaneous occurrence of the diseases results from similar lesions as the familial form. In an attempt to address this, Lewis et al. (3) divided the reported cases into 3 types: type I, those with two or more siblings with multiple colonic polyps and a malignant brain tumor, with neither the parents or other generations being affected with either; type II, affected individuals having an autosomal dominant colonic polyposis syndrome and polyps occurring in several generations; and type III, isolated nonfamilial cases that could not be classified in the two first groups. This analysis led to the proposal that Turcot’s syndrome was most likely autosomal dominant and that it should be considered as an additional phenotypic variant of familial polyposis. In contrast, Itoh and Ohsato (4) divided the reported cases into 3 groups based on the number or size of colonic polyps and concluded that Turcot’s syndrome was distinct from familial polyposis. Nonetheless, under either consideration, the occurrence of the two rare diseases together seemed stochastically unlikely to be due to chance.

Recently, the Li-Fraumeni syndrome of associated breast and soft tissue tumors has been shown to be often due to the germ line transmission of mutations in the tumor suppressor gene, p53 (5, 6). This gene, which is resident in the chromosome 17p region of the genome, is the most commonly altered target for mutations in human cancer (reviewed in Refs. 7 and 8). Particularly interesting with regard to the phenotype of the Turcot syndrome is the well-established high frequency of p53 alteration in colon carcinomas (9-12) and gliomas (10, 13-18) and it is noteworthy that in the latter these alterations occur early in their malignant progression (13, 15).

Here we have tested the hypothesis that constitutional mutation of the regions of the p53 gene that are commonly altered in sporadic colon and astrocytic tumors is the underlying etiology of the rare Turcot’s syndrome. We describe DNA sequencing analysis of the p53 genes in the normal and tumor tissues of a familial and a sporadic case of this syndrome. The analyses indicate that p53 mutations in exons 5 to 9 do occur in the tumors of these patients but that significant mutations in these evolutionarily conserved regions do not occur in their germ line.

Materials and Methods

Patients and Tissue Samples

Lausanne Patient. The clinical history of Caucasian patient G-A. J. has been described previously (case 4 in Ref. 19; Ref. 20). Briefly he had a family history of gastrointestinal and brain malignancies and was himself affected by a glioblastoma in 1979 (Fig. 1A). Five years later he developed a Dukes stage B adenocarcinoma of the cecum measuring 6 cm with no synchronous polyps (Fig. 1B). Rare foci of invasion were found in the lymphatic vessels but no venous invasion was present. Another 8 years later, he presented with a Dukes stage A adenocarcinoma of the rectosigmoid region measuring 6 cm. Three synchronous polyps were found measuring 0.5 to 1.2 cm; two of them were tubular adenomas and the third one was a hyperplastic polyp. Both colonic carcinomas were histologically well differentiated and the 35 mesenteric lymph nodes that were sampled showed no evidence of metastasis (20). The patient is still alive with no evidence of recurrence (3 years after subtotal colectomy) and has never undergone chemotherapy. It had been previously established that his sister died of a brain tumor, most likely a glioblastoma (case 3 in Ref. 19) and a new extended family history revealed that several other members of the family had gastrointestinal malignancies, although only one of these was of young age (Fig. 2). These features allowed the classification of patient G-A. J. as a familial case of Turcot’s syndrome subclassified as Lewis type I and Itoh and Ohsato group I (3, 4).

Montreal Patient. Patient G. G. (not previously reported) was a 47-year-old Caucasian male working in a fish plant. He developed seizures 10 years prior to admission. The patient’s parents, ages 70 and 78 years, his three sisters, one brother, and his daughter are alive and have no clinical evidence of neurological or abdominal diseases. Two other brothers died by accident. Patient G. G. presented with a tumor mass in the brain occupying the left
Fig. 1. H & E-stained formalin-fixed, paraffin-embedded sections of the glioblastoma (A) and colon carcinoma of 1981 (B) of the Lausanne patient and the low grade astrocytoma (C) and colon carcinoma (D) of the Montreal patient. Arrowheads in D, clusters of poorly differentiated adenocarcinoma cells invading the lamina propria and the submucosa. A, B, and D, × ~100; C, × ~260.

Fig. 2. Pedigree of the family of the Lausanne patient. □, ○, individuals without evidence for malignant disease including their offspring; □, glioblastoma at age 21 years, colon carcinoma Dukes stage B at age 26 years, colon Dukes stage A and colon polyps at age 35 years; ●, brain tumor at age 16 years; □, stomach cancer at age 37 years; □, rectal cancer at age 75 years; ●, intestinal cancer at age 78 years.

Frontal lobe and extending to the corpus callosum. Surgery was performed, and a low grade astrocytoma was removed (Fig. 1C). Two months later the patient was found to have an 8-cm tumor mass in the ascending colon. A right hemicolectomy was performed demonstrating a poorly differentiated adenocarcinoma (Fig. 1D) invading the full thickness of the colonic wall. There was conspicuous invasion of veins and lymphatic vessels by the tumor. In addition, examination of the colonic mucosa away from the tumor showed 21 adenomatous polyps including one with focal early invasive carcinoma. Metastasis was present in all ten mesenteric lymph nodes that were removed. There was no evidence of liver metastasis and therefore the carcinoma was classified as Dukes stage C. Flow cytometry performed on a cell suspension prepared from the fresh colonic specimen showed an aneuploid cell population with a DNA index of 1.9 compared to the diploid peak of the same sample. Furthermore, there was a high proliferation index with 25% S + G2/M fraction. Postoperatively the neurological condition of the patient deteriorated and he died 3 weeks after the resection of the colonic tumor. Although no autopsy was performed his death was most likely due to disseminated intravascular coagulation. Patient G. G. was recognized as a sporadic case of Turcot’s syndrome and subclassified as Lewis type III and Itoh and Ohsato group I (3, 4).

Tissue Sample. DNA from lymphocytes and from paraffin-embedded tissue samples of the astrocytoma, glioblastoma, and the non-polyposis colon cancers were extracted by standard methods (21). A primary culture of the second colon carcinoma of the Lausanne patient was established and could be maintained for 4 months. It showed slowly growing, well differentiated adenocarcinomatous cells that were also used for DNA extraction. Histologies of each of the tumors are shown in Fig. 1.

Amplification and Sequencing of the p53 Locus

Lausanne Patient. One µg of genomic DNA was denatured for 5~8 min at 98°C and then amplified for 20~30 cycles at 94°C for 40 s, 52°C for 1 min, and 74°C for 2 min with 1 unit of Thermus aquaticus DNA polymerase (Replitherm; Epicentre Technologies, WI) in 10 mM Tris-HCl, pH 8.3~1.5 mM MgCl2-50 mM KCl-0.005% Tween 20-0.005% Nonidet P-40-0.01% gelatin in the presence of 1 µM oligonucleotide primer sets p53IN4AL (5’-AGAGGAATTCTTCTTCCTCTCAGTAC-3’) and p53IN7BL (5’-TTGAGGATCCCATGTCAGTGGCTCCTGACCTG3’) for exons 5, 6, and 7 or p53IN7AL (5’-AGAGGAATCTTCTTCTCTCTGCAGTAC-3’) and p53IN9BL (5’-TTGAGGATCCCAAGACTTAGTACCTGAA-3’) for exons 8 and 9. Three independent polymerase chain reaction-amplified DNA sequences were mixed and the amplified fragment of 1100 base pairs was isolated and cloned into the EcoRI/


**Results and Discussion**

We focused our attention on exons 4 or 5 to 9 of the *p53* gene since these genomic regions contain the great majority of sporadic *p53* mutations found in brain, colon, and other tumor types (7, 8). These hot spots overlap the highly evolutionarily conserved regions that are likely involved in *p53* function (22). In order to minimize the chances of our detecting an amplification-generated artifact in the sequence, we mixed the amplification products of three independent analyses, picked at least six independent clones from each, and determined their sequence individually whenever possible.

**Lausanne Patient.** The DNA sequence obtained with DNA from paraffin-embedded colon adenocarcinomas that occurred in both 1981 (14 clones sequenced) and 1990 (27 clones sequenced) showed the presence of 3 different alleles (Fig. 3). One allele (7 of 14 and 10 of 27 clones sequenced) had 3 mutations: a GAC (Asp) to GGC (Gly) transition at codon 228, an AAC (Asn) to AGC (Ser) transition at codon 235, and disruption of the splice acceptor site of intron 6 by a G to T point mutation (AG to AT). The second allele (2 of 14 and 5 of 27 clones sequenced) had a GAC (Asp) to GGC (Gly) transition at codon 173, an AAC (Asn) to AGC (Ser) transition at codon 235, and disruption of the splice acceptor site of intron 6 by a point mutation (AG to AT). The third allele (5 of 14 and 12 of 27 clones sequenced) had a GAC (Asp) to GGC (Gly) transition at codon 173, an AAC (Asn) to AGC (Ser) transition at codon 235, and disruption of the splice acceptor site of intron 6 by a point mutation (AG to AT), suggesting the possible generation of aberrant *p53* transcripts from this allele. The second allele (2 of 14 and 5 of 27 clones sequenced) had a GAC (Asp) to GGC (Gly) transition at codon 228. The third allele (5 of 14 and 12 of 27 clones sequenced) was wild type, suggesting either that tumor cells with different combinations of mutant and wild type alleles coexisted in the tumor or that normal tissue was present within the specimens used for DNA sequencing. Interestingly neither the 3 missense mutations nor the splice acceptor site mutation have been described previously for colon cancer (7, 8, 23). The occurrence of the same mutated alleles in the colon carcinomas from 1981 and 1990 (operated in two different hospitals, thus excluding trivial sample mixing) suggests that these tumors had a common clonal origin.

In order to determine if any of these mutations was present in the germ line of this patient who had a cancer-prone familial history (Fig. 2), we determined the nucleotide sequence of these regions of the *p53* gene in the DNA of his paraffin-embedded glioblastoma (12 clones sequenced) and his peripheral blood lymphocytes (6 clones sequenced). Neither these nor any other mutations of the *p53* gene were detected in either tissue (Fig. 4). In addition, to test whether the patient could be a mosaic with mutations only in the endodermal layer, we amplified the DNA for exon 7 from a sample of normal colon epithelium and found no mutations (5 clones sequenced). This strongly
suggests that germ line mutations in exons 5 to 9 of the p53 gene do not underly the Turcot's syndrome in this patient.

Montreal Patient. The histopathological changes found in the colonic tumor of patient G. G. were remarkable for tumor dedifferentiation and widespread vascular invasion, consistent with the aggressive biological and clinical behavior which terminated in rapid death. In order to determine whether small deletions or point mutations of the p53 gene were present, we sequenced the majority of exon 4 to exon 9 sequences in PCR-derived clones from normal colon, astrocytoma, and colon adenocarcinoma (Fig. 4), as well as exons 5 to 6 of an adenomatous colon polyp (data not shown). In each tissue type (16 clones sequenced totally), a G to A change was observed at position 224 at the intron 4 to exon 5 boundary (Fig. 3). This change occupies the n position of the 3' acceptor splice site consensus sequence y_{14}^t y_{15}^n CAG/g (24) where y = C or T (pyrimidine), t = thymidine, and n represents any nucleotide. Position —¿I (n) is considered to be a functionally irrelevant position and its alteration would not be expected to lead to a splicing alteration. We surmise that this base substitution represents a DNA sequence polymorphism that is neutral in effect. Precedent for a functionally neutral constitutional polymorphism in the p53 gene has been reported in a breast cancer family (25).

A point mutation at codon 282 (CGG to TGG) was found in 1 of 4 clones for the astrocytoma which results in an 2S2Arg to Trp transition (Fig. 3); a double mutation at this amino acid has previously been reported in a small cell lung carcinoma (23). This mutation was not observed in the normal colon (7 clones sequenced) or the colon adenocarcinoma (3 clones sequenced), where all clones were found to be wild type in sequence in the corresponding region (Fig. 4).

Although PCR amplification of the p53 gene in the paraffin-embedded tissues of this patient yielded only a limited number of clones for each tissue type, the data suggest that a mutation may exist in the p53 gene in the astrocytoma and that it is not inherited. In order to determine whether mutations exist in the colon adenocarcinoma outside the sequenced regions or may have been undetected by sequencing due to the limited number of clones available, we analyzed p53 expression by immunohistochemistry using monoclonal antibody PAb1801. Frozen tissue was unavailable for the brain tumor. Immunostaining of the frozen tissue sections prepared from the colonic tumor of patient G. G. showed strong nuclear staining in approximately one-half of the tumor cell nuclei (data not shown). The high expression indicated by staining with this antibody suggests that a mutant p53 was likely expressed in this tissue and that it may have played a role in the progression of this tumor.

In conclusion, the results obtained with tissues from these two patients [who fulfill the criteria of Turcot's syndrome according to its original description (2)] strongly preclude the p53 gene as the underlying factor in germ line predisposition to the syndrome (this analysis could not rule out that the patients were mosaics of normal and mutant alleles in a proportion too low to be detected). However, diverse mutations were shown to occur in the tumor specimens of these patients suggesting that p53 plays a role in the progression of the disease. It should be noted that although the number of cases we have been able to examine is small, only about 55 total cases have been reported in the literature. It may yet be that Turcot's syndrome is genetically heterogeneous with some cases arising by a germ line p53 mutation and others, like those we have examined here, by mutations in other loci. One potential candidate is the adenomatous polyposis coli gene which is the germ line cause of polyposis coli (26-28); its sequence remains to be examined in Turcot's syndrome patients.

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References

p53 MUTATIONS IN TURCOT’S SYNDROME

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