SMAD4-related Familial Juvenile Polyposis Syndrome with Colon Cancer

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Abstract. Background: Juvenile polyposis syndrome (JPS) is a rare autosomal dominant disorder characterized by the development of multiple hamartomatous polyps in the gastrointestinal tract with an increased risk of malignancy. SMAD4 germline mutations account for about a third of JPS. Patients and Methods: We describe, in the same family, the morphological and genetic aspects of two cases of JPS with colon cancer in one patient. Results: Both cases were characterised by diffuse colorectal and gastric involvement by typical juvenile polyps as well as "atypical" multilobulated and densely epithelial polyps with some dysplastic areas. A germline mutation of SMAD4 was demonstrated in both cases. SMAD4 protein and DNA analyses were performed on the colonic adenocarcinoma showing a lack of expression of SMAD4 protein and loss of heterozygosity at the SMAD4 locus. Conclusion: These two exceptional familial cases underline the fact that the morphological features of JPS associated with SMAD4 mutations are different from those found in non SMAD4 mutated cases: polyps are more widespread in the upper GI tract with massive gastric polyposis and they have a dense epithelial component. This study also confirmed that SMAD4 genetic analysis is useful for the diagnosis of JPS and may be predictive of an increased risk of malignancy through inactivation of both alleles of SMAD4.

Juvenile polyps may affect up to 2% of children and adolescents and are the most common type of gastrointestinal polypoid lesions in childhood (1). Most are solitary and confined to the rectosigmoid region. Histologically, they are characterized by cystically dilated epithelial crypts and an inflammatory component. They are usually described as hamartomas in the literature and they do not appear to carry an increased risk of malignancy. They must be distinguished from polyps arising in the setting of juvenile polyposis syndrome (JPS) which have a malignant potential.

JPS is a rare autosomal dominant condition defined by: (i) more than five juvenile polyps of the colorectum; and/or (ii) juvenile polyps throughout the gastrointestinal tract; and/or (iii) any number of juvenile polyps with a family history of juvenile polyposis (1). JPS polyps, although usually histologically benign, may sometimes look dysplastic (2) and JPS has long been known to carry an elevated risk of cancer of the gastrointestinal tract (1,3).

SMAD4 is a tumor suppressor gene that is critical for transmitting signals from transforming growth factor (TGF) beta and related ligands (4,5). In pancreatic and sporadic colorectal cancer, inactivation of the SMAD4 gene through homozygous deletion or intragenic mutation frequently occurs in association with malignant progression (6-9). Mutation of this gene is occasionally seen in other human cancers (10). Germline mutations in SMAD4 account for about a third of JPS cases (11,12) while some other JPS cases have been associated with BMPRL1A mutations (13) or PTEN mutations (14,15).

In a familial JPS, a germline point mutation in the SMAD4 gene was found in two first degree relatives (father and daughter) both suffering from widespread and florid polyposis with upper gastrointestinal involvement. The father developed a colon adenocarcinoma with SMAD4 allelic loss.

Abbreviations: JPS, juvenile polyposis syndrome; PCR, polymerase chain reaction; LOH, loss of heterozygosity; TSG, tumor suppressor gene.

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and lack of SMAD4 protein expression. These observations confirm the peculiar pathological features of the polyps in SMAD4-related JPS cases and their increased risk of malignancy through inactivation of both SMAD4 alleles.

**Patients and Methods**

**Clinical history.** This was analysed for patient 1 (the father) and patient 2 (the daughter).

**Pathological and immunohistochemical analysis.** Surgical and endoscopic samples of juvenile polyps in both patients and of adenocarcinoma in patient 1 were fixed in formalin, embedded in paraffin and stained with hematoxylin and cosin. SMAD4 and p53 protein immunohistochemistry was performed on normal colonic mucosa, benign juvenile polyps and colonic adenocarcinoma with an indirect immunoperoxidase method using clone B-8 (Santa Cruz Biotechnology ; dilution 1/100) and clone DO-7 (Dako ; dilution 1/100) as primary antibodies directed against SMAD4 and p53, respectively. Cytoplasmic SMAD4 immunostaining was scored as either present or strongly decreased / absent as compared with normal epithelial cells. Nuclear p53 immunostaining was scored as the percentage of stained cells in three different fields at magnification 250.

**Germline mutation analysis.** Blood samples of both patients were collected in order to search for germline mutations for SMAD4, BMPR1A and PTEN genes. Coding exons and adjacent intronic regions of the 3 genes, including consensus splicing sequences, were screened by sequencing after PCR amplification of genomic DNA. Mutations were identified using the phredPhrap and consed package.

**Allelic loss analysis.** This analysis was performed in patient 1. Epithelial carcinomatous areas and normal areas were microdissected on paraffin sections with a laser microbeam microdissection system (PALM, Bernried, Germany). Tissue fragments were immersed overnight in lysis buffer (50 mM Tris-HCl pH 7.5, 1mM EDTA, 0.5% Tween20, 0.2mg/ml proteinase K).

We analyzed the allelic profiles with the automated DNA analysis system ALFExpress II (Amersham Pharmacia Biotech, Uppsala, Sweden).

**Results**

**Clinical history.** A 30-year-old male (patient 1) presented in 1963 with rectal bleedings. Barium enema showed multiple colorectal polyps. Biopsies followed by electrocoagulation of the polyps were performed by rectoscopy. In 1973, a control barium enema showed polyps in the right colon and a larger mass that was diagnosed as adenocarcinoma and treated by right hemicolecction and adjuvant chemotherapy. Clinical follow-up was marked by recurrences of colonic and rectal benign polyps. In 1998, a control gastrointestinal endoscopy showed diffuse polypoid hyperplasia of the gastric folds. No further treatment was performed.

The familial investigation showed that patient 1’s daughter (patient 2) also had a large number of polyps in the stomach, colon and rectum and had suffered from abdominal pain and rectal bleedings since the age of 11. Patient 2 successively underwent colectomy (1973), proctectomy (1994) and gastrectomy (1998). She did not develop gastrointestinal cancer (last examination in 2003).

The follow-up period for both patients has been almost 40 years and no polyp has been observed in the small bowel. There was no tumor outside the intestinal tract. The physical examination revealed no mental retardation, nor dysmorphism. Both patients are alive and well.

**SMAD4 germline mutation.** A frame-shift germline mutation was detected in both patients at exon 11 of the SMAD4 gene (536 insT, CTA > CTTA) resulting in a truncated protein.

**Pathological features.** Most of the colorectal polyps of both patients had a typical juvenile appearance, macroscopically pedunculated with spherical head, macroscopically composed of normal or cystically dilated crypts lying in a dense inflammatory lamina propria with ulcerated surface. About 20% of the colonic polyps of both patients had an "atypical" presentation at gross examination (Figure 1): they appeared as multiple and closely packed lobes attached to a single long stalk and measured up to 10 cm. Microscopically, each polyp lobe contained either typical juvenile areas or more epithelial areas with a high proportion of hyperplastic glands.

In a few colonic polyps, often those with "atypical" gross features, there were microscopic areas of low-grade epithelial dysplasia with nuclear crowding and hyperchromasia. A single polyp of patient 1 (Figure 1) had a typical aspect of villous adenoma with high-grade dysplasia and a 2.5 cm adenocarcinoma. This well-differentiated tumor infiltrated the polyp base and the muscularis propria, with two lymph node metastases (stage pT2N1).

In the stomach of both patients, sessile or pedunculated polyps ranging from 0.5 to 5 cm extended from the cardia to the pylorus. Microscopically, they had a typical juvenile appearance with numerous glands, lined by a columnar foveolar epithelium.

**Immunohistochemistry.** SMAD4 cytoplasmic immunostaining was present in epithelial cells in non polypoid areas of both patients but absent in polyps of both patients and in adenocarcinoma of patient 1 (Figure 2).

p53 immunostaining was strongly positive in over 80% epithelial cells in low-grade dysplastic areas in both patients and in the malignant tumor (Figure 3), but absent in non dysplastic polyps and in normal areas.
Allelic loss analysis. Patient 1 was homozygous for D18S363 and heterozygous for D18S46. Microdissected carcinomatous cells showed LOH at D18S46 with almost complete disappearance of one allele when compared to normal tissue (Figure 4).

Discussion

We describe two familial cases of JPS with SMAD4 germline mutation and with peculiar morphological features. One patient developed a colon adenocarcinoma associated with loss of the second SMAD4 allele.

The classification of the polyposis in our cases had not been clearly established for several years. After the initial symptoms, the finding of grossly unusual "atypical" polyps and microscopically dysplastic areas in some polyps suggested other diagnoses: familial adenomatous polyposis, hamartomatous polyposis, hyperplastic polyposis. SMAD4 genetic testing has therefore been helpful for the diagnosis of JPS in these two patients (17).

Some polyps in both patients were macroscopically "atypical" multilobulated and microscopically hyperplastic. A previous study (18) of four SMAD4-related JPS patients compared to seven non SMAD4-related JPS patients showed that many polyps arising in the former patients were more epithelial with elongated crypts. The "atypical" polyps already described in 1988 by Jass et al. (1) in some JPS were possibly associated with SMAD4 mutation.

Another genotype-phenotype correlation has been reported in JPS: Friedl et al. (19) showed that SMAD4-related JPS had a remarkable prevalence of massive gastric polyposis as compared to BMPRIA-related cases or cases without identified mutations. We made similar findings in our patients who both presented with widespread and florid gastric polyposis. It is therefore likely that SMAD4-related JPS have a specific pathological phenotype.

We found low-grade dysplastic foci in some polyps in both patients. This finding is rare in sporadic juvenile polyposis but has been reported in approximately a third of JPS patients (2) and foci of low-grade dysplasia may be demonstrated in 50% of "atypical" multilobulated polyps (1). SMAD4 does not seem to play a major role in dysplasia since polyps from both mutation carriers and non mutation carriers had similar frequencies of dysplasia (18). Adenomatous polyposis coli gene could play a more important role in the emergence of dysplasia in JPS polyps (2). TP53 is a crucial cell regulator and tumor suppressor gene (TSG) that plays an important role in the colorectal adenoma-carcinoma sequence (20). TP53 mutations can lead to p53 stabilisation and accumulation that may be detected by immunohistochemistry. In our patients, we observed a diffuse and strong p53 immunostaining in dysplastic areas and in carcinomatous areas. Similarly, Wu et al. (2) showed that p53 overexpression may be observed in dysplastic juvenile polyps but a diffuse and strong staining was found in only a minority of patients with dysplastic polyps. It seems that, as in sporadic colorectal cancer, p53 alterations may play a role in the neoplastic transformation in JPS, especially in the late stages of tumor progression.

SMAD4 was first identified as a tumor suppressor gene in pancreatic cancer (21). Approximately 55% of pancreatic cancers show either homozygous deletion or intragenic mutation of the SMAD4 gene (10,22). In sporadic colorectal cancer, the frequency of SMAD4 gene mutations increases with the progression of tumors, ranging from 10% in intramuscular carcinoma to 35% in carcinoma with metastases (23). Loss of the second allele was observed in 95% of late stage carcinomas with SMAD4 mutations. In other studies, SMAD4 point mutations (8,9) or loss of SMAD4 immunostaining (24) were also more prevalent in late stages colorectal cancer.

It has long been known that JPS carries an elevated risk of malignant neoplasia, approximately 30-40% for colorectal carcinoma and 10-15% for upper gastrointestinal carcinoma (1,3,25). In our patient with germline SMAD4 mutation, we showed the presence of SMAD4 allelic loss and loss of SMAD4 protein expression in the colon adenocarcinoma, suggesting that the "second hit" may have contributed to the tumor progression. Alterations of both SMAD4 alleles or loss of protein expression have also been shown in benign polyps and in small intestinal carcinomas in SMAD4-related JPS (18,26). Therefore, SMAD4 probably acts as a gatekeeper TSG, not only in sporadic colorectal cancer, but also in SMAD4-related JPS. It is still unknown whether SMAD4-related JPS carries a higher risk of malignancy than non SMAD4-related JPS. Whatever the answer to this question, genetic testing among relatives of SMAD4-related JPS patients should lead to a closer clinical and colonoscopic surveillance for individuals bearing the mutation.

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References


Figure 1. (A) "Atypical" lobulated juvenile polyp (from patient 2 colectomy specimen). (B) Long stalk of an "atypical polyp" (arrowhead) and adenocarcinoma (asterisk) infiltrating the base of a villous polyp (arrow) (from patient 1 right hemicolecctomy specimen).

Figure 2. (A) Smad4 cytoplasmic immunostaining in normal epithelial cells of patient 1. (B) Loss of Smad4 expression in adenocarcinoma of patient 1.

Figure 3. Strong p53 nuclear expression in dysplastic mucosa.

Figure 4. Allelic loss at D18S46 in microdissected adenocarcinoma cells of patient 1.