Mutations of adenomatous polyposis coli (APC) gene are uncommon in sporadic desmoid tumours

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Summary Desmoids are locally aggressive, non-metastasizing soft-tissue tumours, whose aetiology is still unclear. In patients affected with familial adenomatous polyposis (FAP), the incidence of desmoids is much higher than in the general population. The APC gene, which is responsible for FAP, is involved in the development of desmoids associated with this syndrome. In this study 16 sporadic and four FAP-related desmoids were analysed in order to investigate the possible involvement of APC in non-syndromic cases also. The 5′ end (exons 1–11) and the coding portion of exon 15 of APC were screened using the in vitro synthesized-protein assay (IVSP). Exons 5, 6, 8–14, and a region of exon 15 spanning codons 1036–1634 were investigated by single-strand conformation polymorphism (SSCP) analysis. APC germline mutations were identified in all FAP patients, but not in sporadic cases. Somatic mutations were found in three FAP-associated desmoids (75%) and two sporadic tumours (12.5%). In one of the latter cases, both alleles were affected. These findings indicate a limited role of the gene in the development of desmoid tumours outside FAP.

Keywords: desmoid; APC; DNA mutation

Desmoid tumours, also known as aggressive fibromatoses (Mackenzie, 1972), are generally considered to be soft-tissue proliferations that do not metastasize, even if they have a marked tendency towards local invasion and a significant risk of recurrence. The neoplastic nature of desmoids has recently been assessed by molecular studies, which demonstrated that these pathological entities are indeed clonal processes (Li et al. 1996; Alman et al. 1997a; Lucas et al. 1997). Whereas desmoids are rare in the general population, representing less than 0.1% of all human tumours, with an incidence of 2–4 cases per million per year (Pack and Ehrlich, 1944; Reitamo et al. 1986), they occur with elevated incidence (8–12%) in patients affected with familial adenomatous polyposis (FAP) (Jones et al. 1986; Gurbuz et al. 1994). FAP is an autosomal dominant genetic condition, characterized by the development of hundreds to thousands of colorectal adenomas (polyps), which almost invariably lead to carcinomas, if prophylactic colectomy is not performed (Bussey, 1975). This syndrome may be considered as a growth disorder affecting multiple body sites, being characterized by the occurrence, in addition to colorectal polyps, of a variety of extracolonic lesions, including thyroid and adenocortical tumours, epidermoid cysts, osteomas and desmoid tumours (Campbell et al. 1994). The latter predominantly occur intra-abdominally or in the abdominal wall, usually after surgery (Jones et al. 1986; Gurbuz et al. 1994), and represent an important cause of morbidity and mortality in FAP (Clark and Phillips, 1996).

The gene responsible for FAP, termed APC for adenomatous polyposis coli, maps to the long arm of chromosome 5 (q21–22) and was isolated in 1991 (Groden et al. 1991; Joslyn et al. 1991; Kinzler et al. 1991; Nishisho et al. 1991).

In addition to germline mutations in individuals affected with FAP, somatic inactivating mutations of the APC gene have been identified in a high proportion of colorectal adenomas and carcinomas, both in FAP patients and sporadic cases (Miyoshi et al. 1992; Powell et al. 1992; Miyaki et al. 1994). This indicates that the suppression of APC activity is a key step in early phases of colorectal carcinogenesis. Although somatic mutations are dispersed along the entire coding region of APC, more than 80% of them are clustered between codons 1281 and 1554.

The complete inactivation of the APC gene, through the mutation or the loss of the constitutionally wild-type allele, was found to be necessary for the development of desmoid tumours that occur in FAP patients (Miyaki et al. 1993; Sen-Gupta et al. 1993; Palmirotta et al. 1995). Also, in these tumours the somatic mutations were found to cluster in a restricted region of APC (codons 1399–1584). The involvement of APC in non-FAP-related desmoids also is indirectly suggested by the finding of deletions affecting chromosome 5q in a subgroup of these tumours (Bridge et al. 1992, 1996). More recently, Alman et al. (1997) reported the identification of APC mutations in three of six sporadic aggressive fibromatoses analysed in exon 15.

In this study, we screened the entire coding sequence of APC in desmoid tumours from patients both with and without FAP in order to investigate the occurrence in the two groups of a possible common molecular mechanism.

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Table 1 Clinical, pathological and genetic characteristics of desmoid cases

| Case no. | Patient sex | Age at diagnosis | Tumour site* | Tumour sizea | APC mutationsa | Anamnestic data |
|----------|-------------|------------------|--------------|--------------|----------------|-----------------|
| 2322     | M           | 20               | Thorax wall  | NA           | –              | Total colectomy at 24 |
|          | 24          |                  | Intra-abdominal | 7           | –              |
|          | 27          |                  | Intra-abdominal recurrence* | 10 | G              |
|          | 28          |                  | Intra-abdominal recurrence | 18 |               |
| 2428     | M           | 15               | Abdominal wall | 11 | G.S            | Restorative proctocolectomy at 12 |
| 2443     | F           | 51               | Intra-abdominal | 11 | G.S            | Hysterectomy at 47; restorative proctocolectomy at 51 |
| 2444     | M           | 30               | Intra-abdominal | 7 | G.S            | Total colectomy at 28 |

Non-FAP-related cases

| Case no. | Patient sex | Age at diagnosis | Tumour site* | Tumour sizea | APC mutationsa | Anamnestic data |
|----------|-------------|------------------|--------------|--------------|----------------|-----------------|
| 2321     | F           | 54               | Right axillary region* | 9 | NI             | –              |
|          | 56          |                  | Recurrence   | 7 | –              |
| 2323     | F           | 53               | Thoraco-abdominal wall | 5 | S              | –              |
| 2324     | F           | 33               | Abdominal wall | 19 | S              | Desmoid development during pregnancy |
| 2325     | F           | 24               | Left shoulder; abdominal wall | 16, 10 | – | Congenital neuropsychic deficit |
|          | 25          |                  | Right thorax wall*, right arm | 10; 12 | NI |
|          | 26          |                  | Shoulder and abdominal recurrences | 4,7 | –|
|          | 28          |                  | Thorax and abdominal recurrences | 25; 25 | – |
| 2429     | F           | 42               | Intra-abdominal | 17 | NI             | Synchronous pancreatic cystadenoma |
| 2430     | M           | 18               | Right hand    | NA | –              | –              |
|          | 19          |                  | Axillary region | NA | – |
|          | 20          |                  | Axillary region recurrence* | 5 | NI |
|          | 21          |                  | Axillary region recurrence | 9 | – |
| 2431     | F           | 22               | Left thigh, posterior aspect | 7 | – |
|          | 31          |                  | Recurrence*   | 3.5 | NI |
| 2432     | F           | 15               | Left thigh, posterior aspect | 23 | NI | Previous local trauma |
| 2434     | F           | 23               | Left thorax wall | NA | – | Left breast excision for fibroadenoma at 20; multiple bilateral ovary cysts at 22 |
|          | 25          |                  | Recurrence*   | 20 | NI |
| 2435     | F           | 57               | Lumbosacral region | 2.5 | NI | – |
| 2437     | F           | 84               | Buttock      | 9 | NI |
| 2438     | M           | 19               | Left axillary region | NA | – |
|          | 25          |                  | Recurrence    | NA | – |
|          | 27          |                  | Recurrence    | 8 | – |
|          | 28          |                  | Recurrence*   | 12 | NI |
| 2439     | M           | 29               | Dorsal cervical region | 5 | – |
|          | 30          |                  | Recurrence*   | 10 | NI |
| 2440     | M           | 65               | Left shoulder | 5.5 | – |
|          | 67          |                  | Recurrence    | 5.5 | – |
|          | 69          |                  | Recurrence*   | 9 | NI |
| 2441     | F           | 81               | Right dorsal lumbar region | 11 | NI | Surgical treatment for breast carcinoma at 73 |
| 2442     | M           | 53               | Intra-abdominal | 12 | NI | Rectum resection for adenocarcinoma at 49; two intestinal polyps endoscopically resected at 51 |

*In cases where more than one tumour occurred, an asterisk indicates the surgical specimen that was analysed in the present study. *Length of larger axis (cm); NA, data not available. ^G, germline; S, somatic; NI, none identified.

MATERIALS AND METHODS

Patient samples

Twenty consecutive cases of desmoid tumours, surgically treated between October 1992 and January 1995 at the Istituto Nazionale Tumori of Milan, were analysed in this study. Four were from FAP patients and 16 were from patients without clinical evidence of FAP by endoscopic examination, and with no cases of FAP or desmoids reported among relatives. Patients’ sex, age at diagnosis, anatomical site of tumours, tumour size and available anamnestic data are reported in Table 1. The samples were frozen in liquid nitrogen immediately after surgery and stored at –80°C until use. When possible, peripheral blood leucocytes (PBLs) were also obtained.

Molecular analysis

DNA and RNA purification and cDNA synthesis were performed as previously described (Pensotti et al. 1997). Polymerase chain reaction–single-strand conformation polymorphism (PCR-SSCP) analysis was carried out using the primers and amplification conditions reported by Groden et al. (1991). PCR products were then heat denatured, loaded on 20% homogeneous Phast-gels (Pharmacia Biotech), run on a Phast System™ apparatus under non-denaturing conditions and visualized by silver staining. In vitro synthesized-protein (IVSP) assay was performed as described elsewhere (Powell et al. 1993). Four overlapping fragments, spanning APC codons 686–1217 (S2), 1099–1693 (S3), 1555–2256 (S4) and 2131–2843 (S5), and covering the entire
Two mutations were identified in sporadic desmoid 2324. One consisted in the deletion of 23 bp from codon 1142 to codon 1149. The other was a nonsense mutation affecting codon 1469. Analysis of PBL DNA of the patient revealed that both mutations were somatic. In order to verify whether the two mutations lay on the same or on different alleles, a region spanning codons 1099–1693, corresponding to IVSP segment S3, was amplified by PCR from tumour DNA and cloned into plasmid vectors. Sequence analyses revealed that the mutations segregated into different recombinant clones (Figure 2). This demonstrated that the identified mutations affected different alleles in desmoid 2324 DNA.

PBL DNAs of eight individuals (the four FAP patients and sporadic cases no. 2324, 2325, 2431 and 2442) were available for LOH analysis. All subjects were informative, i.e. constitutional heterozygous, at the DSS346 locus. Two intragenic polymorphisms, one in exon 11 (Groden et al. 1991), and one in exon 13 (Fodde et al. 1992) were also analysed by SSCP. Two patients, one with FAP (no. 2444) and one sporadic (no. 2324), were informative for both polymorphisms and maintained heterozygosity in tumour DNA, whereas the other six cases were constitutionally homozygous for both polymorphisms (data not shown).

**RESULTS**

The APC gene was investigated in the 20 desmoid tumours included in the study by a combination of two methods (Figure 1). IVSP was used to screen the entire coding portion of exon 15 and, in 12 cases where RNA could be obtained and transcribed into cDNA, a region at the 5' end of the gene including exons 1–11. In addition, SSCP was used to screen exons 5, 6, 8–14 and a portion of exon 15 from segment 15E to segment 15I (codons 1036–1634), according to the subdivision established by Groden et al. (1991). The latter included the mutation cluster region (MCR) of colorectal tumours and the region where all somatic mutations so far reported in FAP-associated desmoids lie.

Mutations were detected in all four cases from FAP patients and in two sporadic tumours (Table 2).

Desmoids from FAP patients carried germline APC mutations, one in exon 5 and three in exon 15. These were identified during a systematic investigation of a large panel of individuals affected with the syndrome (data unpublished). In addition to these mutations, three FAP-related desmoids were found to carry somatic APC mutations, i.e. mutations not present in the corresponding PBL DNA. One mutation consisted in a 5-bp deletion affecting codons 1309–1311, which represents the germline mutation most frequently detected in FAP individuals (Miyaki et al. 1995). The other two were frameshifts: a 1-bp deletion and 1-bp insertion at codons 1534 and 1558 respectively.

In tumour 2323, a sporadic case, a nonsense mutation at codon 1450 was identified. This mutation was not present in the constitutional DNA.

**DISCUSSION**

The screening of the APC gene in FAP- and non-FAP-related desmoid tumours revealed substantial differences between the two groups. Germline APC mutations were identified in all four FAP patients. In contrast, no constitutional abnormalities of the gene were found in sporadic desmoids. It has been reported recently that germline APC mutations are responsible for an inheritable form of susceptibility to desmoids, which may occur in individuals that do not carry the high number of colonic polyps (>100) characteristic of FAP (Eccles et al. 1996; Scott et al. 1996). However, our results suggest that the majority of desmoid tumours that arise in patients without evidence of FAP are not due to constitutional defects in the APC gene.

At the somatic level, APC mutations were found in desmoids both from FAP patients and sporadic cases, but at significantly different frequencies. Three of the four FAP-associated desmoids (75%) carried somatic mutations predicted to lead to the truncation of APC protein products. Although not formally demonstrated, it
is likely that these mutations affected the constitutionally wild-type alleles, thus leading to the complete inactivation of APC, as documented in previous studies (Miyaki et al. 1993; Sen-Gupta et al. 1993; Palmirrotta et al. 1995). On the other hand, truncating APC mutations were observed in only 2 of the 16 sporadic cases (12.5%), a proportion significantly lower than in FAP patients ($P = 0.03$, Fisher’s exact test), and only in one of these did we detect the inactivation of both alleles.

The fraction of mutated sporadic desmoids observed in this study was lower than that reported by Alman et al. (1997b), who identified APC mutations in three of six cases (50%). However, this difference was not significant ($P = 0.10$, Fisher’s exact test), and might be caused by a sampling bias.

It seems unlikely that the low rate of APC mutations detected in sporadic cases, in comparison with FAP-related ones, is attributable to a reduced sensitivity of the screening protocol employed, as both groups were analysed using the same approach. However, it cannot be excluded, at least in theory, that sporadic desmoids carry APC mutations that lie preferentially in those regions of the gene that were not analysed in this study, including regulatory sequences. It is also possible that in sporadic desmoids the APC gene is affected by mutations that are not detectable by the techniques that were used. For example, missense mutations in exonic regions that were analysed only by IVSP would have been missed, as this method only detects nonsense and frameshift mutations. These possibilities, however, would be in contrast with previous observations, which suggest that the development of desmoids mediated by the APC gene requires the presence of at least one allele carrying a truncating mutation near or beyond codon 1444 (Palmirrotta et al. 1995). In fact, our results are in keeping with this hypothesis, as in all cases, both FAP and sporadic, in which APC alterations were identified, at least one allele was mutated, either at germline or somatic level, in a region spanning codons 1450–1558.

Total or partial APC gene deletion, a possibility suggested by cytogenetic observations (Bridge et al. 1992, 1996), should also be considered. Unfortunately, this could not be adequately examined in this study, as the search for allele losses affecting APC could be performed only in four of the sporadic tumours. All were found to be heterozygous for the flanking markers DSS346, but only one case, no. 2324 with somatic mutations in both APC alleles, was informative for the two intragenic polymorphisms investigated.

Even considering the above-mentioned technical limitations, our data suggest that mutations in the APC gene contribute to the development of only a small fraction of sporadic desmoids. The protein encoded by APC takes part in co-ordinated pathways that

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Table 2 Mutations of the APC gene in desmoid tumours

| Case no. | Mutation type | Codons affected | Nucleotide change | Consequence of mutation |
|----------|--------------|----------------|------------------|-------------------------|
|          | G            | 1538           | del(GA)          | Frameshift to stop at codon 1542 |
| 2322     | G            | 935            | TAC→TAA         | Stop at same codon       |
| 2428     | S            | 1558           | ins(A)          | Frameshift to stop at codon 1559 |
| 2443     | G            | 213            | CGA→TGA         | Stop at same codon       |
| 2444     | S            | 1534           | del(G)          | Frameshift to stop at codon 1564 |
|          | S            | 1464–1465      | del(AGAG)       | Frameshift to stop at codon 1471 |
|          | S            | 1309–11        | del(AAAGA)      | Frameshift to stop at codon 1312 |

| Non FAP-related cases |
|-----------------------|
| 2323                  | S            | 1450          | CGA→TGA         | Stop at same codon       |
| 2324                  | S            | 1142–1149     | del 23 bp       | Frameshift to stop at codon 1146 |
| 2325                  | S            | 1469          | CAA→TAA         | Stop at same codon       |

1 G. germline; S. somatic.
2 cDNA sequence Gen Bank accession no. M74068.
control cell to cell adhesion and cell migration (Nathke et al. 1996; Barth et al. 1997). These functions are mediated through binding to different cellular proteins, one of which, the β-catenin, appears to be down-regulated by APC itself (Munemitsu et al. 1995). It is conceivable that disturbances in these cellular pathways may promote the uncontrolled growth of mesenchymal cells, which gives rise to desmoid formation. In principle, different genetic mechanisms may be responsible for these disturbances. In FAP patients the inactivation of both APC alleles is selected for, because of the presence of germline mutations of the gene. In sporadic cases, other genetic alterations may occur with similar probabilities to APC mutations. These alterations might affect genes whose products interact directly or indirectly with the APC protein. Among these, an obvious candidate is the β-catenin gene (CTNNB1), which was recently reported to be mutated in colon cancer and in melanoma cell lines (Morin et al. 1997; Rubinfeld et al. 1997). However, genes mapped to chromosomes 8 and 20, that have been found to be frequently trisomic in sporadic desmoids (Fletcher et al. 1995; Mertens et al. 1995; Qi et al. 1996), should also be considered.

The identification of inactivating APC mutations in desmoids, although limited to a small proportion of cases in sporadic tumours, might have important consequences for the treatment of these neoplasias. At present, the chemotherapy of desmoids is mainly based on the use of non-steroidal anti-inflammatory drugs (NSAIDs) (Clark and Phillips, 1996). Among these, one of the most commonly employed is sulindac. Recently, sulindac was shown to increase the expression of APC mRNA in vitro (Schnitzler et al. 1996), and it has been suggested that this effect might explain, at least in part, the growth inhibition properties of the drug. If this were true, one should expect that sulindac has no or little effect in inducing the regression of desmoids that do not express wild-type APC protein. However, no data are at present available to confirm this hypothesis.

Finally, it must be noted that the two sporadic desmoids in which mutations of APC were detected were also the only two non-FAP-related tumours that occurred in the abdominal wall. Interestingly, 10 out of the 15 FAP-associated desmoids that have been reported to carry somatic APC mutations were also localized in the abdominal wall (Miyaki et al. 1993; Sen-Gupta et al. 1993; Palmirotta et al. 1995 and present study). This might suggest that the presence of somatic APC mutations in desmoid DNA, rather than being dependent on the germline status of the patients, reflects the anatomical site of tumour appearance. However, somatic mutations of APC were also reported in FAP-related desmoids that originated intra-abdominally (Miyaki et al. 1993 and present study), which is by far the most frequent site of occurrence of desmoids in FAP patients (Jones et al. 1986; Gurbuz et al. 1994), but not in the two intra-abdominal sporadic cases (no. 2429 and 2442) analysed in this study.

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