Superficial Fibromatoses are Genetically Distinct from Deep Fibromatoses

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Whereas deep fibromatoses (abdominal, extra-abdominal, mesenteric) display locally aggressive behavior, superficial fibromatoses typically remain small and less likely to recur despite essentially identical morphology. Somatic *β-catenin* or *APC* gene mutations have been reported in ≤74% of sporadic deep fibromatoses and in virtually 100% of Gardner syndrome–associated fibromatoses, whereas genetic events in superficial fibromatoses remain less well characterized. We performed immunohistochemical staining for β-catenin on 29 superficial fibromatoses (22 palmar, 5 plantar, 1 penile, and 1 infantile digital fibromatosis) and 5 deep fibromatoses. Mutations of *β-catenin* and *APC* genes were analyzed in cases of superficial fibromatoses by direct DNA sequencing of the *β-catenin* gene on Exon 3 encompassing the GSK-3 36 phosphorylation region and of the *APC* gene on the mutation cluster region. Nuclear accumulation of β-catenin was present in 86% (25/29) of superficial fibromatosis cases ranging from 5 to 100% of nuclei (mean, 13%; median, 10%), though in a minority of nuclei in most examples. Deep fibromatoses had 60 to 100% nuclear staining in all five cases. No somatic mutations of *β-catenin* or *APC* genes were identified in any of the superficial fibromatoses. In contrast to deep fibromatoses, superficial fibromatoses lack *β-catenin* and *APC* gene mutations; the significance of focal nuclear β-catenin accumulation is unclear. This difference may account inpart for their divergent clinical manifestations despite their morphologic resemblance to deep fibromatoses.

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The fibromatoses are classified as superficial and deep (1). The superficial fibromatoses encompass small lesions of the hands (palmar fibromatoses/Dupuytren's contractures, knuckle pads, infantile digital fibromatoses), feet (plantar fibromatoses/Ledderhose's disease), and penis (penile fibromatoses/Peyronie's disease; 2). Palmar fibromatosis is the most common of these, occurring in 1 to 2% of the population with a male predominance, frequently presenting bilaterally. Plantar fibromatoses occur in a younger age group and are also prone to bilaterality. As the name implies, infantile digital fibromatoses arise in the digits of infants and young children and are characterized by peculiar inclusion-like condensations of cytoplasmic actin (3). Penile fibromatoses are uncommon and tend to present in association with other superficial fibromatoses. The deep fibromatoses (aggressive fibromatoses, musculoaponeurotic fibromatoses, desmoid tumors) are rarer, encountered in two to four individuals per million population per year (4). In the pediatric population, there is a female predominance, and most lesions are extra-abdominal. In patients in their late twenties, there is a female predominance, and lesions are typically of the abdominal wall, whereas in later adult years, there is no sex predisposition and there are proportionally fewer abdominal tumors (5). Tumors are large, and local control can prove difficult, but despite their capacity for local aggression, deep fibromatoses do not metastasize.

Because fibromatoses may be a component of the Gardner syndrome (familial adenomatous polyposis or FAP; 6–8), it is not surprising that virtually all Gardner syndrome–associated lesions harbor mutations of the *APC* gene (9–15). This observation has led to the study of *APC* mutations in sporadic desmoid tumors. Because one function of the *APC* gene involves regulation of the cellular level of

β-catenin, the interaction between these two molecules has been explored in tandem, and although sporadic desmoid tumors may have *APC* mutations (16, 17), they are more likely to have *β-catenin* mutations (18, 19). It appears that the majority of desmoid tumors, both familial and sporadic, have mutations of one of these two genes.

Recently, standard karyotyping analysis of a large series of superficial and deep fibromatoses has revealed clonal chromosomal aberrations in about half of deep fibromatoses and 10% of superficial lesions (20). Loss of 5q, which houses the *APC* gene, was detected in two of the deep fibromatoses in the latter series but not in superficial lesions. Examples of penile fibromatoses were not included, but cytogenetic aberrations have been described in these (21).

Given the fact that superficial fibromatoses are phenotypically similar to deep fibromatoses in that they are both composed of sweeping fascicles of fibroblasts and myofibroblasts but biologically distinct, we studied β-catenin protein accumulation and *β-catenin* and *APC* mutations in a series of these lesions.

MATERIALS AND METHODS

Case Selection

Random cases of superficial fibromatoses with sufficient paraffin-embedded material accessioned between 1994 and 2000 were retrieved from the archives of the Johns Hopkins University Department of Pathology. These years were selected because recent material is less prone to DNA degradation than material from older cases. These included 22 palmar fibromatoses from 21 patients (both of the bilateral lesions were tested in one patient), 5 plantar fibromatoses, 1 infantile digital fibromatosis, and 1 penile fibromatosis. The palmar fibromatoses were from 17 men and 4 women, with a median age of 61 years (range, 23–79 y, mean, 58 y), and bilateral lesions were known to be present in four of the patients. The plantar fibromatoses occurred in three women and two men, at a median age of 47 years (range, 26–60 y, mean, 43 y). The penile fibromatosis was from a 57-year-old man, and the infantile digital fibromatosis was from an 8-year-old boy. None of the patients was known to have the Gardner syndrome. All cases were evaluated by immunohistochemical staining for β-catenin as described below. Five deep fibromatoses were also stained with the β-catenin antibody.

Immunohistochemistry for β-Catenin

Immunoperoxidase stain using diaminobenzidine as the chromogen was performed on the Techmate 1000 automatic staining system (BioTek Solutions, Tucson, AZ). Deparaffinized sections of formalin-fixed tissue were stained with β-catenin antibody (mouse monoclonal antibody, Becton Dickinson Transduction Laboratories, Lexington, KY) at 1:500 dilution after heat-induced antigen retrieval. The percentages of nuclei expressing β-catenin were estimated in each lesion by light microscopy. Normal endothelial cells were used as negative internal controls, and juvenile nasopharyngeal angiofibromas with known β-catenin mutations as positive controls (22).

DNA Extraction

Microdissection of superficial fibromatoses from hematoxylin- and eosin-stained slides for DNA extraction was performed from formalin-fixed, paraffin-embedded specimens. Microdissection was performed using 27-gauge tuberculin needles on un–cover slipped 5-μmsections stained with hematoxylin and eosin under a standard Olympus microscope using the 4× objective. Most of the specimens consisted of lesional tissue alone, but normal skin was dissected in two cases, and skin appendages in four additional cases to serve as nonlesional controls. Genomic DNA was extracted as described elsewhere (23).

Mutation Analysis of the *β-Catenin* Gene

Direct sequencing of Exon 3 of the *β-catenin* gene was performed on 26 superficial fibromatosis cases (19 palmar, 5 plantar, 1 penile, and the infantile digital fibromatosis). Genomic DNA from each sample was amplified by polymerase chain reaction (PCR) using the following primer pair: 5'-ATGGAACCAGACAGAGGGGC-3' and 5'-GCTACTTGTTCTGAGTGAAG-3'. These amplified a 200-bp fragment of Exon 3 of the *β-catenin* gene that encompasses the region for GSK-3β phosphorylation. PCR reaction was performed under standard conditions in a 50-μL volume using PCR Master (Boehringer Mannheim, Mannheim, Germany) and 1 μM of both 5' and 3' oligonucleotides with 40 cycles (94° C for 1 minute, 58° C for 1 minute, and 72° C for 2 minutes). PCR products were treated with shrimp alkaline phosphatase and Exonuclease I (Amersham, Buckinghamshire, United Kingdom) before sequencing. Treated PCR products were sequenced directly with SequiTherm EXCEL II DNA Sequencing Kit (Epicentre, Madison, WI) using the internal primers: 5'-AAAGCGGCTGTTAGTCACTFF-3' and 5'-GACTTGGGAGGTATCCACATCC-3'. Oligonucleotides were end-labeled with (γ-^{32}P)-ATP (NEN DuPont, Boston, MA) using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). These methods have been previously used in our labora-

tory and are known to readily detect mutations (22, 24, 25).

Mutation Analysis of the *APC* Gene

Mutation analysis of the *APC* gene was performed on 20 superficial fibromatosis cases (16 palmar, 3 plantar, 1 penile, and the infantile digital fibromatosis). Four sets of oligonucleotide primers (A1: 5'-CAGACTTATTGTGTAGAAGA-3' and A2: 5'-CTCCTGAAGAAAATTCAACA-3' for Codons 1260 to 1359; B1: 5'-AGGGTTCTAGTTTATCTTCA-3' and B2: 5'-TCTGCTTGGTGGCATGGTTT-3' for Codons 1339 to 1436; C1: 5'-GGCATTATAAGCCCCAGTGA-3' and C2: 5'-AAATGGCTCATCGAGGCTCA-3' for Codons 1417 to 1516; D1: 5'-ACTCCAGATGGATTTTCTTG-3' and D2: 5'-GGCTGGCTTTTTTGCTTTAC-3' for Codons 1497 to 1596) were used to amplify the mutation cluster region of the *APC* gene (23). PCR reaction was performed under standard conditions in a 50-μL volume using PCR Master and 1 mM of both 5' and 3' oligonucleotides with 40 cycles (94° C for 1 minute, 55° C for 1 minute, and 68° C for 2 minutes for APC-B, C, and D primer pairs and 94° C for 1 minute, 52° C for 1 minute, and 68° C for 2 minutes for APC-A). PCR products were purified using shrimp alkaline phosphatase and exonuclease I. Purified PCR products were sequenced directly with SequiTherm EXCEL II DNA Sequencing Kit using the same primers as for DNA amplification. These methods have been previously used in our laboratory and are known to readily detect mutations (22, 24, 25).

RESULTS

Nuclear Accumulation of β-Catenin

Deep fibromatoses had 60 to 100% nuclear staining in all five cases tested (Fig. 1, A and B). Nuclear accumulation of β-catenin was present in 86% (25/29) of superficial fibromatosis cases ranging from 5 to 100% of nuclei (mean, 13%; median, 10%). With the exception of one case (Case 7), all had a low level of expression. These included 22 palmar fibromatoses from 21 patients (both of the bilateral lesions were tested in one patient), 5 plantar fibromatoses, 1 infantile digital fibromatosis, and 1 penile fibromatosis. The single penile fibromatosis did not express β-catenin, and expression in the infantile digital fibromatosis was focal, although varying degrees of nuclear accumulation were seen in the palmar and plantar lesions (Table 1, Fig. 1, C-F). Endothelial cell nuclei and the nuclei of vascular smooth muscle cells showed cytoplasmic staining but no nuclear β-catenin accumulation, and skin appendages and overlying skin lacked expression when these structures were present.

Somatic *β-Catenin* and *APC* Gene Mutations in Superficial Fibromatoses

No somatic mutations of *β-catenin* ($n = 26$) or *APC* ($n = 20$) genes in the mutation cluster region were identified in any of the superficial fibromatoses (Table 1).

DISCUSSION

The *β-catenin* and *APC* genes are closely interrelated and play a significant role in epithelial neoplasms, best studied in colon cancer (18, 26–39), but also in deep fibromatoses (19, 40) and sarcomas (41). β-catenin is an intracellular protein that is regulated by the APC protein. The latter complexes with glycogen synthase 3β (GSK-3β) and thus controls degradation of β-catenin. Mutations of the *APC* gene or the *β-catenin* gene itself can thus result in the nuclear accumulation of β-catenin protein (42, 43). Because β-catenin is a component of the wnt-signaling pathway controlling cell proliferation, its unregulated accumulation leads ultimately to unchecked cell proliferation and neoplasia. A recently described component of the wnt-signaling pathway is AXIN, and mutations of this group of molecules may be responsible for a subset of colorectal carcinomas through activation of β-catenin signaling (44–48).

The superficial fibromatoses are quite similar to deep fibromatoses on histomorphologic grounds but differ substantially in their growth characteristics. Because the deep fibromatoses are known to have mutations of either the *APC* or *β-catenin* genes, (9, 11, 13–19, 40, 49–52) and the status of these genes in superficial fibromatoses was not characterized, we studied the *β-catenin* status of a group of superficial fibromatoses. We found that despite some degree of nuclear accumulation of β-catenin by immunohistochemical staining, none of the studied superficial fibromatoses had detectable *APC* or *β-catenin* mutations. Our results suggest that the APC/β-catenin pathway may play a role in the different growth characteristics of the superficial *versus* deep fibromatoses and that these two categories of fibromatoses are truly genetically distinct. Our results are also consistent with the recent standard karyotyping series of superficial and deep fibromatoses, which showed greater percentages of anomalies in deep lesions and loss of 5q (the site of the *APC* gene) only in deep lesions (20).

The cause for the nuclear accumulation of β-catenin in many of our cases by immunohistochemistry is not entirely clear but might be explained by any of several mechanisms. First, superficial fibromatoses could harbor alterations of a gene other than *APC* or *β-catenin* in the β-catenin–signaling pathway that was not studied. *AXIN* genes

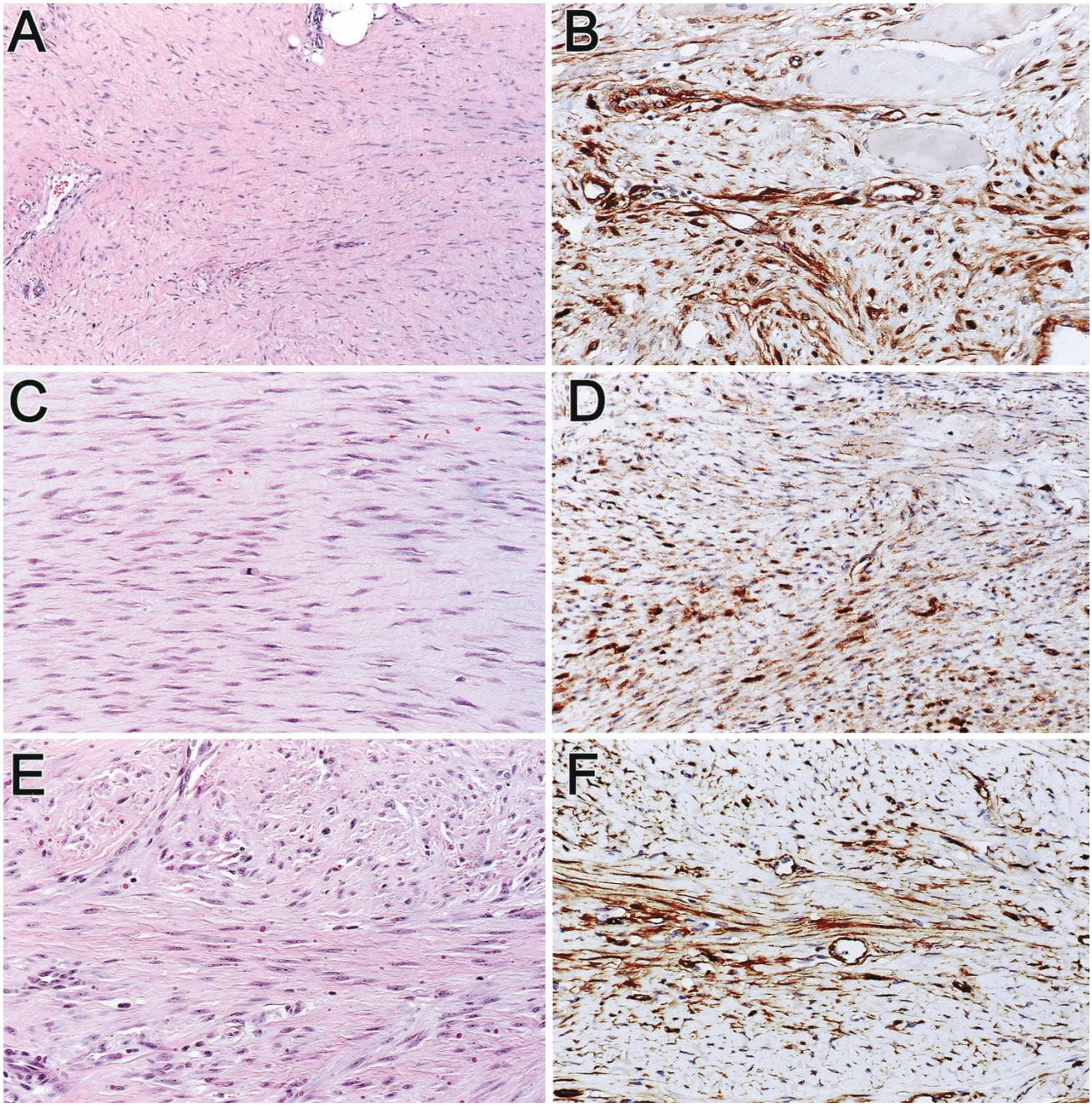

FIGURE 1. **A** and **B**, deep fibromatosis (desmoid tumor), hematoxylin and eosin (H&E) (**A**) and β-catenin immunohistochemistry (**B**). Note that most of the nuclei (estimate: 80%) show β-catenin accumulation, whereas the endothelial cell nuclei and infiltrated skeletal myocyte nuclei are negative. **C** and **D**, plantar fibromatosis. These superficial fibromatoses are frequently mitotically active (**C**, H&E). On the immunohistochemical preparation (**D**), scattered nuclei (estimate: 10 to 15%) display nuclear β-catenin accumulation, and cytoplasmic staining is focally present. No mutations were identified in either the *β-catenin* or *APC* genes. **E** and **F**, infantile digital fibromatosis showing the characteristic cytoplasmic inclusions (**E**, H&E). Scattered nuclei (estimate: 10 to 15% in this field) also show β-catenin accumulation by immunohistochemistry (**F**), although no mutations were identified in either the *β-catenin* or *APC* genes.

have recently been shown to be mutated in a subset of hepatocellular carcinomas showing nuclear β-catenin accumulation but lacking *β-catenin* gene mutations (53). Furthermore, immunohistochemical evidence of β-catenin accumulation does not always correlate well with genetic events (24, 54–56). Second, because *APC* is a large gene and only a small fraction of the gene was evaluated, encompassing the mutation cluster region for colorectal adenomas, it is conceivable that other mutations were present in this gene that resulted in β-catenin accumulation. Although the latter possibility cannot be excluded, we have found the techniques used in this series successful in detecting mutations in other organ systems (22, 24, 25, 57), and the same techniques have been applied to the deep fibromatoses, and mutations have been detected in these (9–15, 17, 40, 49, 51, 52). In contrast to the case with

TABLE 1. β-Catenin Expression in Superficial Fibromatoses

Case No.	Type	Age (yr)/Sex	β-Catenin Immunostain; Estimated % Nuclear Staining	β-Catenin Gene Mutations	APC Gene Mutations
1	I.D.F.	8/m	5	−	−
2	Palmar	23/m	5	−	NA
3	Palmar	46/m	10	−	−
4	Palmar	52/m	10	−	NA
5	Palmar	61/m	10	−	NA
6	Palmar	50/m	5	−	−
7	Palmar	52/f	100	NA	NA
8	Palmar	67/f	1	−	−
9	Palmar	62/m	5	−	−
10	Palmar	65/m	5	−	NA
11	Palmar	41/m	20	−	−
12	Palmar	79/m	5	−	−
13	Palmar	51/m	10	−	−
14	Palmar	62/f	10	NA	NA
15	Palmar	64/m	1	−	−
16	Palmar	64/m	20	−	−
17	Palmar	71/m	5	−	−
18	Palmar	56/m	5	−	−
19	Palmar	56/m	5	−	−
20	Palmar	65/m	10	−	−
21	Palmar	65/f	5	−	−
22	Palmar	64/m	10	−	−
23	Palmar	58/m	5	−	−
24	Penile	57/m	0	−	−
25	Plantar	26/f	20	−	NA
26	Plantar	60/m	10	−	NA
27	Plantar	28/f	10	−	−
28	Plantar	47/m	20	−	−
29	Plantar	55/f	5	−	−

IDF, infantile digital fibromatosis; f, female; m, male; −, no mutations detected; NA, not available.

the *APC* gene, the majority of *β-catenin* mutations are detectable by sequencing of the GSK-3β phosphorylation region. As such, we believe that these findings reflect true biologic differences between superficial and deep fibromatoses.

Among patients with familial adenomatous polyposis (FAP), intestinal and extraintestinal neoplasms typically arise through biallelic (germline then somatic) inactivation of the *APC* gene, whereas the corresponding tumors in non-FAP patients occur either through somatic biallelic *APC* inactivation or somatic mutation of a single *β-catenin* allele. As the various FAP-associated tumors have been studied, somatic alterations of the APC/β-catenin pathway have been initially detected in familial examples and then subsequently demonstrated in the sporadic counterparts. The first studied tumors were gastrointestinal adenomas (30, 38, 39, 58), followed by desmoid tumors (16, 18, 19), medulloblastomas (59), childhood hepatoblastomas (60, 61), and gastric fundic gland polyps (24, 25). More recently, the same story has unfolded in juvenile nasopharyngeal angiofibromas (22, 62), which occur more frequently in FAP patients than in controls. Sporadic tumors lack *APC* mutations (63) but are highly likely to have *β-catenin* mutations (22). It is of interest that superficial fibromatoses are not known to be increased in FAP, even in highly specialized variant disease featuring specific mutations associated with hereditary desmoid tumors (9, 14, 49). It has been estimated that FAP patients in general have an 852-fold increased risk of developing desmoids, typically intraabdominal lesions (64). However, Couture *et al.* have recently presented a unique French-Canadian kindred harboring a germline mutation of Codons 2643 to 2644 of the APC gene (49). These patients have a penetrance of desmoid tumors approaching 100% and have cutaneous cysts, but few manifest colon polyposis. Noteworthy is that among the fibromatoses found in these specialized carriers, virtually all were axial, and none were superficial. These observations of familial lesions suggest that the pathobiology of deep and superficial fibromatoses differs and that they are truly two divergent and probably biologically unrelated processes. The absence of both *β-catenin* and *APC* gene mutations in the studied superficial fibromatoses in contrast to common mutations of these genes in deep fibromatoses reaffirms that the existing classification reflects a biologic distinction that can be confirmed on genetic grounds.

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