Mesenchymal Stromal Cell Mutations and Wound Healing Contribute to the Etiology of Desmoid Tumors

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Abstract

Desmoid tumors are nonmalignant neoplasms of mesenchymal origin that mainly contain fibroblast lineage cells. These tumors often occur in patients with familial adenomatous polyposis (FAP) coli who have germ line mutations in the APC gene. Given emerging data that has implicated multipotent mesenchymal stromal cells (MSC) in the origin of mesenchymal tumors, we hypothesized that desmoid tumors may arise in patients with FAP after MSCs acquire somatic mutations during the proliferative phase of wound healing. To test this idea, we examined 16 desmoid tumors from FAP-associated and sporadic cases, finding that all 16 of 16 tumors expressed stem cell markers, whereas matching normal stromal tissues were uniformly negative. Desmoid tumors also contained a subclass of fibrocytes linked to wound healing, angiogenesis, and fibrosis. Using an MSC cell line derived from an FAP-associated desmoid tumor, we confirmed an expected loss in the expression of adenomatous polyposis coli (APC) and the transcriptional repressor BMI-1 while documenting the coexpression of markers for chondrocytes, adipocytes, and osteocytes. Together, our findings argue that desmoid tumors result from the growth of MSCs in a wound healing setting that is associated with deregulated Wnt signaling due to APC loss. The differentiation potential of these MSCs combined with expression of BMI-1, a transcriptional repressor downstream of Hedgehog and Notch signaling, suggests that desmoid tumors may respond to therapies targeting these pathways. Cancer Res; 72(1): 346–55. ©2011 AACR.

Introduction

Desmoid tumors, also known as aggressive fibromatosis, are mesenchymal tumors that occur sporadically or are associated with the heritable colorectal cancer syndrome, familial adenomatous polyposis (FAP). Desmoid tumors exhibit locally destructive growth, with a dense infiltrative character that can produce disfigurement, functional deficits, and death (1). Treatment often involves surgical excision, which is associated with a high recurrence rate (2). Although several studies reported successful regression of desmoid tumors with drug treatments and radiation therapy, effective systemic therapy has not been reported.

Tumors arising in patients with FAP show loss of adenomatous polyposis coli (APC) tumor suppressor function (7, 8). This leads to high intracellular β-catenin levels and is correlated with constitutive activation of Wnt signaling. In sporadic desmoid tumors, specific point mutations in the CTNNB1 gene that stabilize β-catenin protein achieve a similar result (9, 10).

Desmoid tumors can arise at sites of wound healing and show histologic features observed in dermal fibroproliferative disorders such as keloids and hypertrophic scarring (11, 12). Normal wound healing is a tightly regulated, self-limited process that produces rapid defect coverage to protect from further harm, then regenerates and remodels tissues at the injury site. In response to tissue stress or injury, mesenchymal cells from various sources are mobilized and recruited to wounds, where they engraft and promote healing (13–15). These cells include hematopoietic stem cell (HSC)-derived monocyte precursors, which comprise a small fraction of circulating nucleated cells that also home sites of tissue injury, engulf, and differentiate into CD34+ fibrocytes. During wound healing, these pluripotent cells execute tissue remodeling and differentiate into endothelial cells, fibroblasts, and myofibroblasts (16–18). During the resolution phase of normal wound healing, recruited stem/progenitor cells undergo terminal differentiation or apoptosis. However, under conditions of chronic inflammation or tumor progression, these activated cells persist. For example, both mesenchymal stromal cells (MSC) and fibrocytes are found in keloids and hypertrophic scars (19–21). Together, these multipotent cells cooperate synergistically to support angiogenesis, a hallmark of
accelerated wound healing and fibrosis (22–24). Desmoid tumors exhibit features consistent with chronic wound healing, including increased angiogenesis and proliferation of fibroblast-like cells within a collagen matrix (25, 26). Desmoid tumors also express genes characteristic of myofibroblasts, further indicating that persistent recruitment of monocyte precursors and defective wound healing resolution play significant roles in desmoid tumor neoplasia (27).

Because of their association with wound healing, MSCs are implicated in desmoid tumor formation. Primary fibroblast cell lines have been derived from desmoid tumors; however, the growth conditions used did not specifically select or expand MSCs (28, 29). A recent report described the culture of putative MSCs from mouse desmoid tumors; however, these cells were not fully characterized (30, 31). We hypothesized that desmoid tumors arise after MSCs acquire somatic mutations during the proliferative phase of wound healing in genes that increase the transcriptional potential of β-catenin–Tcf/Lef. To explore this idea, we examined the expression of stem cell markers in archived human desmoid tumor specimens and established a desmoid tumor–derived MSC line from a patient with FAP. Our findings implicate MSCs in the etiology of desmoid tumors and suggest novel targets for the systemic treatment of this disease.

Materials and Methods

Human desmoid tumor specimens and normal MSCs

Human desmoid tumors and matching adjacent fascia from a subset of the collection were obtained from surgical specimens excised from patients under an approved protocol by the Institutional Review Board at the Brigham and Women’s Hospital. Tumors were fixed in 10% buffered formalin and embedded in paraffin for immunohistochemical analysis. Normal first-passage adipose–derived MSCs were a generous gift from Dr. Martin L. Yarmush, Shriners Hospital for Children, Boston, MA.

Immunohistochemistry

Serial sections (4 μm) of preserved desmoid tumors were deparaffinized in a xylene and graded ethanol series. Antigen retrieval was carried out in pH 6.0 citrate buffer (Millipore) by heating for 2 minutes in a decloaking chamber (Biocare Medical). Peroxidase activity was quenched using the Dual Enzyme Block (Dako North America, Inc.) for 15 minutes at room temperature. All primary antibody reactions were carried out overnight at 4°C. Antibodies are listed in Supplementary Table S1. Biotinylated horse anti-mouse antibodies (IgG H+L; Vector Laboratory) were used at room temperature for 30 minutes. Antibodies were diluted in Antibody Diluent Reagent Solution (Invitrogen). ABC Reagents (Vector Laboratory) were applied for enhancement for 15 minutes. Positive staining was detected with diaminobenzidine using the Liquid DAB+ Substrate Chromagen System (Dako North America, Inc.) and countered stained with hematoxylin. Dehydrated slides were cover slipped with Permount (Fisher). Images were acquired using an Olympus BX40 microscope. CD73+ and CD34+ cells were counted at 4 representa-

tive high-powered fields (40×) using Image J software. The Student t test was used for statistical analysis.

For immunofluorescent staining, cells were plated on 4-well Lab-Tek II Chamber slides and allowed to attach for 2 days. Cells were fixed in 4% formaldehyde for 30 minutes at room temperature, then washed and blocked with blocking buffer (1× PBS, 5% normal goat serum, 0.3% Triton X-100) for 1 hour at room temperature. Reactions used antibodies placed in antibody buffer [1× PBS, 1% bovine serum albumin (BSA), 0.3% Triton X-100]. Cells were washed and incubated in fluorochrome-conjugated secondary antibodies (Invitrogen) for 1 hour at room temperature in the dark. Slides were cover-slipped with Prolong Gold Antifade Reagent (Invitrogen). Similar procedures were used after deparaffinizing and rehydrating sectioned desmoid tumors. Imaging used a Nikon Eclipse TE2000-S inverted microscope with a Spot RT Slider Camera and Spot Software (version 4.6 for Windows; Diagnostic Instruments, Inc.).

Cell culture and cell morphology analysis

To establish a desmoid tumor cell line, a fresh tumor sample was immediately placed in HEPES-buffered saline supplemented with 15% FBS and antibiotics. In the laboratory, the tumor was minced and placed in tissue culture dishes submerged in MesenPRO RS medium and incubated in a humidified 5% CO2 incubator. Cell culture medium and related reagents were purchased from Invitrogen. Tumor debris and unattached cells were discarded after 4 days. A single attached cell was isolated and expanded with fresh medium provided every 4 to 5 days. At approximately 80% confluence, cells were trypsinized and expanded. Characterizations were conducted on passage 1 to 3 cells. Cell density and viability (Trypan Blue exclusion) were determined using a hemocytometer. To characterize the morphology of the desmoid tumor–derived cells, phase contrast imaging used a Nikon TE2000-E microscope and a Hamamatsu OrcaER camera at 10× magnification.

Cell differentiation

Cells were plated on 4-well Lab-Tek II Chamber Slides (Fisher) and incubated for 2 days in MesenPro MSC medium. For adipogenic differentiation, cells were placed in StemPro adipogenesis differentiation medium. For osteogenic and chondrogenic differentiation, cells were incubated in either StemPro osteogenesis differentiation medium or StemPro chondrogenesis medium. The desmoid tumor–derived cells required 5 to 6 weeks for differentiation and were provided fresh medium every 3 to 4 days. For osteogenic differentiation, immunofluorescence histochemistry used osteopontin and RUNX2 antibodies. Similar procedures were executed for chondrogenic or adipocyte differentiation but used connective tissue growth factor (CTGF) and SOX9, or PPARY and c/EPBα antibodies, respectively. After fixing cells in 4% formaldehyde for 10 minutes, standard von Kossa, Alcian blue, and Oil Red O staining reactions were carried out.

Fluorescent-activated cell-sorting analysis

Cells were grown to near confluence and passaged, as described. After centrifugation, cell pellets were suspended in
4% formaldehyde and fixed for 10 minutes at 37°C, chilled on ice for 1 minute, and permeabilized for 30 minutes at 4°C by adding ice-cold 100% methanol to prechilled cells for a final concentration of 90%. A total of 5 × 10^5 cells in separate aliquots were washed and pelleted by centrifugation in dilution buffer (0.5% BSA in 1× PBS). Primary antibody was added at appropriate dilutions; reactions proceeded for 1 hour at room temperature and then were washed in dilution buffer. Fluorochrome-conjugated secondary antibodies diluted in dilution buffer were added appropriately and reactions were incubated for 30 minutes at room temperature. Cells suspended in PBS were analyzed using an Accuri C6 Flow Cytometer; data analysis was performed with CFlow Software.

**Results**

**Human desmoid tumors express stem cell markers**

To investigate the presence of MSCs in desmoid tumors, immunohistochemical analysis was conducted on 16 different paraffin-embedded tumor specimens and 5 matched normal adjacent tissues. Clinical features of the patients with desmoid tumor are provided in Supplementary Table S2. Within all 16 tumors, cells were positively stained for the obligate MSC markers, CD73 (Ecto-5'-nucleotidase) and CD90 (Thy-1; Fig. 1A and B). Cells in desmoid tumors also expressed the stem cell marker and Polycomb group transcriptional repressor, BMI-1 (Fig. 1C). Staining for stem cell markers in matched normal connective tissue yielded negative results (Supplementary Fig. S1). Immunohistochemistry and immunofluorescent histohemistry of paraffin-embedded desmoid tumors also showed coexpression of CD73, CD90, BMI-1, and CD44 within the same cells (Fig. 1D–F). CD44 is a stem cell marker that directs MSC homing to anatomic locations other than bone marrow (32). The number of CD73⁺ cells varied among desmoid tumor specimens, but the difference was not statistically significant (Supplementary Fig. S2).

**Desmoid tumor–derived cells show characteristics of MSCs**

Freshly removed desmoid tumor cells were cultured in MSC-specific medium. First-passage desmoid tumor–derived cells had a large, flat polyploid morphology (Supplementary Fig. S3A). Prominent cytoskeletal structures spanning the length of cells were visible under the light microscope. These cells grew more slowly and were more resistant to detachment by Trypsin-EDTA (0.05%) treatment than normal human fat–derived MSCs (data not shown). Supplementary Figure S3B and S3C shows first-passage desmoid tumor–derived cells at increasing densities. At higher density, cells aligned with one another in a polarized fashion forming long ridges with cell bodies stretched in opposite directions. At this time, cell clusters spontaneously differentiated into neuronal-like cells, a result consistent with reports that both desmoid tumors and MSCs express neuronal genes (Supplementary Fig. S3D; refs. 27, 33).

Upon passage, the neuronal-like cells did not attach and were subsequently lost. Although all subsequent characterizations were carried out using desmoid tumor–derived cells passaged less than 3 times, this cell line was maintained for at least 10 population doublings and appeared virtually immortal consistent with the self-renewal capability of stem cells.

Cultured desmoid tumor cells were characterized by fluorescent immunohistochemistry to assess obligate MSC markers. The cell line coexpressed CD73, CD90, and CD105 (Endoglin; Fig. 2A and B). Appropriately, the transmembrane endothelial precursor marker, CD34, was not expressed in the desmoid tumor–derived cells (Fig. 2C). In addition, the desmoid tumor–derived cells were positively stained with antibodies for TGFβ1, 2, and 3 and the active form of integrin β1, and displayed a prominent F-actin cytoskeleton upon staining with Alexa Fluor 568-labeled phalloidin (Supplementary Fig. S4A and S4B). To prove that these desmoid tumor–derived cells were authentic MSCs, fluorescent-activated cell-sorting (FACS) analysis of surface antigen profiles confirmed that...
approximately 90% of the population coexpressed MSC positive, but not negative markers (Fig. 2D).

**CD34+ fibrocytes also populate desmoid tumors**

Monocyte precursors home to sites of tissue injury and differentiate into CD34+ fibrocytes (17). Because MSCs and HSC-derived progenitors maintain close physical contacts, we anticipated that CD34+ fibrocytes would be found in desmoid tumors. We conducted fluorescent immunohistochemistry for CD34 on our archived desmoid tumor collection and the matching set of adjacent normal tissues. Consistent with a wound healing origin, CD34+ fibrocytes were present in tumors but not in matched normal tissue (Fig. 3A). The number of CD34+ cells did not vary significantly between FAP and sporadic desmoid tumors (Supplementary Fig. S2).

CD34+ fibrocytes give rise to endothelial cells that stimulate angiogenesis. The TGFβ signaling receptor, ALK1, and CD105 accessory protein are coexpressed in endothelial cells and promote angiogenesis during embryonic development (34). Expression of ALK1 in normal adult vasculature is minimal, but is induced during wound healing and tumor progression (35). Fluorescent immunohistochemistry assessed ALK1 and CD105 coexpression in desmoid tumors (Fig. 3B). Among many cells expressing CD105, a subset coexpressed ALK1. This result is consistent with the observation that fibrocytes express both CD34 and CD105, whereas MSCs lack CD34 expression (24). As expected, neither CD105 nor ALK1 was expressed in the matched normal tissues from patients with desmoid tumor (Fig. 3B).

**Trilineage mesenchymal differentiation of desmoid tumor–derived MSCs**

In addition to FACS, multipotency must be shown to confirm MSC status. The desmoid tumor–derived cells were cultured for 5 to 6 weeks in differentiation medium, and then assayed for lineage-specific markers to confirm differentiation into chondrocytes, adipocytes, and osteocytes (Fig. 4). Chondrocyte differentiation requires activity of the transcription factor, SOX9, and chondrocytes specifically express CTGF. Fluorescent immunohistochemistry showed that the desmoid tumor–derived cells grown in chondrocyte differentiation medium coexpressed these markers, and confirmed nuclear localization of SOX9 (Fig. 4A). Also, collagen proteoglycans present in the chondrocytes yielded the appropriate result when fixed desmoid tumor–derived cells were subjected to Alcian blue staining (Fig. 4B). Osteocyte differentiation requires activity of the osteoblast-specific transcription factor RUNX2, and osteopontin is a glycoprotein selectively secreted by osteocytes. Fluorescent immunohistochemistry showed that the desmoid tumor–derived cells grown in osteocyte differentiation medium expressed these markers with nuclear and cytoplasmic localization of RUNX2 (Fig. 4C). In addition, a black precipitate characteristic of osteocyte mineralization was evident when

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Figure 2. Desmoid tumor–derived MSCs coexpressed positive but not negative MSC markers. Fluorescent immunohistochemistry was conducted on early passage desmoid tumor–derived MSCs showing coexpression of the MSC markers (A) CD73 (green) and CD90 (red) as well as (B) CD73 (green) and CD105 (red), but not expression of the negative endothelial precursor marker, CD34 (C). Original magnification was 40×. FACS analysis of these cells showed coexpression of the obligate MSC markers CD73, CD90, and CD105, but not CD34 (D). The gated population is outlined in red on the top, left dot plot. Sorting showed that 99.1% of cells were CD73+, CD34–; 99.3% were CD73+, CD90+; and 89.3% were CD73+, CD105+.
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Figure 3. Desmoid tumors contained CD34⁺, CD105⁺, and ALK1⁺ cells. Representative fluorescent immunohistochemistry images are shown of formalin-fixed, paraffin-embedded desmoid tumors stained for CD34 (red; A, top). Matched normal adjacent tissue did not display any CD34⁺ cells (A, bottom; original magnification, 40×). Representative fluorescent immunohistochemistry images of formalin-fixed paraffin-embedded archival desmoid tumors stained for (B, top) ALK1 (green) and CD105 (red). Matched normal adjacent tissue showed only AKL1⁺ and CD105⁺ cells (B, bottom). Open arrows indicate CD105⁺/ALK1⁻ cells; gray filled arrows indicate dual positive cells. The original magnification was 20×.

these cells were subjected to von Kossa staining (Fig. 4D). Adipocyte differentiation requires the activities of transcription regulators PPARγ and c/EBPα. Fluorescent immunohistochemistry showed that the desmoid tumor–derived cells grown in adipocyte differentiation medium expressed these markers with nuclear localization of c/EBPα. In addition, lipid droplets were present with Oil Red O staining (Fig. 4E and F). Taken together these data confirmed development of a multipotent MSC line from a human desmoid tumor.

MSCs expressed the N-terminus but not the C-terminus of APC protein

The germ line of patients with FAP contains a mutation in one APC gene that typically truncates the carboxy-terminus of the encoded protein. Deregulated Wnt signaling occurs in APC-associated tumors including desmoid tumors when tumor-engendering stem cells sustain LOH of the wild-type APC allele (7). We confirmed that MSCs from both the original desmoid tumor and the desmoid tumor–derived cell line were APC deficient by conducting fluorescent immunohistochemistry to detect the carboxy- versus amino-terminus of APC. This assay showed that although the truncated APC product was present, MSCs in both the desmoid tumor and the cell line lacked the APC carboxy-terminus antigen, consistent with somatic LOH (Fig. 5A and B). As a positive control, immunohistochemistry conducted in parallel on first-passage normal human adipose–derived MSCs confirmed the presence of both terminal ends of APC protein in these nontumor cells (Fig. 5C).

Wnt signaling is activated in APC-deficient MSCs

APC loss leads to activation of Wnt signaling as shown by the accumulation of nuclear β-catenin. Previously, we showed that abundant nuclear β-catenin was present in our archived desmoid tumor library (36). Nuclear β-catenin was also detected in desmoid tumor–embedded MSCs, suggesting β-catenin stabilization (Fig. 6A). Immunostaining of desmoid tumors for the proliferation marker, Ki-67, showed that CD73⁺ MSCs were among the Ki-67⁺ population (Fig. 6B).

Notch and Hedgehog pathways are activated in desmoid tumor–derived MSCs

Activation of Notch and Hedgehog signaling is implicated in the development of multiple cancers. To investigate the presence of Notch signaling in desmoid tumorigenesis, immunostaining of desmoid tumors showed that MSCs expressed Notch-1 and its activation target, Hes-1 (Fig. 7A and B). Consistent with our findings in tumor specimens (Fig 1C and D), we also found that the desmoid tumor–derived cells expressed BMI-1, as well as its upstream Hedgehog activation target, Gli-1 (Fig. 7C and D). Notch signaling is presumed to play a positive role in regulating BMI-1 function because transcriptional repression of this target is relieved by γ-secretase inhibitors.

Discussion

In this study, we examined a tumor of mesenchymal origin to obtain insight into stromal cell interactions and desmoid tumorigenesis. We showed that both MSCs and HSCs are present in human desmoid tumors but not in matching adjacent normal tissues. We also produced a human desmoid tumor–derived MSC line and showed that both the original tumor and cell line contained truncated APC protein. Furthermore, we showed the presence of nuclear β-catenin within these APC-deficient MSCs, denoting active Wnt signaling. Taken together, our findings suggest that desmoid tumors may arise from MSCs following APC mutation and subsequent Wnt signaling deregulation.

Desmoid tumors are relatively acellular neoplasms that do not metastasize (1, 2). They are composed of cells of fibroblast lineage, and exhibit a marked fibrotic response with significant vascularity. Importantly, their histologic characteristics are indistinguishable from that of nonneoplastic lesions associated with abnormal wound healing, including hypertrophic scars or dermal keloids (19–21). There are data suggesting that a wound healing response may induce desmoid tumor formation (11, 12). Indeed, clinicians treating patients with FAP have noted a tendency for surgery to increase desmoid tumor growth, and it is not uncommon for sporadic desmoid tumors to present initially within surgical scars.

Tumorigenesis shares characteristics observed in wound healing. For instance, symmetrical stem cell division, a process yielding stem cell duplication rather than the stem...
cell and its committed progenitor, is a prominent feature of the proliferative phase of normal wound healing. Tumors are initiated by symmetrical stem cell division that entails fixation of a mutation inactivating a critical tumor suppressor gene, such as $APC$, or activating a specific oncogene, such as $CTNNB1$ (7–12). Tumor growth proceeds with continued self-renewal of the mutant stem cell and expansion of its progeny. Because desmoid tumors have a monoclonal origin (25, 26), it is feasible that this initiating mutation occurs in an MSC recruited or activated in situ during the proliferative response to tissue stress or injury. In this scenario, the specific mutation allows stem cell growth without the normal instructive positional, spatial, and mechanical cues provided by the tissue microenvironment (37). In particular, signaling via Wnt, Hedgehog, and Notch pathways governs normal adult stem cell behavior in vivo. Appropriate responses in MSCs to input from these physical or paracrine cues may be compromised specifically by APC loss and contribute to desmoid tumor etiology.

MSCs are multipotent cells that reside in most adult tissues. Although normally quiescent, they serve in normal tissue homeostasis by contributing to the maintenance of stromal elements. During wound healing, these multipotent cells aid tissue regeneration. HSC-derived monocyte precursors circulate in the blood, and are distinguished from MSCs by expression of CD34, a marker found on short-term reconstituting HSCs and their multipotent progenitors (16, 17). MSCs and HSCs cohabit the same niche in bone marrow, and a relationship of MSC-dependent support of HSCs is illustrated in coculture studies, which show that expansion of HSCs in vitro is stimulated by the addition of MSCs (38, 39). In a systemic response, MSCs and HSC-derived monocyte precursors are recruited to sites of tissue injury where both cell types proliferate. In a wound healing context, monocyte precursors differentiate into multipotent CD34$^+$ fibrocytes, and, depending on the availability of certain cytokines, differentiate into endothelial cells or myofibroblasts (24). Fibrocytes and myofibroblasts produce extracellular matrix components such as collagens and express matrix metalloproteinases, factors needed for tissue remodeling during wound healing (16, 17). In circumstances where the proliferative phase of wound healing fails to terminate, myofibroblasts also express fibrogenic factors such as TGF$\beta$ and inflammatory mediators such as the prostaglandin, PGE$_2$.

Here, we showed that MSCs are present in desmoid tumors and may contribute to the etiology of these neoplasms. This latter claim is supported by the absence of APC$^{-/-}$ protein in both the FAP-associated desmoid tumor and its derived MSC line, indicating clonal expansion of a cell that acquired APC LOH (Fig. 5). Absent APC function, $\beta$-catenin is constitutively stabilized (Fig. 6). Although we previously noted abundant

![Desmoid tumor–derived MSCs exhibited tripotent differentiation capability. Desmoid tumor–derived cells were incubated in differentiation media, and confirmation of differentiation was obtained by expression of chondrocyte markers CTGF (green) and Sox9 (red), overlay shows yellow stain (A) plus positive Alcian blue staining (B); osteocyte markers RUNX2 (green) and osteopontin (green; C) plus positive von Kossa staining (D); and adipocyte markers PPAR$\gamma$ (green) and c/EBP$\alpha$ (red; E) plus positive Oil Red O staining (F). Original magnification was 40x.](https://www.aacrjournals.org/canres/doi/abs/10.1158/0008-5472.CAN-11-2819)
nuclear β-catenin in desmoid tumors and stromal cells, here we observed few proliferating MSCs (Ki-67⁺; ref. 36). Hoos and colleagues corroborated our findings and showed that Ki-67 expression was not upregulated in desmoid tumors, in contrast with other malignant tumors (40). Although our data suggest that Wnt signaling does not drive desmoid tumor proliferation, we propose that excess Wnt signaling maintains MSCs in an immature state. Ross and colleagues showed that enforced Wnt signaling inhibits terminal differentiation of preadipocytes (MSC progenitors) and a similar conclusion was found in HSCs (41, 42).

Our desmoid tumor–derived MSC line also exhibited stem cell behavior (Figs. 2 and 4). Therefore, notwithstanding APC loss or stabilized β-catenin protein, mutant MSCs associated with desmoid tumor formation presumably remain nonautonomous for growth and maintenance, and require both supporting cells and a niche microenvironment. Similar to conditions in the bone marrow, we found that MSCs resided with CD34⁺ fibrocytes in desmoid tumors, suggesting that these latter cells may provide support for the MSC tumor cells (38). MSCs and CD34⁺ fibrocytes also coexist in keloids and hypertrophic scars, which desmoid tumors resemble histologically (19–21). Our findings suggest that desmoid tumor etiology resembles defective wound healing, and the presence of immature stromal progenitor cells in both dermal tumors and desmoid tumors may reflect Wnt signaling–dependent inhibition of differentiation (41, 42). Furthermore, studies show that homing of HSC-derived progenitors to peripheral tissues under conditions of chronic inflammation causes fibrosis (43). It is therefore possible that the high levels of fibrosis and angiogenesis found in desmoid tumors derive from a tumor-associated inflammatory response, with persistent recruitment of CD34⁺ fibrocytes.

Under conditions of tissue homeostasis, canonical Wnt signaling is tightly controlled, permitting proliferation of committed progeny from adult stem cells, differentiation, and niche exit. However, it is likely that deficient negative regulation of Wnt signaling increases the self-renewal of tumor-initiating MSCs that arise in FAP-associated desmoid tumors with APC loss and in sporadic desmoid tumors with stabilizing CTNNB1 mutations (7–12). Three Wnt target genes are relevant. Secreted frizzled-related protein1 (sFRP1) is expressed in desmoid tumors and encodes a soluble Wnt receptor antagonist (27). Although its loss allows constitutive Wnt signaling and drives cancer growth, SFRP1 gene expression, resulting in canonical Wnt pathway inhibition, increases noncancerous stem cell self-renewal (44). SFRP1 expression is downstream of Gli-1 activity, which we showed was expressed in desmoid tumor–derived MSCs (Fig. 7D). Importantly, SFRP1

Figure 5. MSCs expressed only the APC-N-terminus consistent with somatic APC⁺ LOH. Fluorescent immunohistochemistry was conducted on paraffin-embedded desmoid tumors and desmoid tumor–derived cells for both the APC (N-15) and (C-20) termini. Normal human adipose–derived MSCs were used as controls. In MSCs from both the desmoid tumor (A) and cell line (B), staining was positive for the N-terminus (red) but not the C-terminus. In contrast, staining was positive for both N- and C-termini of APC protein in normal human adipose–derived MSCs (C). Original magnification was 10× and 20×. Overlay shows a purple nuclear stain.
expression regulates β-catenin activity in HSCs, and in desmoid tumors may promote the retention and maintenance of CD34+ fibrocytes (45). We also showed that desmoid tumors contained CD73+ MSCs expressing CD44 (Fig. 1F). CD44, a hyaluronic acid adhesion receptor, is upregulated in APC-deficient cells and potently affects stem cell niche production and homing of HSC-derived progenitors (46).

Our main goal is to identify aspects of desmoid tumor biology that will define effective therapies for this challenging disease. Our results enable predictions about potential therapies for desmoid tumors that target either MSCs or CD34+ fibrocytes. We found that desmoid tumor-derived MSCs expressed BMI-1 and the Hedgehog signaling effector, Gli-1 (Fig. 7C and D). BMI-1 is a transcriptional repressor downstream of Hedgehog and Notch pathways. Drugs for these targets include Notch pathway γ-secretase inhibitors and Hedgehog inhibitors. Endothelial cells specifically use the TGFβ pathway to stimulate angiogenesis via CD105, ALK1, and BMP9 under conditions of wound healing and tumor progression (35). We showed that within desmoid tumors, a population of cells coexpressed ALK1 and CD105 (Fig. 3B); therefore, survival of CD34+ fibrocyte-derived endothelial cells in desmoid tumors may be inhibited by antiangiogenesis therapies directed against ALK1 (47). If desmoid tumors depend on maintenance and proximity of both pluripotent MSCs and CD34+ fibrocytes, then therapies aimed at differentiating or reprogramming these cells might disturb the niche requirements of one or both. We showed that desmoid tumor-derived APC-deficient MSCs were capable of terminal differentiation. Therefore, differentiation therapies such as PPARγ agonists or retinoids may be effective (48, 49). Finally, our work

Figure 6. MSCs showed nuclear β-catenin localization. Immunohistochemistry was conducted on paraffin-embedded desmoid tumors and showed accumulation of nuclear β-catenin in MSCs, indicating active Wnt signaling (A). Original magnification was 10× and 40×. Fluorescent immunohistochemistry of desmoid tumor sections stained for Ki-67 (red) and CD73 or CD105 (green) identified dually positive cells (B).

Figure 7. Notch and Hedgehog pathways were upregulated in desmoid tumors. Fluorescent immunohistochemistry was conducted on paraffin-embedded desmoid tumors and showed positive expression of the Notch target gene Hes-1 (green; A) and Notch-1 (red; B). Colocalization with BMI-1 (red) produced yellow nuclei (A). Desmoid tumor-derived cells also expressed the transcriptional repressor BMI-1 (C). Colocalization of red staining of BMI-1 with the blue counter stain produced purple-colored nuclei. Similarly, purple nuclei were evident when the MSCs were stained with antibodies for the Hedgehog transcriptional activator, Gli-1 (red; D).
shows that tumor MSC lines can be generated from human desmoid tumors, thereby providing a resource that can accelerate preclinical drug testing.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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