

Aggressive Fibromatosis (Desmoid Tumor) Is Derived from Mesenchymal Progenitor Cells

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Abstract

The cellular origins from which most tumors arise are poorly defined, especially in mesenchymal neoplasms. Aggressive fibromatosis, also known as desmoid tumor, is a locally invasive soft tissue tumor that has mesenchymal characteristics. We found that aggressive fibromatosis tumors express genes and cell surface markers characteristic of mesenchymal stem cells (MSC). In mice that are genetically predisposed to develop aggressive fibromatosis tumors (*Apc*^{wt/1638N}), we found that the number of tumors formed was proportional to the number of MSCs present. *Sca-1*^{-/-} mice, which develop fewer MSCs, were crossed with *Apc*^{wt/1638N} mice. Doubly mutant mice deficient in *Sca-1* developed substantially fewer aggressive fibromatosis tumors than wild-type (WT) littermates, but *Sca-1* deficiency had no effect on the formation of epithelial-derived intestinal polyps. MSCs isolated from *Apc*^{wt/1638N} mice (or mice expressing a stabilized form of β -catenin) induced aberrant cellular growth reminiscent of aggressive fibromatosis tumors after engraftment to immunocompromised mice, but WT cells and mature fibroblasts from the same animals did not. Taken together, our findings indicate that aggressive fibromatosis is derived from MSCs, and that β -catenin supports tumorigenesis by maintaining mesenchymal progenitor cells in a less differentiated state. Protecting this progenitor cell population might prevent tumor formation in patients harboring a germline APC mutation, where fibromatosis is currently the leading cause of mortality. *Cancer Res*; 70(19); OF1-9. ©2010 AACR.

Introduction

Aggressive fibromatosis, also known as desmoid tumor, is a locally invasive soft tissue lesion arising in connective tissues. Although these lesions infiltrate into surrounding normal tissues, they do not metastasize to distant sites. Histologic and cytologic analyses of the tumors reveal that they are composed of bipolar fibroblastic cells that express the intermediate filament vimentin but lack expression of epithelial markers such as E-cadherin. The location, cellular morphology, and histologic profile of these tumors suggest that they derive from mesenchymal sources; however, the aggressive fibromatosis cell of origin has yet to be definitively elucidated (1, 2).

Previous studies identified the molecular etiology of aggressive fibromatosis (3, 4). These lesions can occur as sporadic tumors or as part of preneoplastic conditions, such as familial adenomatous polyposis (FAP) and familial infiltrative fibromatosis. Patients with FAP develop gastrointestinal

lesions that typically progress to cancer by the third decade of life, but with the advent of early colectomy as a treatment, the most common cause of mortality in afflicted patients is aggressive fibromatosis tumors (5, 6). In both FAP and familial infiltrative fibromatosis, lesions are associated with a mutation in the *APC* gene, whereas in sporadic aggressive fibromatosis, most tumors contain mutations in *CTNNB1*, the gene that codes for β -catenin (7, 8). In both cases, genetic aberrations cause stabilization of β -catenin and activation of β -catenin-mediated T-cell factor (TCF)/lymphoid enhancer factor-1-dependent transcription (9, 10). The *Apc*^{wt/1638N} mouse harbors a targeted mutation in the *Apc* gene, resulting in the expression of a truncated nonfunctional version of the APC protein, consequently leading to upregulated β -catenin-mediated, TCF-dependent transcription. These mice developed large numbers of aggressive fibromatosis tumors and gastrointestinal lesions and, as such, are a well-characterized animal model that closely approximates the human disease (11, 12).

Some mesenchymal neoplasms are associated with specific translocations that result in the expression of functional fusion proteins that often contribute to oncogenesis. For instance, rearrangement of the *EWSRI* gene on chromosome 22 to the *ETS* gene family member *FLI-1* on chromosome 11 generates the EWS-FLI-1 fusion protein commonly found in Ewing's sarcoma. In addition, chromosomal translocations resulting in the generation of the FUS-CHOP fusion protein are observed in myxoid liposarcomas. Interestingly, overexpression of either EWS-FLI-1 or FUS-CHOP proteins in murine mesenchymal stem cells (MSC; also known as mesenchymal

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stromal cells due to the heterogeneity of the population) results in the induction of tumors, strongly resembling the neoplastic lesions from which they are affiliated (13, 14). After long-term *in vitro* culture, both murine and human MSCs can undergo spontaneous transformation, producing tumors resembling fibrosarcomas (15, 16). In addition, conditional expression of a translocation specific to synovial sarcomas in muscle progenitors, but not mature myoblasts, has the capacity to induce the formation of synovial sarcomas in mice (17), further supporting the notion that oncogenic changes in mesenchymal precursors give rise to mesenchymal neoplasms.

Mesenchymal tumors display cellular heterogeneity and contain a subpopulation of side population (SP) cells, which show an enhanced tumor-initiating potential. These observations suggest that this tumor type may be organized into a cellular hierarchy, with SP cells behaving like cancer stem cells driving tumorigenesis in established lesions (18, 19). However, these findings suggest that established tumors may be driven by a subpopulation of stem-like cells, but neither prove nor insinuate that mesenchymal neoplasms are derived from a normal mesenchymal progenitor that has sustained oncogenic mutations, nor do they suggest that cells with stem-like characteristics contribute to *de novo* tumor formation.

Here, we use a mouse model of FAP and mesenchymal precursor cells from these mice and from mice expressing an activated form of β -catenin to investigate the influence of mesenchymal progenitor cells (MPC) on the formation of aggressive fibromatosis.

Materials and Methods

Primary human tumors

Primary human aggressive fibromatosis tumors were obtained at the time of surgery, and all material was used with informed patient or guardian consent. They were dissociated into single cells as previously reported (18). Cells from four tumors were examined, all from sporadic lesions involving the extremity. Two were from males (ages 11 and 23) and two from females (ages 16 and 25). Dissociated cells were not cultured but rather used immediately afterwards for flow cytometry analysis or for expression array analysis. Ethical approval was obtained for all human tissue samples collected. Four tumors were used for this analysis.

Flow cytometry

For staining of MSC markers, 1.0×10^6 dissociated cells were resuspended in 100 μ L of PBS supplemented with 2% fetal bovine serum (FBS). Antibodies used consisted of phycoerythrin (PE)-conjugated CD146 (Becton Dickinson), PE-conjugated CD44 (Becton Dickinson), PE-conjugated CD166 (Becton Dickinson), PE-conjugated CD29 (Becton Dickinson), and 0.1 μ g/ μ L of Stro-1 (R&D Systems). For visualization of Stro-1 staining, after incubation with primary antibody, cells were stained for 30 minutes at 4°C with FITC-conjugated IgM (The Jackson Laboratory). After staining, cells were washed twice with PBS and then counterstained with 1 μ g/mL of

propidium iodide (PI; Molecular Probes). PI-positive (non-viable) cells were excluded from analysis. Cell staining was quantified using LSRII flow cytometer (Becton Dickinson).

Genome-wide transcriptional profiling

RNA was extracted from cell pellets using RNeasy Micro kit (Qiagen) according to the manufacturer's instructions. RNA quality was assessed with a Bioanalyzer (Agilent Technologies), and cDNA was generated and hybridized onto Affymetrix Human Genome U133.0 2.0 gene chips. To compare our tumor expression data with control samples, an analysis of gene expression was performed using Parktec Genotyping Suite and Ingenuity Systems Software. To compare expression analysis, we used expression profiles from the Gene Expression Omnibus database (20). The data from the Gene Expression Omnibus database were subjected to background correction by weighting on the *GAPDH* value of each array. For normal fibroblasts, data from normal human fibroblasts (accession number GDS2535) were used from three independent samples, and expression arrays were combined to generate a single weighted data set for normal human fibroblasts. For MSC gene expression, data from accession number GDS3062 were used from three independent MSC samples, and these data were combined to a single weighted data set. A heat map of the weighted values was constructed using the web-based program CIMminer (21) using the expression profile from the MSCs as the reference data in an unsupervised manner.

Generation of genetically modified mice

The generation and phenotype of the *Apc*^{wt/1638N} and *Sca-1*^{-/-} mice have been previously reported (11, 22). These mice were crossed to produce *Apc*^{wt/1638N}/*Sca-1*^{+/-}, *Apc*^{wt/1638N}/*Sca-1*^{+/-}, and *Apc*^{wt/1638N}/*Sca-1*^{-/-} mice using a previously reported breeding strategy so that the phenotype in *Sca-1*-deficient mice was always compared with *Sca-1* wild-type (WT) littermates (23, 24). The *Catnb*^{lox(ex3)} allele (25) contains loxP sites flanking exon 3, which, when exposed to Cre recombinase, results in expression of a functional β -catenin protein missing the NH₂-terminal phosphorylation sites and as such is a constitutively stabilized, transcriptionally active protein. Mice expressing the *Catnb*^{lox(ex3)} allele were crossed with the Tg(cre/Esr1) mouse, which expresses Cre recombinase driven by a tamoxifen-inducible promoter (26). Tamoxifen drives efficient Cre-mediated recombination in almost all cells and does not react with native receptors, thus causing expression of a stabilized β -catenin protein. Cells from the *Catnb*^{lox(ex3)} mouse were also treated with an adenovirus expressing Cre recombinase and green fluorescent protein (GFP) or an adenovirus expressing only GFP, as in our previous work (27), to allow tracking *in vivo*. All mice were onto a C57BL/6 background.

Analysis of tumors in mice

Generation of the *Apc*^{wt/1638N} and *Sca-1*^{-/-} mouse crosses allowed for the comparison of littermate controls between the various genotypes. Fifteen male and 15 female mice of each genotype were sacrificed at 6 months of age, and the

number of aggressive fibromatosis tumors, intestinal polyps, and skin cysts was scored by an observer blinded to the genotype as previously reported (24). For histology, representative tumor samples were formalin fixed, paraffin embedded, sectioned, and stained with H&E. To determine the number of cells present in the tumors, a blinded study was performed, counting the number of nuclei present in stained sections.

Cell culture

MSCs were isolated as previously described (28). Briefly, mice were euthanized at 8 weeks of age, femurs and tibias were removed, and the bone marrow was aspirated. Recovered cells were plated in MesenCult MSC basal medium for mouse MSCs supplemented with MSC stimulatory supplements (StemCell Technologies). After 72 hours, the medium was changed to remove nonadherent cells. For colony-forming unit (CFU) studies, the recovered bone marrow cells were counted and plated at 9×10^5 nucleated cells/cm². To measure the number of CFU-fibroblastic (CFU-F), stromal cells were cultured for 7 days, after which they were stained with crystal violet [0.05% (w/v) in methanol; Sigma], and colonies >1 mm were counted (29). To determine if the MSCs derived from mice expressing the *Catnb*^{lox(ex3)} allele have altered differentiation characteristics, their ability to differentiate down adipocytic or osteogenic lineages was determined as previously reported (30–32). Adipogenic differentiation was induced by using medium supplemented with isobutyl methylxanthine, indomethacin, insulin, and dexamethasone, and the phenotype was quantitated by detection of Oil Red O–positive cells. Osteogenic potential was determined by adding dexamethasone to the medium, and the phenotype was confirmed using alkaline phosphatase immunohistochemistry. The number of CFUs of each type was measured in the same manner as for CFU-F. Primary dermal fibroblast cell cultures were also prepared from these mice in an identical manner as previously reported (33).

Cell grafting

Cells (3.0×10^4 , 3.0×10^5 , or 3.0×10^6) were resuspended in 50 μ L of 1 \times PBS supplemented with 2% FBS. This suspension was then mixed with 50 μ L of Matrigel (Becton Dickinson) and s.c. injected into immunodeficient nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice as previously described (18, 34). Cells for grafting were derived from WT mice, mice expressing the *Catnb*^{lox(ex3)} allele, and *Apc*^{wt/1638N} mice. Both MSCs, isolated as above, and primary fibroblast cultures from the three genotypes were grafted. In some cases, cells were infected with an adenovirus expressing GFP to allow tracking of the cells. Mice were observed for 12 weeks, after which they were euthanized and tumor formation was assessed. Tumors were removed and samples were paraffin embedded, formalin fixed, sectioned, and stained for H&E. An antibody to GFP was used to determine the origin of tumors that formed in select cases.

Western blot analysis

Western blot analysis was undertaken as previously reported using an antibody for total β -catenin (BD Bio-

sciences) and an antibody to actin (as a loading control; Calbiochem) using previously reported techniques and conditions (8, 9).

Statistical analysis

The mean, SD, and 95% confidence interval were calculated for each data set using Microsoft Excel. Regression analysis was calculated using Microsoft Excel. Data are presented as the mean and 95% confidence interval. The two-tailed Student's *t* test was used to calculate differences between groups of data. Statistical significance is given as $P < 0.05$.

Results

Aggressive fibromatosis tumors express similar genes and markers as MSCs

We examined human aggressive fibromatosis tumor cells for the expression of cell surface markers known to be present on MSCs. Dissociated cells from aggressive fibromatosis tumors were stained with antibodies to CD146, CD44, CD166, CD29, CD15, and Stro-1, and the expression of these cell surface markers was analyzed using flow cytometry. A subpopulation of aggressive fibromatosis cells expressed these markers, suggesting a role for MSCs in the etiology of this tumor type. Thirteen percent ($\pm 6\%$) of cells stained for CD146, 3% ($\pm 3\%$) for Stro-1, 52% ($\pm 15\%$) for CD44, 3% ($\pm 2\%$) for CD166, 97% ($\pm 22\%$) for CD29, and 39% ($\pm 11\%$) for CD15 (\pm data are given as the 95% interval from the four tumors). A heat map of expression arrays from the four aggressive fibromatosis samples was compared with that of MSCs and normal fibroblasts available from public databases. The data from the MSCs were used as the index for ordering the genes. The expression profile from the aggressive fibromatosis cells shared similarities in expression levels with close to two thirds of the MSCs genes, but less than a quarter of the normal fibroblast cell genes, as illustrated by a heat map, as shown in Fig. 1. Thus, human aggressive fibromatosis tumor cells share similarities in the expression of genes with MSCs.

A positive correlation between the number of CFU-F and the number of aggressive fibromatosis tumors that develop in *Apc*^{wt/1638N} mice

To study the role of MSCs in tumor development *in vivo*, we used a mouse that develops aggressive fibromatosis. *Apc*^{wt/1638N} mice carry a targeted mutation at codon 1638 of the murine *APC* gene. Mice heterozygous for the *Apc*^{1638N} mutation develop large numbers of aggressive fibromatosis with complete penetrance, and more tumors form in male mice than in female mice (11). The inherent variability in the number of aggressive fibromatosis tumors (between 10 and 45 tumors at 6 months of age) that form in each individual male *Apc*^{wt/1638N} mouse allowed us to determine if a relationship existed between the numbers of tumors and the number of MSCs. CFU-F, which has the potential to differentiate into various mesenchymal lineages, is a surrogate measure of MSCs present in the bone marrow, and as such,

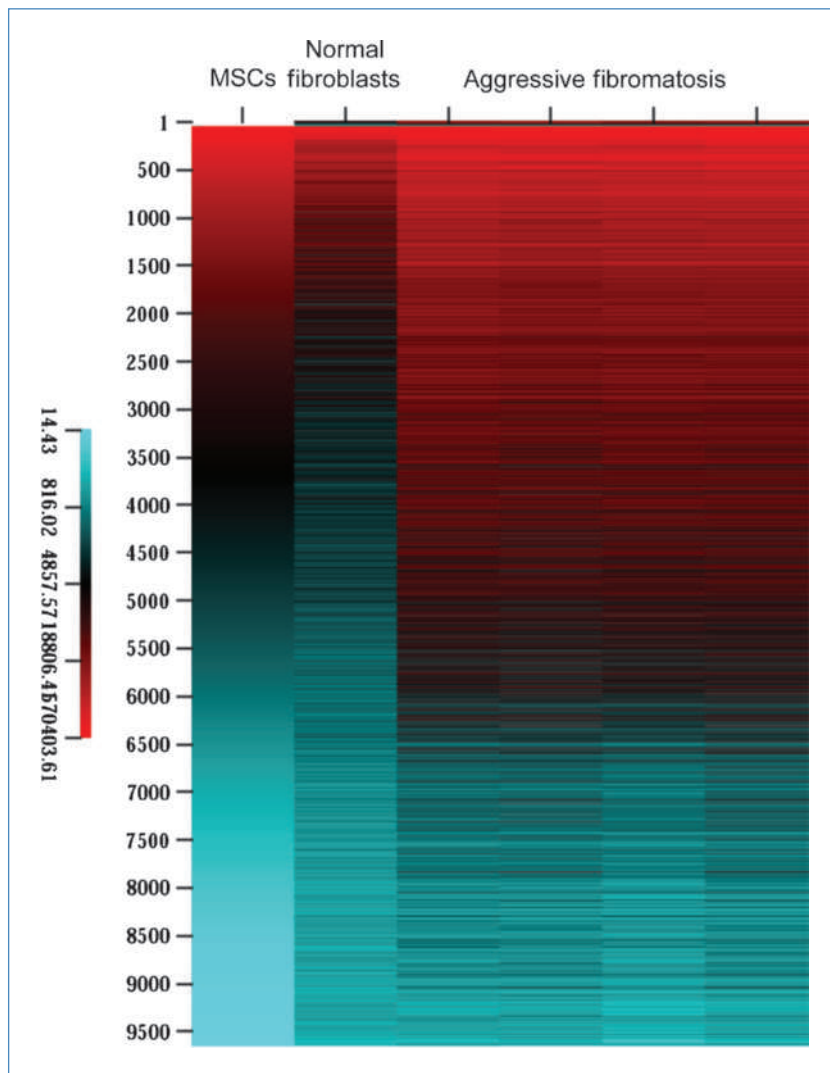


Figure 1. Human aggressive fibromatosis tumors express genes characteristic of MSCs. A heat map of expression arrays from four aggressive fibromatosis samples compared with that of the MSCs and normal fibroblasts. The data from the MSCs were used as the index for ordering the genes. The expression profiles from the aggressive fibromatosis cells share more similarities in expression levels with MSCs than genes expressed by normal fibroblasts.

CFU-Fs can be used as an estimation of MSCs present. Ten $Apc^{wt/1638N}$ male mice were sacrificed at 6 months of age, and femoral bone marrow was removed and plated to assess CFU-F formation. We found that mice with low numbers of tumors had fewer CFU-Fs compared with high numbers of tumors (Fig. 2).

Mice deficient in mesenchymal progenitors develop fewer aggressive fibromatosis tumors

Mice lacking *Sca-1* exhibit fewer numbers of MSCs due to reduced capacity to self-renew, as marked by progressively fewer CFU-F with age (22). Using a previously described breeding strategy, we mated $Apc^{wt/1638N}$ mice to $Sca-1^{-/-}$ mice and generated $Apc^{wt/1638N}$ mice that were either WT, heterozygous, or completely lacking the *Sca-1* gene (24). $Apc^{wt/1638N};Sca-1^{-/-}$ had fewer numbers of CFU-F compared with $Apc^{wt/1638N};Sca-1^{+/+}$ littermate controls (Fig. 3A).

At 6 months of age, mice were sacrificed and tumor development was assessed. We found that $Apc^{wt/1638N};Sca-1^{+/+}$

mice had the greatest numbers of tumors, averaging 24 tumors per male mice. In contrast, $Apc^{wt/1638N};Sca-1^{-/-}$ littermate controls developed significantly fewer tumors, averaging 12 tumors per male mouse (Fig. 3B). Consistent with previously published reports, male mice developed more tumors than female mice (6, 9, 10). However, as with the males, $Apc^{wt/1638N};Sca-1^{+/+}$ female mice formed more tumors than $Apc^{wt/1638N};Sca-1^{-/-}$ littermate controls (Fig. 3B). Tumors from each genotype were also stained with H&E. We found that tumors derived from $Apc^{wt/1638N};Sca-1^{-/-}$ had fewer cells compared with $Apc^{wt/1638N};Sca-1^{+/+}$ mice (Fig. 3C and D). In addition, tumors from $Apc^{wt/1638N};Sca-1^{+/+}$ mice were larger than from $Apc^{wt/1638N};Sca-1^{-/-}$ mice. For female mice, the difference was $16.5 (\pm 4.7) \text{ mm}^3$ versus $8.3 (\pm 3.6) \text{ mm}^3$ mean volume per tumor, and for male mice, the difference was $22.5 (\pm 6.8) \text{ mm}^3$ versus $12.8 (\pm 4.2) \text{ mm}^3$ (both $P < 0.05$). Thus, reducing the number and self-renewal capacity of MSCs affects both the number of aggressive fibromatosis that forms and the cellularity of the tumors.

Epithelial-derived tumors are not affected by a reduction in MSCs

In addition to the formation of mesenchymal neoplasms, *Apc*^{wt/1638N} mice also develop intestinal polyps and skin cysts (12). Interestingly, whereas the numbers of aggressive fibromatosis were reduced in *Apc*^{wt/1638N};*Sca-1*^{-/-} mice when compared with *Apc*^{wt/1638N};*Sca-1*^{+/+} littermate controls, no differences were observed in the numbers of intestinal polyps or skin cysts (Fig. 4) in either male or female mice, thus showing that in *Apc*^{wt/1638N}, epithelial neoplasms were not affected by the ablation of *Sca-1*.

Mesenchymal precursors from *Apc*^{wt/1638N} and mice expressing the *Catnb*^{lox(ex3)} allele have the capacity to initiate tumor formation

We next determined whether MSCs with an oncogenic mutation have tumorigenic potential. To address this question, we isolated bone marrow stromal cells and dermal fibroblasts from 8-week-old *Apc*^{+/1638N}, *Apc*^{+/+}, and *Catnb*^{tm2Kem} mice. Cells from the genotypes were s.c. injected into immunodeficient NOD/SCID mice. For *Catnb*^{lox(ex3)} mice, cells were examined with and without activation of the conditional activated β -catenin allele, and an elevated level of β -catenin was observed in cells in which the conditional allele was activated. After 12 weeks, mice were sacrificed and tumor formation was assessed. We detected aberrant cellular growth in mice injected with 3.0×10^5 or 3.0×10^6 MSCs derived from *Apc*^{+/1638N} and *Catnb*^{lox(ex3)} mice in which the conditional allele was activated (Fig. 5). In contrast, none of the mice injected with cells derived from WT mice generated tumors, nor did mice in which dermal fibroblasts were injected develop tumors. Histologic examination of the lesions revealed the presence of cells of mesenchymal origins, specifically cells resembling those found in the bone marrow. Examination

of tumors from *Catnb*^{lox(ex3)} mice expressing GFP showed that the tumors expressed GFP, verifying they were of donor mouse origin. We observed that MSCs with oncogenic mutations have the capacity to deregulate cellular growth, and the resulting tumors are reminiscent of aggressive fibromatosis tumors.

Mesenchymal precursors from mice expressing the *Catnb*^{lox(ex3)} allele have a decreased differentiation ability

To determine if β -catenin stabilization altered the ability of MSCs to differentiate, stromal cells from *Catnb*^{lox(ex3)} mice expressing either the WT or the stabilized allele were grown in the presence of differentiation medium, and the number of osteoblastic and adipocytic CFUs that formed was compared between cells expressing a β -catenin-stabilized allele and a WT allele. Whereas stabilization of β -catenin resulted in a larger number of CFU-F, it resulted in a smaller number of CFU-osteoblastic and CFU-adipocytic (Fig. 6). This shows that β -catenin maintains MSCs in a less differentiated and more proliferative state.

Discussion

Cells from aggressive fibromatosis tumors express cell surface markers and genes expressed in mesenchymal progenitors. However, this observation does not imply that tumors arise from normal stem cells, as neoplastic cells can express genes that are characteristically expressed in progenitor cell types (13, 14). As such, we sought to identify the cells responsible for oncogenesis before tumor formation. We observed a positive correlation between MSC numbers and aggressive fibromatosis tumor formation and that the loss of MSCs caused a reduction in the numbers of aggressive fibromatosis tumors in mature mice. We also showed that MSCs derived from mice predisposed to aggressive fibromatosis tumor formation have the capacity to initiate aberrant cellular growth, resulting in a tumor that shares cytologic similarities to aggressive fibromatosis in immunocompromised mice. Taken together, these findings raise the likelihood that development of aggressive fibromatosis is influenced by a primitive mesenchymal precursor. The observation that MSC numbers did not affect the formation of intestinal neoplasms, which derive from epithelial precursors, strengthens the specificity of the association between aggressive fibromatosis and MSCs.

Recent studies show that primary MSCs transduced to express an oncogenic mutation can form sarcomas (35, 36). Mesenchymal cells in peripheral tissues also can derive from circulating precursors, as has been shown for adipocytes (37). These findings raise the possibility that these tumors arise from circulating MSCs harboring a mutation, resulting in β -catenin stabilization that hones to peripheral sites, causing aggressive fibromatosis. This possibility can be tested by determining if a mutated form of β -catenin into human MSCs will result in aggressive fibromatosis tumors, and by performing bone marrow and MSC transplantations from mice harboring mutation resulting in

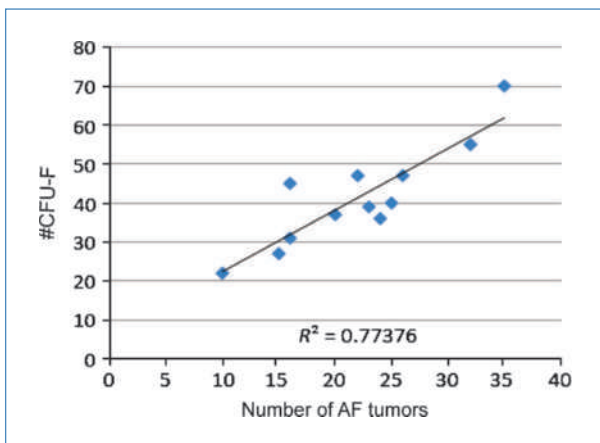


Figure 2. A positive correlation between the numbers of aggressive fibromatosis (AF) tumors that form and CFU-F in mice. *Apc*^{wt/1638N} mice with fewer numbers of CFU-Fs develop fewer numbers of aggressive fibromatosis. *Apc*^{wt/1638N} mice were sacrificed at 6 mo of age, and tumors were counted. In tandem, CFU-F from each sacrificed mouse were assessed. Data represent a total of 15 male mice, and trend line represents the linear regression.

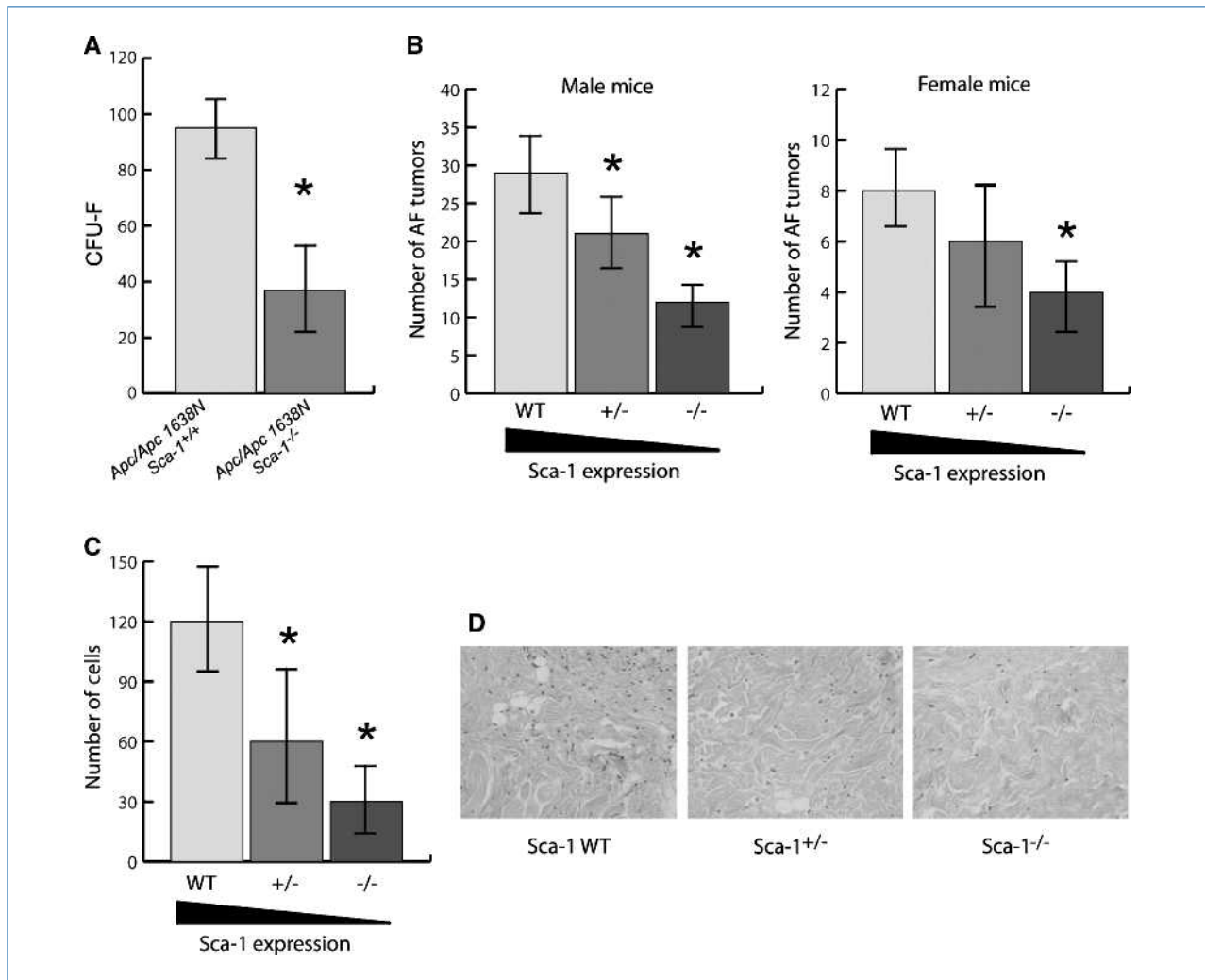


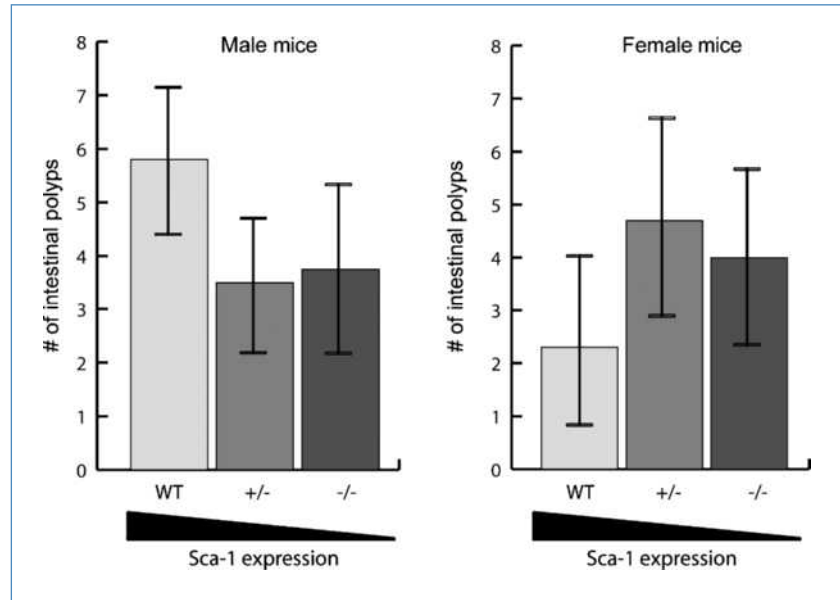
Figure 3. Loss of Sca-1 reduces numbers of aggressive fibromatosis tumors in *Apc^{wt/1638N}* mice. A, there were fewer numbers of CFU-F in *Apc^{wt/1638N}; Sca-1^{-/-}* mice when compared with *Apc^{wt/1638N}; Sca-1^{+/+}* mice. Columns, mean number of CFU-F isolated from three independent mice from each genotype; bars, 95% confidence intervals. B, *Apc^{wt/1638N}; Sca-1^{+/+}*, *Apc^{wt/1638N}; Sca-1^{+/-}*, and *Apc^{wt/1638N}; Sca-1^{-/-}* mice were sacrificed at 6 mo of age, and aggressive fibromatosis tumors were scored. In both males and females, *Apc^{wt/1638N}; Sca-1^{-/-}* mice formed fewer tumors than *Apc^{wt/1638N}; Sca-1^{+/+}* mice. Columns, mean number of tumors per mice (total of 15 mice per given genotype); bars, 95% confidence intervals. C, tumors derived from *Apc^{wt/1638N}; Sca-1^{-/-}* mice have decreased cellularity when compared with *Apc^{wt/1638N}; Sca-1^{+/+}* littermate controls. Columns, mean number of nuclei counted in each field of vision at $\times 400$ magnification (three mice for each given genotype and four fields for each section were counted); bars, 95% confidence intervals. D, representative H&E slides of the tumors from each genotype show diminished numbers of nuclei in tumors derived from *Apc^{wt/1638N}; Sca-1^{-/-}* mice when compared with *Apc^{wt/1638N}; Sca-1^{+/+}* mice. An asterisk above a data point shows a significant difference from *Sca-1^{+/+}* mice.

β -catenin stabilization to determine if such transplanted circulating cells can form aggressive fibromatosis tumors. In our study, we did not transduce a mutated form of β -catenin into human MSCs, nor did we undertake bone marrow and MSC transplantations. Such future studies would more clearly define the cell of origin of aggressive fibromatosis tumors.

Not only did we show a reduction in the number of tumors from *Apc^{wt/1638N}; Sca-1^{-/-}*, but we also showed that tumors derived from these mice are smaller and contain fewer cell numbers when compared with *Apc^{wt/1638N}; Sca-1^{+/+}* mice. Mice lacking *Sca-1* develop age-dependent osteoporosis and this phenotype is caused not by the loss of differentiated

osteoprogenitors but rather by a deficit in the numbers of MSCs. Interestingly, no differences in MSC frequency were detected in young *Sca-1^{-/-}* mice; however, aged *Sca-1^{-/-}* mice have much lower numbers of MSCs when compared with their WT counterparts. This deficit and the subsequent development of age-dependent osteoporosis are attributed to an impairment in the capacity of MSCs to self-renew (22, 38). It has been postulated that Sca-1 may play a role in balancing the signals between differentiation and self-renewal in stem cells (39). Given these observations, decreased tumor cellularity and tumor size in *Apc^{wt/1638N}; Sca-1^{-/-}* mice may be attributed to a diminished capacity of oncogenic MPCs to self-renew.

Figure 4. Loss of Sca-1 does not alter the number of gastrointestinal tumors formed by *Apc*^{wt/1638N} mice. *Apc*^{wt/1638N}, *Sca-1*^{+/+} and *Apc*^{wt/1638N}, *Sca-1*^{+/-} mice develop similar numbers of epithelial-derived lesions. Mice from each of the three genotypes were sacrificed at 6 mo of age, and the number of intestinal polyps and skin cysts was counted. No significant differences were observed in the numbers of intestinal polyps or skin cyst between *Apc*^{wt/1638N}, *Sca-1*^{+/+}, *Apc*^{wt/1638N}, *Sca-1*^{+/-}, and *Apc*^{wt/1638N}, *Sca-1*^{-/-} mice. Columns, mean number of tumors per mice (15 mice per given genotype); bars, 95% confidence intervals.



MSCs are multipotent with the capacity to differentiate into muscle, fat, bone, cartilage, and fibroblastic cells. Isolation of single cell-derived clonal populations reveals that lineage commitment is not a random process, but rather, a

cellular hierarchy exists, with the quinti-potent, self-renewing MPC at the apex and the restricted fibroblast at the base (40). Deregulation of these well-orchestrated events dramatically alters the lineage commitment of cells within this hierarchy.

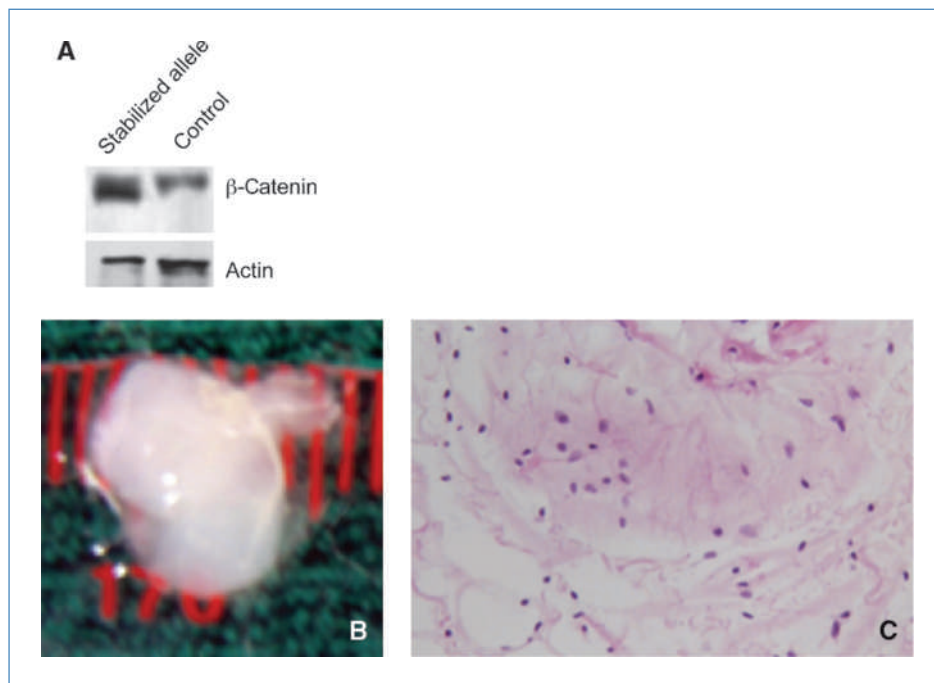


Figure 5. Stromal cells from *Catnb*^{lox(ex3)} and *Apc*^{wt/1638N} mice have the potential to induce aberrant cellular growth. Bone marrow stromal cells isolated from *Catnb*^{lox(ex3)}, *Apc*^{wt/1638N}, and *Apc*^{wt/wt} mice were s.c. injected into NOD/SCID mice. After 3 mo, mice were sacrificed and tumor formation was assessed. Only MSCs isolated from *Apc*^{wt/1638N} and *Catnb*^{lox(ex3)} mice had the potential to induce aberrant cellular growth. A, Western blot analysis showing increased β-catenin protein from *Catnb*^{lox(ex3)} mice in which the conditional allele is activated. A slightly smaller size to the protein product expressed from the alleles in which exon 3 is deleted is shown, and a 3-fold increase in protein level was detected using a pan-β-catenin antibody as determined using densitometry. B, gross appearance of the lesions that formed. C, representative H&E staining of lesions was performed for histologic examination. These growths have cytologic characteristics similar to those found in aggressive fibromatosis.

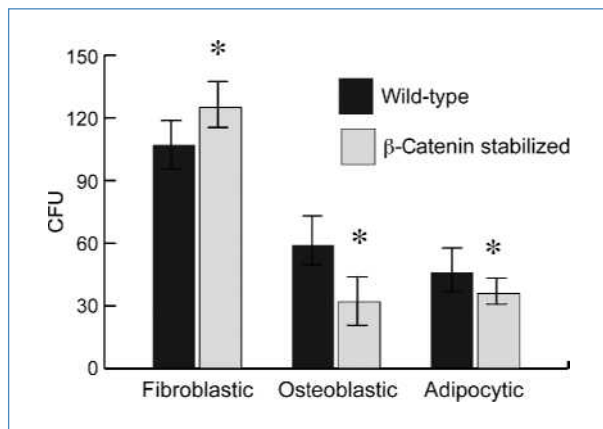


Figure 6. Stromal cells from *Catnb^{lox(ex3)}* mice have a decrease in their ability to differentiate. Stromal cells from mice expressing the *Catnb^{lox(ex3)}* allele develop larger numbers of CFU-F, but fewer numbers of CFU-osteoblastic and CFU-adipocytic. Columns, mean; bars, 95% confidence intervals. An asterisk above a data point shows a significant difference from cells from WT mice.

The *Wnt*/ β -catenin pathway is implicated in regulating this differentiation process and is also a known molecular determinant of aggressive fibromatosis tumor formation. Therefore, in the case of aggressive fibromatosis, distortion of this signaling pathway may tilt a large proportion of mesenchymal precursors to forfeit toward the committed fibroblast, a defining feature of aggressive fibromatosis tumors. The reduction in the number of MSCs in *Apc^{wt/1638N};Sca-1^{-/-}* may therefore lower the numbers of cells mandated down an impaired differentiation pathway.

A role for β -catenin altering MSC differentiation is supported by other *in vitro* studies (41), as well as by *in vivo* studies in repair processes. Indeed, during fracture repair, elevated β -catenin results in cells maintaining a fibroblast-like phenotype, rather than developing an osteoblastic phenotype (42). Thus, in aggressive fibromatosis, a MPC with a mutation resulting in β -catenin stabilization maintains the cell with a proliferative advantage in an undifferentiated state, resulting in a fibroproliferative tumor. Our data suggesting that β -catenin stabilization inhibits differentiation, maintaining cells in an undifferentiated form, support this notion. Pharmacologic agents to modulate β -catenin signaling are under development, and one such agent, lithium, is already available for clinical use (43, 44). This raises the possibility that β -catenin modulation could be used to prevent the development of aggressive fibromatosis in susceptible individuals.

Depletion of *Sca-1*-positive cells in the mammary gland results in the loss of regeneration potential in mammary gland reconstitution experiments, showing the ability of *Sca-1* to prospectively identify a population of mammary epithelial cells (45). Interestingly, in mouse models of breast cancer, overexpression of components of the *Wnt* signaling pathway results in an expansion of *Sca-1*/keratin 6 progenitor cells. In contrast, in mouse models where tumorigenesis is driven by the overexpression of H-ras, Neu, or middle T antigen, no expansion of *Sca-1*/keratin 6 progenitor cells is detected (46, 47).

The molecular etiology of aggressive fibromatosis is well known, where the upregulation of β -catenin signaling is a hallmark of these neoplasms. Taken together, this suggests that the β -catenin pathway may contribute, in part, to tumorigenesis by expanding the progenitor cell compartment, allowing for an increase in the number of cells that may be responsible for tumor maintenance. As such, loss of *Sca-1* results in fewer numbers of progenitors susceptible to this aberrant expansion.

The loss of *Sca-1* may diminish the number of aggressive fibromatosis tumors through multiple mechanisms. First, the reduction in the number of mesenchymal progenitors, which act as a potential candidate cell of origin for these neoplasms, results in fewer cells with the potential to form tumors. This may be particularly important in mesenchymal neoplasms susceptible to β -catenin signaling, such as aggressive fibromatosis, for this loss is amplified in the tumorigenic process by reducing the number of progenitor cells available for both cellular expansion and distorted lineage commitment. Second, in *Sca-1* null mice, the remaining MSCs have an impaired ability to self-renew, resulting in the eventual loss of tumors by targeting those cells responsible for the maintenance of the malignant tissue. This provides strong evidence that functional MSCs are important in the development and progression of aggressive fibromatosis.

Whether aggressive fibromatosis originates from a progenitor cell that acquires an oncogenic mutations or whether it arises from a differentiated cell that acquires a mutation, giving it a stem-like phenotype, has yet to be elucidated; however, these data show an important influence of MPCs in this neoplasm. The identification of the primary cell of origin in aggressive fibromatosis is a key step toward an understanding of the pathology of this disease. Here, we provide evidence to support a model in which cells with MSC-like characteristics play a role in both the initiation and maintenance of aggressive fibromatosis tumors, raising the intriguing possibility that protecting the stem cells in patients with FAP can prevent aggressive fibromatosis and understanding MSC biology can be used to develop new treatments.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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