Gene expression in aggressive fibromatosis

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Aggressive fibromatosis represents a group of tumors with heterogeneous patterns of biologic behavior. In this study, gene expression in 12 samples of aggressive fibromatosis, as well as that in samples of normal skeletal muscle and a variety of normal tissues, was determined at Gene Logic Inc (Gaithersburg, MD), with the use of Affymetrix GeneChip U_133 arrays containing approximately 33,000 genes. Gene-expression analysis was performed with the Gene Logic Gene Express® software system. Differences in gene expression were quantified as the fold change in gene expression between the sets of fibromatosis tissue and normal skeletal muscle. A set of genes was then identified that was significantly overexpressed in aggressive fibromatosis compared with expression in normal muscle. This set of genes was then further examined for expression in a variety of normal tissues. We identified genes that were selectively overexpressed in aggressive fibromatosis compared with expression in 448 samples comprising 16 different nonneoplastic tissues. In particular, ADAM12, WISP-1, SOX-11, and fibroblast activation protein-α were uniquely overexpressed in aggressive fibromatosis compared with expression in normal tissues. In addition, the technique of Eisen clustering identified 2 distinct subgroups of aggressive fibromatosis with regard to gene expression. We conclude that gene-expression patterns may be useful in the further classification of subtypes of aggressive fibromatosis and that such classification could have clinical significance. (J Lab Clin Med 2004;143:89-98)

Abbreviations: ADAM = a disintegrin and metalloproteinase; AF = aggressive fibromatosis; APC = adenomatous polyposis coli; FAP = fibroblast activation protein; HMG = high-mobility group; IGF = insulin-like growth factor; IGFBP = insulin-like growth factor–binding protein; MFH = malignant fibrous histiocytoma; MMTV = mouse mammary-tumor virus; NOS = not otherwise specified; PDGF = platelet-derived growth factor; TGF = transforming growth factor; TNF = tumor necrosis factor

Aggressive fibromatosis, or desmoid tumor, is a monoclonal proliferation of myofibroblasts with variable collagen deposition that is locally invasive but rarely metastasizes.[1–6] These myofibroblasts have histologic similarities to the proliferative phase of wound healing. AF and fibrosarcomas are part of a spectrum of diseases characterized by abnormal proliferation of fibroblastic cells. AF, unlike fibrosarcoma, is generally characterized by normal expression of Ki-67, Bcl-2, and the retinoblastoma gene product.[7] AF has been associated with trauma, pregnancy, and the use of oral contraceptives.[3] The optimal treatment of AF is a matter of controversy.[8–10]

The β-catenin pathway has been strongly implicated in the pathogenesis of AF. β-Catenin has a nuclear function, in which it binds transcription factors, and a cell-membrane function, in which it is a component of epithelial-cell adherens junctions.[11,12] The authors of a recent study found that AF develops in transgenic mice with stabilized β-catenin,[13] showing that the genes regulated by β-catenin are likely important in the pathophysiology of AF.

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Table I. Characteristics of patients supplying AF samples

Sample no.	Age (yr)	Sex	Location	Previous treatment	Postoperative treatment	Follow-up
F1	68	F	Right side of neck	None	None	No follow-up (5 yr after sampling)
F2	23	M	Right shoulder	Sulindac progression	XRT	No follow-up (5 yr after sampling)
F3	35	F	Rectus sheath	None	None	No follow-up (5 yr after sampling)
F4	13	M	Right buttock	None	None	NED at 18 mo
F5	36	M	Left thigh	None	None	No follow-up (4 yr after sampling)
F6	21	M	Left sarcal	None	None	Recurrence at 1 yr, treated with methotrexate/vinblastine with PD excised; recurred at 6 mo with slow PD over 2 yr
F7	22	F	Left buttock	None	None	Recurrence at 1 yr, treated with methotrexate/vinblastine (lung toxicity), excised, XRT, recurred at 3 yr, reexcised
F8	33	F	Rectus sheath	None	None	NED at 3 yr
F9	33	F	Rectus sheath	None	None	No follow-up (4 yr after sampling)
F10	23	M	Left side of pelvis	None	None	No follow-up (4 yr after sampling)
F11	19	M	Right flank	Methotrexate/vinblastine for 4 mo with progression	None	NED at 29 months
F12	16	M	Left arm	Excised 1 year earlier and recurred	None	No follow-up (4 yr after sampling)

NED, No evidence of disease; *PD,* progressive disease; *XRT,* radiation therapy.

Abnormal growth-factor production has also been associated with AF. In vitro studies have revealed that fibroblasts from plantar fibromatosis produced more PDGF-A and PDGF-B in response to strain than did normal fibroblasts, suggesting that PDGF plays a role in AF.[14] A study comprising 20 patients with plantar fibromatosis revealed coexpression of TGF-α and its receptor, epidermal growth factor receptor, by the myofibroblasts in proliferative fibromatosis, suggesting an autocrine or paracrine role in its pathogenesis.[15] The findings of in vitro studies of fibroblasts from patients with hereditary gingival fibromatosis suggest that TGF-β1 is involved in this disease, acting as an autocrine stimulator of fibroblast proliferation.[16]

To better understand the pathophysiology of AF, we sought to characterize gene expression in this disease. The expression of roughly 33,000 genes in 12 cases of AF and 20 samples of normal skeletal muscle was determined with the use of the Affymetrix microarray technique, and differences in gene expression were analyzed. The expression of genes of interest was then examined in 534 other samples obtained from 23 different types of tissue. We conclude that differences in gene expression may help characterize AF, yield clues to the pathophysiology of this category of tumor, and identify potential targets for therapy.

METHODS

Tissue samples. We obtained tissue from 12 cases of AF (F1– F12; 7 male, 5 female; age range 13 to 68 years, median 23 years; none with Gardner's syndrome; Table I) from the Tissue Procurement Facility of the University of Minnesota. We also obtained 554 samples from 24 different types of tissues: 22 of normal adipose tissue, 25 of normal cervix, 41 of normal colon, 15 of normal kidney, 14 of normal liver, 27 of normal lung, 20 of normal skeletal muscle, 85 of normal myometrium, 38 of normal ovary, 10 of normal skin, 28 of normal small intestine, 8 of normal stomach, 63 of normal thymus, 24 of tonsils with lymphoid hyperplasia, 10 of thyroid gland with nodular hyperplasia, 18 of gallbladder with chronic inflammation, 25 of uterine leiomyoma, 12 of colon adenocarcinoma, 9 of squamous-cell carcinoma of the lung, 8 of renal-cell cancer, 18 of papillary ovarian serous adenocarcinoma, 13 of MFHs, 14 of high-grade sarcomas NOS, and 7 of Wilms tumor. Samples were obtained with the use of protocols approved by the University of Minnesota Institutional Review Board. Tumor and normal samples were identified and snap-frozen in liquid nitrogen within 30 minutes of removal from the patient. Tissue sections of each sample were prepared before freezing and examined under light microscopy after hematoxylin-and-eosin staining to confirm the pathologic nature of the sample. None of the samples was necrotic.

Gene-expression analysis. RNA was prepared and gene expression determined at Gene Logic Inc (Gaithersburg, Md), with the use of Affymetrix GeneChip U_133 arrays containing approximately 33,000 genes. Gene-expression analysis was performed with the Gene Logic Gene Express® software system and the Gene Logic standardization algorithm. A fold-change analysis was performed in which the ratio of the geometric means of the expression intensities of the relevant gene fragments was computed; this ratio was reported as the

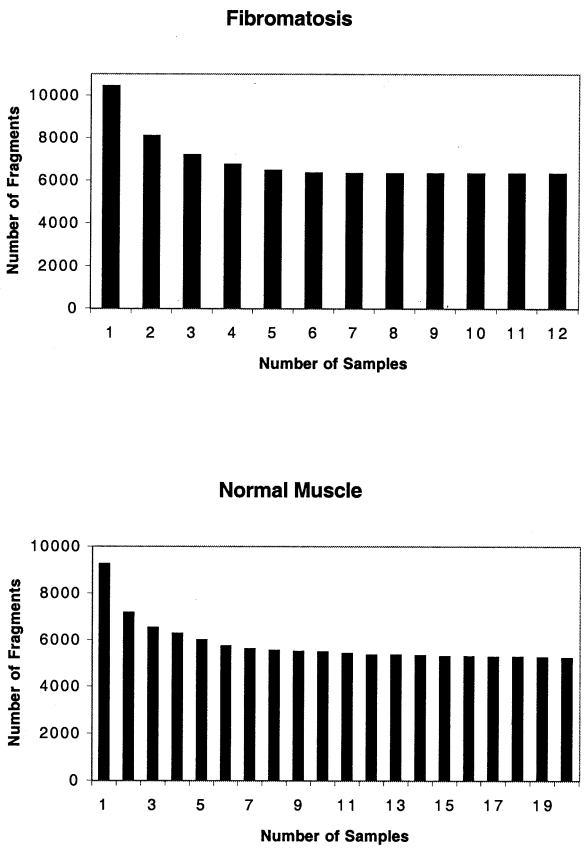


Fig 1. Dependence of the number of gene fragments present in all samples on the number of samples analyzed. The number of fragments present in all samples is shown as a function of the number of samples analyzed in the sample sets. *Top*: AF; *bottom*: normal muscle.

fold change (up or down). Confidence intervals and *P* values on the fold change were also calculated with the use of a 2-sided Welch modified 2-sample *t* test. *P* values of .05 or less were considered significant. Contrast Analyses™ and e-Northern™ analyses were performed with the Gene Logic Gene Express® software system. Clustering was performed with Eisen clustering software and viewed with Tree View software (rana.lbl.gov).

RESULTS

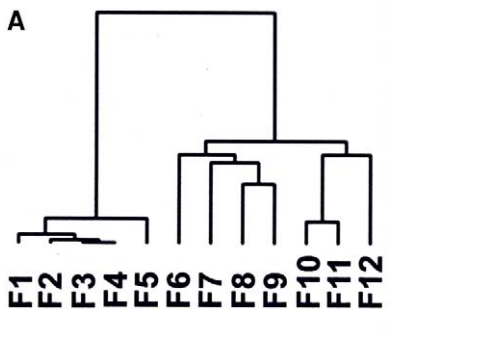
Gene expression in AF samples. Gene expression detected with the use of the Affymetrix GeneChip U_133 chip set was performed on all samples. About 6000 of the roughly 40,000 gene fragments examined were present in all 12 of the samples in the AF set. This number did not vary greatly when 6 or more samples of the set were included in the analysis (Fig 1, *top*). About 5500 gene fragments were present in all of the samples of the normal-muscle set, and little variation was noted when 7 or more samples were included in the analysis (Fig 1, *bottom*).

AF appears as a proliferation of myofibroblast-appearing cells. To identify genes that are overexpressed in AF compared with expression in other tissues, a fold-change analysis was performed that identified a set of 1721 gene fragments that were overexpressed at least twofold more in the set of AF samples than in the set of normal muscle. Contrast Analysis™ with this set of 1721 gene fragments was then performed to identify those genes most overexpressed in the AF set compared with that in normal adipose tissue. The resulting set of 1093 gene fragments was then analyzed with sequential Contrast Analyses™ comparing AF first with normal liver, then with normal myometrium, normal kidney, normal ovary, normal lung, normal colon, normal cervix, normal thymus, normal small intestine, inflamed gallbladder, nodular hyperplasia of the thyroid, and a set of 25 high-grade soft-tissue sarcomas classified as either MFH or high-grade sarcoma NOS. In each analysis, those gene fragments most overexpressed in AF were used for the next Contrast Analysis™. These analyses yielded a set of 186 gene fragments that were selectively overexpressed in AF compared with expression in these other tissues.

The expression of these 186 gene fragments was then examined in the sets of AF and normal tissues through the use of the Gene Logic e-Northern™ analysis software. This analysis provides a graphic representation of the level of gene expression in each sample of a sample set. These analyses suggested that the set of AF samples was heterogeneous in terms of gene expression and might be best analyzed as 2 separate subsets.

Clustering. To better examine the possibility that the set of 12 AF samples could be separated into 2 distinct sets on the basis of gene-expression profiles, clustering was performed with the Eisen clustering software Cluster and a random set of 6000 gene fragments from the Affymetrix U_133 chip set. When clustering was performed with this set of genes, the 12 AF samples clustered into 2 distinct groups, samples F1 to F5 (set A) and F6 to F12 (set B) (Fig 2, *A*). It should be noted that the left-right position around a node on the tree has no significance; rather, it is the length of the line to the node that indicates the similarity of the gene-expression pattern to that of the next cluster. Similarly, samples F1 through F5 formed a separate group from samples F6 through F12 when clustering was performed with the set of 186 gene fragments identified by Contrast Analysis™ as being most overexpressed in the set of AF samples compared with expression in other tissues (Fig 2, *B*). Because of these findings, the AF samples were reanalyzed as 2 distinct sample sets as defined by the clustering.

Gene expression in AF sample sets A and B. About 6500 gene fragments were present in all 5 AF samples



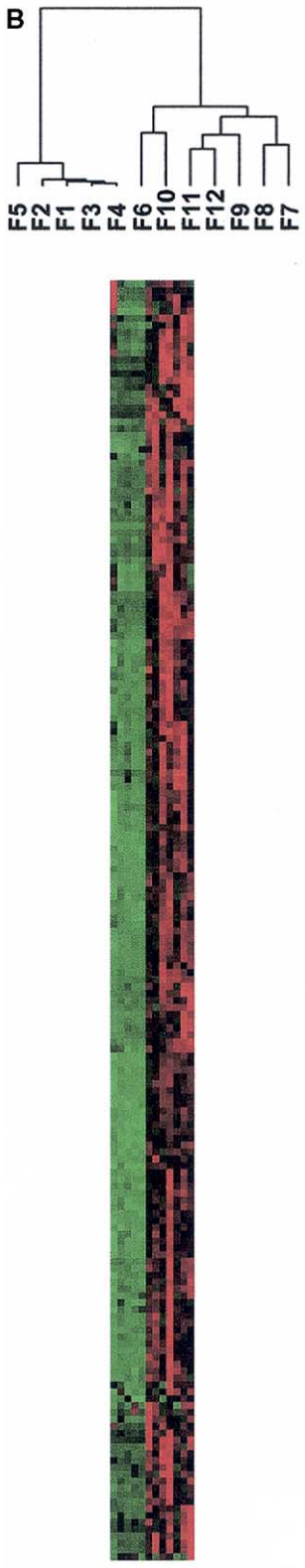


Fig 2. Clustering of gene expression in the AF samples as detected with the Eisen clustering software Cluster. The samples were clustered with the use of a random set of 6000 gene fragments from the U_133 chip set (**A**) or the set of 186 gene fragments most overexpressed in AF compared with expression in nonneoplastic tissues (**B**), as described in the text. The tissue samples in the tree are joined by very short branches if they have gene-expression patterns that are very similar to each other and by increasingly longer branches as similarity decreases. The color of each square represents the ratio of gene expression in the indicated sample relative to the average signal of expression of all genes examined. *Red* indicates gene expression above the median, *green* denotes expression below the median, and *black* indicates expression equal to the median. The intensity of the color reflects the magnitude of divergence from the median. *Columns* represent the indicated tissue sample; *rows* represent individual cDNAs.

in set A (Fig 3, *top*), and about 12,000 gene fragments were present in all 7 samples of AF set B (Fig 3, *bottom*). A fold-change analysis demonstrated that 354 gene fragments were overexpressed at least twofold more in AF set A than in the normal-muscle set, whereas 7529 gene fragments were underexpressed at least twofold less in AF set A compared with expression in normal muscle (Table II). In contrast, fold-change analysis revealed 10,149 gene fragments overexpressed at least twofold more in AF set B than in normal muscle (Table II) and 757 gene fragments underexpressed at least twofold less in AF set B than in normal muscle (Table II).

Genes specific to AF set A. The 354 gene fragments expressed at least twofold more in AF set A than in normal muscle were studied with the use of sequential Contrast Analyses™, as described above, to identify those genes most overexpressed in AF set A compared with expression in 14 other tissues types comprising more than 200 tissue samples. In each analysis, those genes most overexpressed in AF set A were used for the next Contrast Analysis™. These analyses yielded a set of 24 gene fragments that were overexpressed in AF set A compared with expression in these other tissues. In a further exploration of the specificity of expression of these 24 gene fragments, their expression was examined in the 2 sets of AF and the 16 types of nonneo-

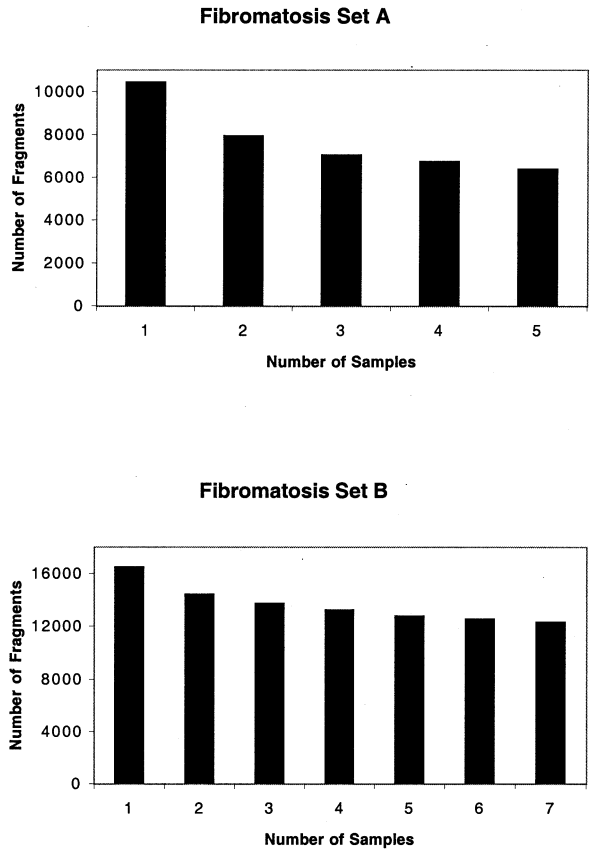


Fig 3. Dependence of the number of gene fragments present in all samples on the number of samples analyzed. The number of fragments present in all samples is shown as a function of the number of samples analyzed in the sample sets. **A**, AF set A; **B**, AF set B.

plastic tissues comprising 448 tissue samples with the use of the Gene Logic e-Northern™ analysis software. This analysis provides a graphic representation of the level of gene expression in each sample of a sample set. No genes were specifically overexpressed in AF set A compared with expression in the other tissues.

Genes specific to AF set B. Similarly, sequential Contrast Analyses™ were performed on the set of 10,149 gene fragments expressed at least twofold more in AF set B than in normal muscle, yielding a set of 170 gene fragments that were selectively overexpressed in AF set B compared with expression in the 14 other normal tissues examined. The expression of these 170 gene fragments was then examined by means of e-Northern™ analysis in the 2 sets of AF samples and 16 nonneoplastic-tissue sets comprising 448 samples. This e-Northern™ analysis identified 40 known gene fragments comprising 30 known genes that were selectively overexpressed in AF set B compared with expression in normal muscle (Table III). The expression of these genes was then examined by means of e-Northern™ analysis in 8 tumor types comprising 106 samples.

Four genes—a disintegrin and metalloproteinase domain 12 (ADAM12), WNT-1–inducible signaling-pathway protein-1 (WISP-1), SRY-box (SOX-11), and FAP-α—were identified as being overexpressed in AF set B compared expression in with normal muscle and also absent or expressed at very low levels in all of the other 16 nonneoplastic tissues, comprising 448 samples, that were examined.

ADAM12 (meltrin-α) was overexpressed in set B compared with expression in all other tissues, although some expression was observed in roughly 60% of the MFH/sarcoma NOS set, and low expression was detected in 4 of 5 of the AF set A samples (Fig 4). ADAM12 expression in the other normal tissues was similar to that in normal muscle (not shown).

WISP-1 was expressed in all AF samples (more highly in set B than set A) and was expressed in few of the normal samples but only at very low levels (Fig 4). WISP-1 expression in the other normal tissues was similar to that in normal muscle (data not shown). It was also expressed in about 60% of the MFH/sarcoma NOS samples and at low levels in all of the Wilms tumors. Wingless-type MMTV integration site family member 5A was also overexpressed in AF set B compared with that in most samples but was expressed in most samples and expressed at similar levels in myometrium and some of the cancer samples (data not shown).

SOX-11 was expressed more highly in AF set B than in set A, but, as previously reported, not in any other normal tissues examined (Fig 4).[17] As previously reported, SOX-11 was also expressed in Wilms tumors, many liposarcomas, and some ovarian cancers.[17]

FAP-α was overexpressed in AF set B compared with that in set A and all other nonneoplastic samples (Fig 4). It was expressed in myometrium and skin, but at much lower levels (not shown). FAP-α expression in the other normal tissues was similar to that in normal adipose tissue (not shown).

Several growth factors and growth-factor receptors, including TGF-β3, angiopoietin-like-2, and TNF (ligand) superfamily member 4 were also overexpressed in AF set B compared with expression in most normal tissues. Serine protease 11 (IGF binding) was overexpressed in set B compared with expression in all samples but was expressed in nearly all samples.

Several genes representing extracellular matrix proteins were overexpressed in AF. Collagen type XI, α1, and aggrecan-1 were expressed more highly in AF set B than in set A and were not expressed in any of the normal tissues. Chondroitin sulfate proteoglycan-2 (versican), spondin-2, and adlican were overexpressed

Table II. Fold-change analysis of gene expression

Fold-change range*	Relative gene expression in AF set A vs normal muscle		Relative gene expression in AF set B vs normal muscle	
	Up in fibromatosis	Down in fibromatosis	Up in fibromatosis	Down in fibromatosis
>100	0	27	0	5
10–100	18	396	412	133
5–10	57	813	1185	141
4–5	39	632	937	53
3–4	61	1295	2082	113
2–3	179	4366	5533	312
1–2	1999	16930	14989	2137

*Number of gene fragments in each indicated range of fold change.
Number of gene fragments in each range of fold change in expression between AF set A and the normal-muscle set, and AF set B and the normal-muscle set. A fold-change analysis of gene expression in the set of AF sets A and B compared with that in the set of normal muscle was performed as described in the Methods. The number of gene fragments in each indicated range of fold change is shown.

Table III. Genes expressed specifically in AF set B

ADAM12
Aggrecan-1
Angiopoietin-like factor 2
Asporin
Biglycan
Clq and tumor necrosis factor–related protein-3
Calcium channel, voltage-dependent, α 2/Δ 3 subunit
cerebral cell adhesion molecule
Chromosome 20 open reading frame 103
Collagen, type III, α 1
Collagen, type V, α 1
Collagen, type XI, α 1
Collagen, type XIV, α 1
Contactin 1
EGF-like domain, multiple 3
EphB3
Fibroblast activation protein, α
Fibulin-6
Neuregulin-1
Neuronal pentraxin II
Nucleolar autoantigen (55 kD) similar to rat synaptonemal complex protein
Sal-like 4 (*Drosophila*)
Short-stature homeobox 2
SOX-11
Stem-cell growth factor
TGF-β3
TNF (ligand) superfamily, member 4 (CD 134 ligand)
Visinin-like 1
VPS10 domain–receptor protein
WNT1-inducible signaling-pathway protein 1 (WISP-1)

Known genes selectively overexpressed in AF set B compared with expression in normal tissues.

in AF set B compared with expression in set A, all normal tissues, and most cancers. Cartilage oligomeric matrix protein (pseudoachondroplasia) was overexpressed in AF set B compared with expression in set A and normal tissues but was expressed in skin as well. Collagen type I α1, type III α1 (Ehlers-Danlos syndrome type IV), type V α1, type V α2, type VI α1, type VI α2, type XII α1, and type XIV α1 (undulin) were overexpressed in AF set B compared with expression in the other tissues studied but were expressed in many tissues, as would be expected. Similarly, biglycan was overexpressed in AF set B compared with expression in the other tissues but was also expressed in many tissues.

Several neural differentiation antigens were overexpressed in AF set B. Neuronal pextraxin II and cerebral cell-adhesion molecule were overexpressed in AF compared with expression in normal tissues, where they were expressed infrequently at low levels. They were expressed more highly in AF set B than in set A and in a small number of the cancer samples. Neurofilament, heavy polypeptide (200 kD) was overexpressed in about 70% of AF set B samples compared with that in all other tissues samples, except about 40% of the normal-ovary set.

Because β-catenin and cyclin D1 have been reported to potentially play a role in AF, we examined the expression levels of these 2 genes as well. β-Catenin mRNA was expressed in all of the AF samples and in all 448 of the nonneoplastic tissues (Fig 5). β-Catenin expression was higher in AF set B than in set A (Fig 5). No clear difference in cyclin D1 expression between AF set A, AF set B, and other tissues was noted (data not shown).

DISCUSSION

In this study, we examined the expression of roughly 33,000 genes in AF. On cluster analysis, the 12 AF samples were clearly delineated into 2 groups. More than 10,000 gene fragments were found to be overexpressed at least twofold more in AF than in normal

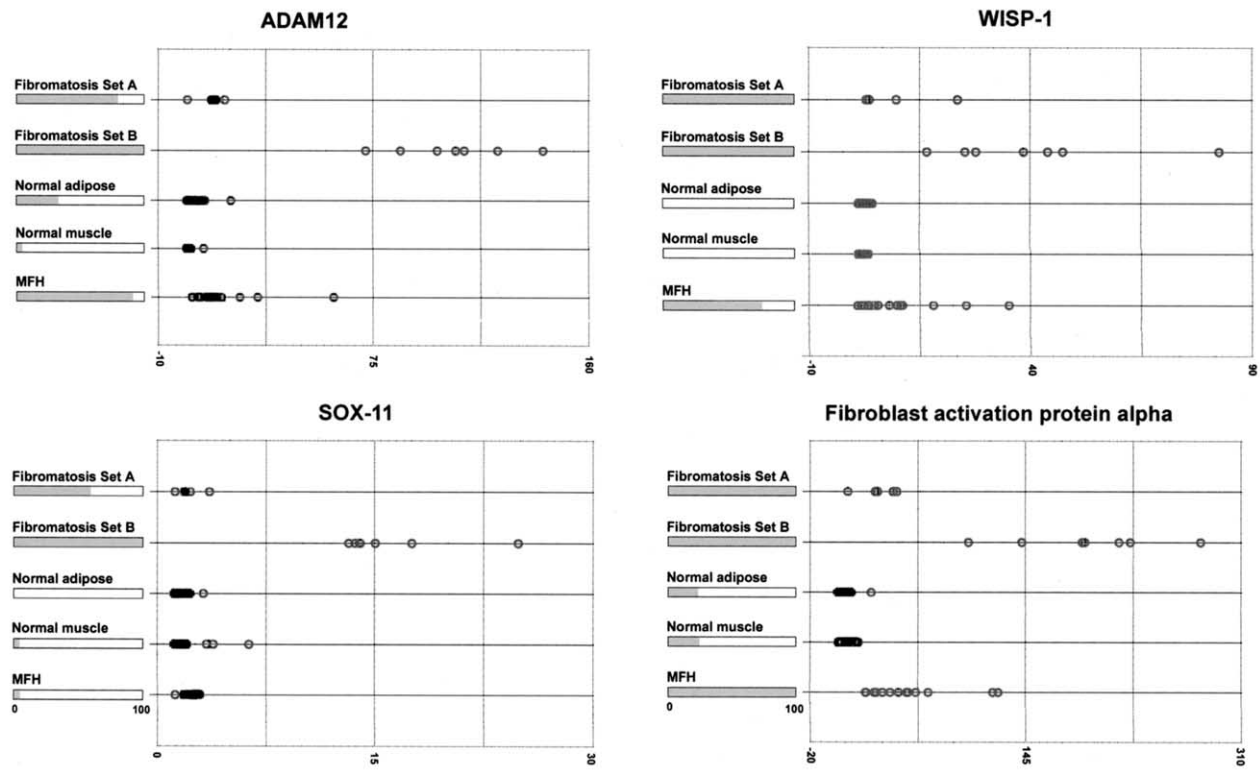


Fig 4. Representative tissue-expression pattern of ADAM12, WISP-1, SOX-11, and FAP-α. Gene expression was examined in a variety of normal and diseased tissue sets, as described in the text. *Left*: Percentage of samples (0–100%) expressing detectable levels of the gene of interest as indicated (*bar graphs*). *Right*: Intensity of gene expression in each sample of the set, plotted as average expression value on a linear scale.

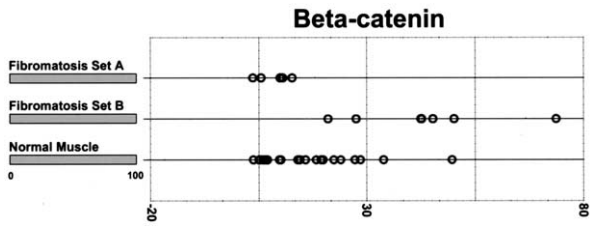


Fig 5. Tissue-expression pattern of β-catenin. We examined expression of the β-catenin mRNA in AF sets A and B and in normal-muscle tissue sets as described in the text. *Left*: Percentage of samples (0–100%) expressing detectable levels of the gene. *Right*: Intensity of gene expression in each sample of the set, plotted as average expression value on a linear scale.

muscle. When the expression of these 10,000 gene fragments was examined in 14 different nonneoplastic-tissue sets, only 170 gene fragments were found to be differentially overexpressed in AF compared with expression in the other tissues. When the expression levels of these 170 gene fragments were graphically displayed in 448 other nonneoplastic-tissue samples from 16 types of tissue, only 30 known genes were found to be preferentially expressed in AF. Four genes—ADAM12, WISP-1, SOX-11, and FAP-α—were selectively overexpressed in AF among the normal tissues examined. Twenty-six other genes were overexpressed in AF compared with expression in the normal-tissue sets, however, they were also expressed to some degree in a variety of the other tissues.

Four genes identified in this study—ADAM12, WISP-1, SOX-11, and FAP-α—were both overexpressed in AF compared with expression in normal muscle and absent or expressed at very low levels in all of the other 16 nonneoplastic tissues examined, comprising 448 tissue samples.

ADAM12, the human homologue of mouse meltrin-α, exists as 2 isoforms: a shorter, secreted form and a longer, membrane-bound form.[18] Both isoforms contain the metalloprotease domain, disintegrin domain, and cysteine-rich domains that characterize ADAMs. In mice, ADAM12 protein expression increases during muscle regeneration but is only present at low levels in normal adult muscle cells.[19] ADAM12 has protease activity for IGFBP-3.[20] IGFBP-3 binds IGFs with high affinity and can thereby modulate IGF actions. ADAM12 may thus regulate IGF activity by altering

IGFBP-3 activity.[20] As such, ADAM12 may regulate IGF activity in AF.

The second gene we found to be specifically overexpressed in AF was WISP-1. Earlier studies have shown that wnt is an oncogene that is activated by insertion of MMTV in MMTV-induced breast cancer.[21] The Wnt genes form a family of protooncogenes that are developmentally regulated. Studies to identify downstream genes involved in wnt signaling have identified WISP-1 and the related genes WISP-2 and WISP-3.[21,22] WISP-1 mRNA is overexpressed in most colon cancers compared with expression in adjacent normal mucosa.[22] The wnt genes encode proteins with features typical of secreted growth factors.

The third gene specifically overexpressed in AF is SOX-11. SRY is the mammalian testis-determining gene located on the Y chromosome. SRY and the SRY box-related (SOX) genes are a subgroup of the HMG proteins that contain a conserved HMG-box type of DNA binding domain.[21,23] SRY and the SOX genes contain a single DNA-binding domain, bind DNA in a sequence-specific manner, and are believed to function as transcription factors. SOX-11 contains putative transcriptional activator or repressor domains.[24] The reported expression pattern of SOX-11 suggests that it is important in the developing nervous system.[24] We previously found that SOX-11 is overexpressed in liposarcoma and Wilms tumors compared with expression in normal tissues and several malignant tumors.[17]

The fourth gene specifically overexpressed in AF is FAP-α. FAP-α is transiently expressed in some fetal mesenchymal cells but generally absent from most normal adult and malignant tissues.[21,25] However, many tumors contain fibroblasts that express FAP-α.[21,25] FAP-α appears to have gelatinase activity[21,26,27] and may mediate the migration of fibroblasts, tumor cells, or both.

The β-catenin pathway has been strongly implicated by others in the pathogenesis of desmoid tumors and AF.[3,11,13,28–33] β-Catenin has a nuclear function, in which it binds transcription factors; and a cell-membrane function, in which it is a component of epithelial-cell adherens junctions.[11,12] The product of the APC gene and β-catenin form a complex with other proteins that results in phosphorylation of β-catenin on serine and threonine. This phosphorylation targets β-catenin for ubiquination, leading to degradation.[13,34,35] Wnt signaling inhibits this phosphorylation of β-catenin. Therefore the overexpression of WISP-1 in AF set B in this study may indicate activity of this signaling pathway in AF. Similarly, inactivation of APC results in increased levels of β-catenin.[36] Stabilized β-catenin translocates to the nucleus, where it binds members of the Tcf-Lef family and forms transcriptional activation complexes.[13] The genes regulated by this complex are not well characterized. Mutations of APC or β-catenin in colon cancer result in β-catenin stabilization, as well as accumulation of β-catenin in the nucleus, where it may act as a transcription factor by binding Tcf-Lef family members.[37]

A recent study of sporadic AF revealed β-catenin mutations in 3 of 12 cases and showed that β-catenin mRNA expression was higher in the β-catenin–mutated group.[29] In this study, β-catenin mRNA expression was higher in AF set B than in set A, which may reflect such differences in the β-catenin gene, although we did not examine mutations.

Ephrin receptor EphB3 was overexpressed in AF in this study. EphB3 is a member of the EPH/ELK receptor family of tyrosine kinases.[21] A recent study showed that β-catenin regulates the expression of the EphB2 and EphB3 receptors and their ligand ephrin B1 in colon cancer and in the intestinal villi.[38] The results of gene-knockout studies suggest that EphB2 and EphB3 are important in organizing cells along the crypt-villus axis and prevent mingling of proliferative and differentiated cell populations.[38]

Many of the other overexpressed genes were growth factors or extracellular matrix proteins. Among these were stem-cell growth factor, angiopoietin-like factor-2, CD134 ligand, and neuregulin 1. Stem-cell growth factor has growth-stimulatory activity at several points in hematopoietic development.[39] Angiopoietin-like factor- 2, originally identified in heart muscle, has effects on endothelial cells and induces sprouting of vascular endothelial cells.[21] TNF ligand superfamily member 4, also known as CD134 ligand, originally identified as a T-cell–activation antigen, is a member of the TNF-receptor family.[21] Neuregulin-1 or heregulin-α, also known as glial growth factor-2, is a ligand for the Her-2/Neu/ErbB family of receptor tyrosine kinases.[21,40–43]

Other genes that we found to be overexpressed in AF are important in neuronal development, including neuregulin-1 (as described above), neuronal pentraxin II, neurofilament protein, heavy polypeptide, amphiphysin, and contactin. Neuregulins and their receptors are critical in neuronal development. Neuronal pentraxin II is a member of the pentraxin family, proteins characterized by their ability to form pentameric complexes and bind a variety of ligands.[21] The neurofilament protein, heavy polypeptide gene is close to the NF2 locus and may regulate interfilament spacing by way of phosphorylation on a repeating motif.[21] Amphiphysin is a synaptic vesicle–associated protein concentrated in nerve terminals.[21] Contactin is a glycosylphosphatidyl-anchored neuronal cell-adhesion molecule.[44] The findings of knockout experiments suggest that contactin

regulates axonal and dendritic interactions in the cerebellum.[45]

When the clustering software Cluster was applied, the AF samples formed 2 distinct groups. The significance of these subclusters is unclear. AF represents a biologically heterogeneous group of tumors, and this heterogeneity may be reflected in the clustering of the AF samples studied here. These groups were not correlated with sex, age, medications, smoking history, or other diagnoses. It is interesting to note that 4 of the patients in set A have not returned for follow-up, whereas the other patient is NED at 18 months. It is possible that this reflects a group with a good outcome, given they would likely have returned if their tumors had recurred. In contrast, 2 of the 7 patients in set B demonstrated recurrence at 1 year, 1 had progressive disease on chemotherapy, and 1 had a recurrence from previous surgery. A larger number of samples will need to be examined to determine whether these groups have different clinical outcomes.

In summary, we have identified differences in gene expression between AF and normal tissues. We also identified genes expressed uniquely in AF among these samples. Differences in gene expression in different AF tumors may yield clues to their pathogenesis and may be useful in diagnosis and studies of the basic biology of AF. Analysis of a larger number of AF samples and correlation of biological phenotypes with gene-expression patterns may identify clinically meaningful characteristics of the 2 subsets identified here.

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