

**The Impact of Advances in Molecular Genetic Pathology on the  
Classification, Diagnosis and Treatment of Selected Soft Tissue  
Tumors of the Head and Neck**

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## **Abstract**

Recent advances in molecular pathology have had a significant impact on the diagnosis, classification, and treatment of soft tissue tumors. The practical application of these discoveries promises to assist greatly in the evaluation and treatment of soft tissue neoplasms in the head and neck region—an area characterized by exceedingly complex anatomy that often restricts the ample sampling of lesions and complete surgical resection. This reviews details some ways in which molecular techniques have strengthened conventional diagnostic and management approaches to low-grade fibromyxoid sarcoma, angiomatoid (malignant) fibrous histiocytoma, and dermatofibrosarcoma protuberans, all of which may involve the head and neck region.

Keywords: sarcoma, molecular genetics, angiomatoid, fibromyxoid, dermatofibrosarcoma

## **Introduction**

The past 10-15 years have seen a number of important advances in our understanding of the classification, diagnosis and etiopathogenesis of soft tissue tumors. Although important advances continue to be made in traditional clinicopathological (morphological) study and immunohistochemistry, the greatest breakthroughs have been in molecular pathology and genetics. This article will thus focus on 3 soft tissue tumors, low-grade fibromyxoid sarcoma, angiomatoid (malignant) fibrous histiocytoma, and dermatofibrosarcoma protuberans, tumors which highlight some of the ways in which advances in molecular genetics have improved our ability to diagnose and treat these rare mesenchymal lesions, while at the same time raising intriguing new questions. The differential diagnosis of these tumors is covered in widely available textbooks and will not be discussed.

## **Low-grade fibromyxoid sarcoma**

### *Clinical features*

Low-grade fibromyxoid sarcoma (LGFMS), initially described by Evans in 1987, is a rare soft tissue tumor characterized by a deceptively banal appearance and potential for late metastases. LGFMS most often occur in young adults, but affect children in ~20% of cases. Most tumors involve the deep soft tissues of the extremities, but LGFMS may occur in any location, including the head and neck, and may on rare occasions involve superficial soft tissues, particularly in children. The overall metastatic risk for LGFMS is approximately 15%, although metastases may occur very late (>20 years after diagnosis), necessitating essentially indefinite clinical follow-up for patients with this disease. In general, LGFMS should be treated as one would other low-grade sarcomas, with wide excision and possible adjuvant radiotherapy. A role for systemic therapy has not been established for LGFMS.

### *Pathological features*

The morphologic diagnosis of LGFMS may be challenging, owing to its typically low cellularity, abundant collagen, and relatively bland cytology. Most cases grow in a circumscribed but subtly infiltrative fashion (Figure 1A), and consist of an admixture of hypocellular, heavily collagenized zones containing widely spaced, monomorphic spindled cells, and more cellular myxoid nodules, containing a greater number of neoplastic cells arrayed about a characteristic curvilinear vasculature (Figure 1B). Clues to the diagnosis of LGFMS include the “abrupt” transition from collagenized zones into myxoid nodules, and the presence of small arteriole-sized vessels with concentric hyalinization, often producing distorted, angulated lumens. Although the cells of LGFMS are almost always monomorphic and bland-appearing, careful inspection invariably shows hyperchromatism and nuclear irregularity. Mitotic activity is typically very low and necrosis is absent. Morphologic features seen in a minority of cases include areas of increased cellularity and nuclear atypia approaching those seen in intermediate to high-grade fibrosarcoma, hyalinization, epithelioid morphology and a cord-like growth pattern (sclerosing epithelioid fibrosarcoma-like), and the presence of giant collagen rosettes (so-called “hyalinizing spindle cell tumor with giant rosettes) (Figure 1C).

### *Immunohistochemical features*

In general, LGFMS show a purely fibroblastic immunophenotype, with expression only of vimentin. Expression of epithelial membrane antigen (EMA) has been documented in up to 40% of LGFMS in one study, a finding that has not been confirmed by other studies. Potential expression of EMA has obvious implications for the distinction of LGFMS from one of its principal morphologic mimics, perineurioma. A very recent study has also reported expression of the perineurial-associated tight junction protein claudin-1 in genetically proven LGFMS, a finding at variance with prior studies and with the known

absence of tight junctions in fibroblastic cells. We have not seen expression of EMA and claudin-1 in LGFMS in our own practices, but recommend caution in the use of these immunohistochemical reagents until further studies have addressed this important issue.

### *Genetic features*

The diagnosis of LGFMS may be very challenging, and there has thus been considerable interest in identifying diagnostically useful genetic markers of this tumor. In 2000 Bejarano and colleagues reported the reciprocal translocation t(7;16) in a rosette-containing LGFMS, a finding quickly followed by a report of a metastatic LGFMS to the lung bearing a ring chromosome derived from chromosomes 7 and 16. These early reports were confirmed by Reid et al, who identified this t(7;16)(q34;p11) in 4 LGFMS (2 with and 2 without giant collagen rosettes), noting that the 16p11 breakpoint was the location of the *FUS* gene, known to be involved in the t(12;16)(q13;p11) characteristic of myxoid liposarcoma. Subsequently Storlazzi and co-workers established that this translocation fused *FUS* to *BBF2H7* (subsequently renamed *CREB3L2*). These findings have been confirmed by a number of subsequent studies, showing *FUS-CREB3L2* gene fusions in approximately 95% of LGFMS, with a variant *FUS-CREB3L1* fusion in <5% of cases. To date, these fusions appear to be highly specific for LGFMS, being present otherwise only in a subset of “sclerosing epithelioid fibrosarcoma”, a rare tumor which may in many instances represent a morphologic variant of LGFMS, rather than a discrete entity. The presence of *FUS-CREB3L2* or *FUS-CREB3L1* fusions can be detected in formalin-fixed, paraffin-embedded sections, by reverse transcriptase polymerase chain reaction (RT-PCR), interphase fluorescent in-situ hybridization (FISH), and DNA-based polymerase chain reaction (Figure 1D).

### **Angiomatoid (malignant) fibrous histiocytoma**

#### *Clinical features*

Angiomatoid (malignant) fibrous histiocytoma (AMFH), a very rare mesenchymal tumor of uncertain differentiation, is considered a tumor of borderline/intermediate malignancy, owing to its significant potential for local recurrence, but <2% risk of metastasis. AMFH most often presents in children and young adults, as a small, superficial mass, occasionally mistaken clinically for a long-standing hematoma or vascular tumor. AMFH may present with systemic symptoms (fevers, weight loss, anemia, polyclonal gammopathy and a Castleman disease-like lymphadenopathy), which are due to cytokine production by the neoplastic cells and resolve following resection. AMFH require prompt wide excision to reduce the risk of local recurrence. Metastases to lymph nodes and distant locations (lung, bone, soft tissue) are seen in <2% of patients; the prognosis for these patients may still be excellent if these metastases can be completely resected. AMFH of the head/neck may have a worse prognosis, owing to difficulties in achieving complete resection in these anatomical locations.

#### *Pathological features*

Grossly, AMFH tend to be relatively well-circumscribed, pigmented and variably cystic. Microscopically, AMFH frequently show a well-formed fibrous pseudocapsule containing lymphoid aggregates and germinal centers, often mimicking a lymph node (Figure 2A). The neoplastic cells themselves are arranged in sheets, short fascicles and distinctive “meningothelial-like” whorls. Intralesional hemorrhage and the formation of pseudovascular spaces are seen in ~50% of cases. The cells of AMFH are histiocytoid to spindled, with variable nuclear pleomorphism, and often contain abundant intracytoplasmic hemosiderin, a valuable diagnostic clue (Figure 2B). Previously traumatized AMFH may consist of tiny, inapparent nodules of tumor cells embedded in a fibrotic background, with abundant hemosiderin and hematoidin deposition. Dramatic

nuclear pleomorphism may occasionally be seen but does not seem to be of clinical import.

#### *Immunohistochemical features*

AMFH shows a unique immunophenotype, with co-expression of desmin, epithelial membrane antigen and CD68 in 50-60% of cases (Figure 2C). CD99 may also be positive. Although expression of muscle actins may be seen, AMFH do not express specific markers of skeletal muscle differentiation, such as myogenin, MyoD1, myoglobin or fast myosin. This is critical in their distinction from rhabdomyosarcoma, a frequent concern in a desmin-positive pediatric neoplasm. Markers of dendritic cell differentiation, such as CD21, CD35, clusterin, and S100 protein are absent.

#### *Genetic features*

In 2000, Waters et al reported the first cytogenetic characterization of AMFH, noting the presence of the translocation t(12;16)(q13;p11) and production of a *FUS-ATF1* fusion gene. This was quickly followed by reports of *EWSR1-ATF1* fusion transcripts in cases of AMFH, resulting from the translocation t(12;22)(q13;q12). Most interestingly, an identical *EWSR1-ATF1* fusion transcript is seen in clear cell sarcoma of soft parts (CCS), a highly malignant soft tissue sarcoma with melanocytic differentiation. For unknown reasons, downstream activation of the microphthalmia transcription factor (MiTF) pathway is seen in CCS, but not AMFH bearing this fusion gene. Given that ATF1 and CREB1 are functionally related proteins with apparently similar functions, it is not too surprising that three large retrospective studies of AMFH have identified *EWS-CREB1* as another fusion gene in AMFH. Again, an identical fusion is seen both in most cases of the exceptionally rare sarcoma known as “gastrointestinal clear cell sarcoma-like tumor” as well as in rare cases of conventional CCS. It now appears that *EWS-CREB1* is the most common fusion transcript in AMFH, with *EWS-ATF1* representing the

second most common genetic event, and *FUS-ATF1* the least common. Very rare AMFH contain rearrangements involving *EWS* without involvement of *CREB1* or *ATF1*, suggesting the existence of additional yet-unknown fusion partners. There does not appear to be an association between the type of fusion transcript and other clinical or pathologic parameters in AMFH. These genetic events may be detected by RT-PCR for the various fusion transcripts or by FISH for *EWS* and *FUS* rearrangements (Figure 2D).

### **Dermatofibrosarcoma Protuberans**

#### *Clinical features*

Dermatofibrosarcoma protuberans (DFSP) are relatively common fibrohistiocytic tumors of intermediate/borderline malignancy, which have significant potential for aggressive local growth and recurrence, but little if any metastatic capacity in their classical form. Although the great majority of DFSP arise in the trunk and proximal extremities, it is also relatively frequent in the head/neck, accounting for upwards of 6% of sarcomas in this location. DFSP may occur in patients of any age, including very young children, but most often involves young to middle aged adults. DFSP usually present as a plaque that may subsequently develop into a nodular or multinodular mass. Occasional cases may present either as a depressed zone of fibrosis mimicking a sclerosing dermatitis (“atrophic pattern”), or as an apparently subcutaneous mass without obvious dermal involvement. DFSP have a very high risk for local recurrence, approaching 20% even with conventional wide excision. In experienced hands Moh’s micrographic surgery has been reported to reduce this risk to 2-5%, and this option should certainly be considered in cosmetically sensitive areas such as the head/neck. It is difficult to determine whether DFSP lacking fibrosarcomatous transformation have any metastatic risk. The metastatic risk for fibrosarcomatous DFSP has varied in reported series, but is most likely in the 15% range.

### *Pathological features*

In its classic form, DFSP is a relatively stereotypical lesion, consisting of an exquisitely storiform proliferation of monotonous, slender spindled cells with dark nuclei and lightly staining cytoplasm, typically showing diffuse infiltration of the subcutaneous adipose tissue (“honeycomb pattern”) (Figure 3A). Mitotic activity is very low and necrosis is absent. Occasional DFSP may deviate from this classical pattern, showing multinucleated giant cells (giant cell fibroblastoma-like), myxoid change, myoid nodules, and pigmented dendritic cells (Bednar tumor) (Figure 3B). Fibrosarcomatous transformation in DFSP most often consists of an “abrupt” nodular transition to a hypercellular, herringbone pattern proliferation of enlarged, hyperchromatic spindled cells with prominent mitotic activity (Figure 3C).

### *Immunohistochemical features*

DFSP show diffuse expression of CD34 in nearly all cases, and are typically negative for other markers, including cytokeratins, Factor 13a, S100 protein, EMA, actins, desmin, etc. Apolipoprotein D, recently identified by gene expression analysis to be highly expressed in DFSP, may be useful in selected cases, but is generally not required for diagnosis. CD34 expression is often (but not always) diminished or absent in the fibrosarcomatous component of transformed DFSP.

### *Genetic features*

In 1990 Bridge et al reported the presence of supernumerary ring chromosomes in DFSP. These ring chromosomes were subsequently shown to be derived from chromosomes 17 and 22. Linear chromosomes involving the long arms of chromosomes 17 and 22 as a result of translocation,  $t(17;22)(q22;q13)$ , were discovered shortly thereafter. These translocations are most often unbalanced. Identical genetic events

have been identified in giant cell fibroblastoma, confirming Enzinger's hypothesis that this represents the juvenile form of DFSP, and in Bednar tumor. These linear and ring chromosomes result in fusion of the *COL1A1* and *PDGFB* genes, with formation of a COL1A1-PDGFB fusion protein. The exact role of this fusion protein in the pathogenesis of DFSP is not yet understood, but it may lead to the aberrant expression of a functional PDGFB-like protein capable of driving proliferation by stimulating cell growth through PDGFR-beta activation in an autocrine or paracrine fashion.

The presence of this COL1A1-PDGFB fusion protein has important implications for the treatment of unresectable and/or metastatic DFSP, inasmuch as imatinib mesylate, a tyrosine kinase inhibitor, has significant activity against activated PDGFR. Maki and colleagues first reported the use of imatinib mesylate in 2 patients with fibrosarcomatous DFSP, with partial therapeutic response. Since this report, several authors have described patients with locally advanced or unresectable metastatic disease with dramatic tumor burden reduction (often >50%) upon treatment with imatinib mesylate.

FISH and RT-PCR may be used to detect these genetic events (Figure 3D). FISH may pose significant challenge in interpretation due to the presence of complex unbalanced translocations. RT-PCR results may also be difficult to interpret, but for different reasons. First, the breakpoint of *COL1A1* can be quite variable and consequently requires the use of various forward primers for *COL1A1*, particularly when RNA is extracted from formalin-fixed paraffin-embedded tissue. Multiplex RT-PCR reactions that employ several primers are more likely to induce false-positive PCR bands.

Moreover, the region between exons 6 and 49 of *COL1A1* contains over 300 triplet repeats, a feature that often results in PCR artifacts. To this end, sequence analysis should be considered for RT-PCR positive bands to confirm results when appropriate.

Approximately 8% of DFSP cases are fusion-negative; it remains to be seen if these rare

cases contain cryptic rearrangements of *COL1A1* and *PDGFB* or altogether different genetic abnormalities.

## **Conclusions**

LGFMS, AMFH and DFSP, soft tissue tumors which may involve the head/neck region, exemplify some of the exciting ways in which advances in molecular genetics have impacted our ability to diagnose, classify, and treat patients with soft tissue tumors. From a diagnostic perspective, FISH for FUS provides a “gold standard” for the diagnosis of LGFMS, a tumor which may otherwise be quite difficult to distinguish from various morphologic mimics, including perineurial tumors, myxomas, and desmoid-type fibromatoses. Similarly, FISH and/or RT-PCR tests may allow for the confident diagnosis of challenging morphologic variants of LGFMS, AMFH and DFSP, including pleomorphic AMFH and myxoid DFSP. With regards to proper classification, the presence of FUS rearrangements in both classical and rosette-containing LGFMS has confirmed earlier morphological observations suggesting that these represented different manifestations of the same entity, much as the presence of identical genetic events in DFSP and GCFB has proven them to represent age specific variants of a single tumor type. In a similar vein, the presence of specific translocations in AMFH suggests that this tumor may be showing a “scrambled phenotype” similar to other translocation-associated sarcomas (i.e. alveolar soft part sarcoma), supporting the WHO’s reclassification of this formerly “fibrohistiocytic” lesion as a tumor of uncertain differentiation. Finally, and perhaps most importantly, our improved understanding of the genetic underpinnings of these and other sarcomas has begun to translate into an improved ability to treat patients, as exemplified by the use of imatinib mesylate for the treatment of DFSP.

It is essential to emphasize, however, that knowledge of the genetic underpinnings of these and other sarcomas must be integrated into the overall clinicopathological

evaluation of a given case, most importantly morphologic evaluation. Nowhere is this better exemplified than in AMFH, a tumor which may share not only one, but two specific translocations with a wholly dissimilar sarcoma, clear cell sarcoma of soft parts.

Similarly, the presence of *FUS* rearrangements not only in LGFMS and AMFH but also in various other sarcomas including myxoid liposarcoma and Ewing sarcoma amply validates the continued primacy of traditional diagnostic methods in the pathological evaluation of mesenchymal neoplasia.

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## Figure Legends

Figure 1: Low-grade fibromyxoid sarcoma, consisting of very bland spindled cells in a fibromyxoid background, with subtle infiltrative growth at the periphery (A). An "abrupt" transition between collagenized zones and myxoid nodules is frequently present (B).

Rosette-containing low-grade fibromyxoid sarcomas are now known to contain the same genetic events as typical low-grade fibromyxoid sarcomas (C). Fluorescent in-situ hybridization for *FUS* rearrangement, positive in a rosette-containing low-grade fibromyxoid sarcoma (D).

Figure 2: At low power, angiomatoid (malignant) fibrous histiocytomas show a fibrous capsule and numerous lymphoid follicles, mimicking a lymph node (A). The neoplastic cells of angiomatoid (malignant) fibrous histiocytoma are distinctly histiocytoid in appearance and often contain intracytoplasmic hemosiderin, a valuable clue (B).

Anomalous desmin expression is seen in ~60% of angiomatoid (malignant) fibrous histiocytomas. Importantly, myogenin and MyoD1 are negative (C). Fluorescent in-situ hybridization for *CREB1* rearrangement, positive in angiomatoid (malignant) fibrous histiocytoma (D).

Figure 3: Dermatofibrosarcoma protuberans, showing a storiform proliferation of monomorphic spindled cells, with "honeycomb" infiltration of fat (A). The diagnosis of myxoid variants of dermatofibrosarcoma protuberans may be difficult, often requiring ancillary immunohistochemistry and molecular testing (B). Fibrosarcomatous change in dermatofibrosarcoma protuberans, resembling adult-type fibrosarcoma (C). Fluorescent in-situ hybridization for *PDGFR-B*, showing increased copy number, a common finding in dermatofibrosarcoma protuberans (D).