**FUS (16p11) Gene Rearrangement as Detected by Fluorescence In-Situ Hybridization in Cutaneous Low-Grade Fibromyxoid Sarcoma: A Potential Diagnostic Tool**

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**INTRODUCTION**

Low-grade fibromyxoid sarcoma (LGMS) is an uncommon sarcoma that was first described by Evans in 1987. It occurs mostly in the subfascial location as deep-seated intramuscular mass in the proximal extremities or trunk of young adults, although a wide age range has been noted including some pediatric cases. Despite its characterization as a cytologically bland spindle cell tumor, LGMS has the potential for aggressive behavior and high rate of metastasis (41% in one series), especially if initially mistaken for a benign mesenchymal tumor.‡ Lane et al described hyalinizing spindle cell tumor with giant rosettes (HSCTGR) as a lesion that shared some of the histologic features of LGMS including a low-grade spindle stroma with myxoid or hyalinized areas; the hallmark histologic difference was the presence of giant collagen-containing rosettes. Given some of the shared histologic features of LGFS and HSCTGR, it has been suggested that these tumors represent a spectrum of the same lesion. This morphologic relationship has been supported by genetic studies demonstrating a t(7;16)(q34:p11) observed in both neoplasms. The translocation results in a chimeric fusion protein derived from the FUS gene on chromosome 16p11 and the CREB3L2 (BBF2H7) gene on 7q34. A rare FUS/CREB3L1 variant translocation resulting from a t(11;16)(p11;p11) has also been described in LGFS. Recently, 2 of the authors (J.C.F.S. and S.D.B.) reported a series of superficial LGFS more commonly affecting children that had a better prognosis than the typical deep LGFS. The incidence of FUS rearrangement in this superficial subset of LGFS is currently unknown.

A fluorescence in situ hybridization (FISH) probe that can identify FUS rearrangements in formalin-fixed paraffin-embedded tissue (FFPET) has become commercially available (Abbott Molecular/Vysis, Des Plaines, IL). Our intention in this study was to determine the frequency of FUS gene rearrangements in this previously reported series of superficial LGFS and to evaluate the utility of the probe in the differential diagnosis of cutaneous fibromyxoid neoplasms.

**MATERIAL AND METHODS**

After receiving institutional review board approval, FFPET samples from superficial LGFS (n = 6) were selected for analysis. Hyalinizing spindle cell tumor with giant rosettes (HSCTGR) (n = 10) and myxomas (n = 10) were used as controls. The frequency of FUS/CREB3L2 rearrangements in this previously reported series of superficial LGFS and to evaluate the utility of the probe in the differential diagnosis of cutaneous fibromyxoid neoplasms.

**Abstract:** Low-grade fibromyxoid sarcoma (LGFS) is a rare, typically deep-seated soft tissue neoplasm with deceptively bland cytology and metastatic potential. A t(7;16)(q34:p11) translocation, yielding a FUS/CREB3L2 fusion gene, has been identified in approximately 80%–90% of deep soft tissue LGFS. Cutaneous fibromyxoid neoplasms occur not infrequently; dermatopathologists rarely consider LGFS in the differential diagnosis, as this lesion is uncommon in the skin. We identified a group of superficial LGFS and a spectrum of other cutaneous fibromyxoid neoplasms and performed fluorescence in situ hybridization (FISH) to assess the frequency of FUS rearrangement. FISH for the chromosomal rearrangement of FUS (16p11), using a dual-color, break-apart probe (Abbott Molecular/Vysis, Des Plaines, IL), was performed on formalin-fixed paraffin-embedded tissue sections from superficial LGFS (n = 6), myxomas (n = 10), and myxofibrosarcoma/myxoid malignant fibrous histiocytomas (myxoid MFH) (n = 5). One hundred nonoverlapping tumor nuclei per case were evaluated for either fused (normal) or split (translocated) signals. Of the LGFS, 4 of 6 (67%) showed a rearrangement of FUS (range: 72%–80% positive nuclei per 100 nuclei). The other neoplasms within the differential diagnosis were devoid of any rearrangement involving FUS (range: 0%–2% positive nuclei per 100 nuclei). Our observed frequency of FUS rearrangement in superficial LGFS is consistent with those published in the literature for more deeply seated lesions. When applied to suspicious superficial myxoid or fibromyxoid neoplasms, the FUS FISH probe in formalin-fixed paraffin-embedded tissue can be a useful ancillary technique for diagnosis of this uncommon and deceptively bland tumor.

**Key Words:** low-grade fibromyxoid sarcoma, hyalinizing spindle cell tumor with giant rosettes, fluorescence in situ hybridization, FUS/CREB3L2

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histiocytoma (myxoid MFH) (n = 5) were identified from the archives of the Armed Forces Institute of Pathology, Indiana University Department of Pathology, and the Cleveland Clinic Department of Anatomic Pathology. Tissue sections stained with hematoxylin and eosin were reviewed independently by experienced soft tissue pathologists (J.C.F.S., J.R.G., and S.B.) blinded to the FISH results to confirm the histologic diagnosis and ensure tissue adequacy.

Unstained 4-μm sections from whole tissue blocks were placed on electrostatically charged slides (SuperFrost; Fisher, Hampton, NH) and evaluated individually using the dual-color, break-apart probe flanking the \( FUS \) (16p11) gene. The \( FUS \) dual-color, break-apart rearrangement probe set contains a 500 kb probe labeled in SpectrumGreen that lies on the telomeric side of \( FUS \) and a second 270 kb probe labeled in SpectrumOrange that lies on the centromeric side of \( FUS \).

The tissues were subjected to FISH analysis in the Cleveland Clinic Molecular Oncologic Pathology laboratory according to the manufacturer’s instructions and established laboratory protocol that has been described in detail previously. The hybridized slides were reviewed on an Olympus IX-50 microscope (Olympus, Tokyo, Japan) at \( \times 100 \) magnification with oil immersion, using a DAPI/Green/Red triple band pass filter set. The tissues were scored by evaluating a minimum of 100 tumor nuclei per sample. Overlapping cells indistinguishable as separate nuclei were excluded from analysis, and only tumor nuclei with 4 signals present were evaluated to avoid a false-positive interpretation that might result from nuclear truncation.

In concert with other FISH assays validated for FFPET in this laboratory, a positive result was reported when greater than 10% of the tumor nuclei had evidence of a rearrangement. The \( FUS \) FISH probes have previously accurately detected translocations in a myxoid liposarcoma cell line (a generous gift from Dr. Nils Mandahl, University Hospital, Lund, Sweden), which has a reverse transcriptase polymerase chain reaction (RT-PCR)–confirmed \( FUS/DDIT3 \) fusion transcript. The probe-specific normal range was established in benign fibroadipose tissue (n = 8) that had been routinely processed. The benign fibroadipose tissue was essentially devoid of any rearrangement involving \( FUS \) (\( FUS \) range: 0–2 positive nuclei per 100 nuclei, mean 1.1, and SD 0.60). This distribution established a probe-specific normal range of less than or equal to 3% (2.3% with 2 SDs from the mean used for the cut off calculation).

### RESULTS

Characteristic histopathologic features of superficial LGFMS were similar to their deep-seated counterparts and included an admixture of heavily collagenized hypocellular zones, alternating with more cellular myxoid nodules, and a proliferation of deceptively bland spindle cells with at least a focal storiform growth pattern arrayed around arcades of curvilinear blood vessels (Fig. 1). All 6 cases (100%) demonstrated histologic features typical of LGFMS. Additionally, 2 cases had features of HSCTGR, 1 case focally demonstrated collagen rosettes, and the other had prominent numerous collagen rosettes, consisting of a central core of hyalinized collagen surrounded by a cuff of epithelioid fibroblasts (Fig. 2). The histologic features of myxoma and myxofibrosarcoma/myxoid MFH cases included for comparison were morphologically similar to those described in detail in a previous study.

The FISH results obtained are presented in Table 1, and representative photomicrographs of the break-apart probe are depicted in Figure 3. Of the 6 cutaneous LGFMS, 4 (67%) showed a rearrangement of \( FUS \) (range: 72%–80% positive nuclei per 100 nuclei). One of the 2 \( FUS \) rearrangement–negative cases was composed predominantly of areas resembling HSCTGR (Fig. 2), and 1 had typical features of LGFMS but with somewhat increased nuclear atypia (Fig. 4). All myxomas and myxofibrosarcomas/MFH were devoid of any rearrangement involving \( FUS \) (range: 0%–2% positive nuclei per 100 nuclei).
DISCUSSION

Recently, Billings et al.\(^{10}\) reported a series of superficial LGFMS with a better prognosis and higher incidence in children than typical deep-seated LGFMS. Due to its rarity, superficial LGFMS is not often considered by dermatopathologists in the differential of cutaneous fibromyxoid neoplasms. In the series published by Billings et al.,\(^{10}\) a benign diagnosis was considered in 9 of 12 cases (75%). Only 3 cases (25%) were submitted with a diagnosis of low-grade sarcoma, with only 1 of 12 (8%) correctly diagnosed as LGFMS. This problem of underrecognition is further confounded when the pathologist is confronted with a small biopsy containing scant lesional tissue. The distinction between LGFMS and some of its benign mimics is particularly important given the potential for local recurrence and metastases, which impacts treatment decisions.

A growing number of sarcomas have been found to possess recurrent and specific chromosomal translocations. These provide attractive diagnostic targets, which can be identified by classical cytogenetics and molecular methods such as RT-PCR and FISH. These ancillary techniques provide diagnostic information, which may be essential for accurate characterization of difficult lesions. In so far as LGFMS possesses a distinct chromosomal translocation not present in entities that fall within the differential, exploitation of this alteration represents an invaluable addition to the diagnostic armamentarium.

A t(7;16)(q34;p11) translocation, resulting in a FUS/CREB3L2 fusion transcript, has been reported in up to 95% of LGFMS cases when combinations of classical cytogenetic and molecular cytogenetic methods are employed.\(^{5,9,12-15}\) A rare FUS/CREB3L1 variant has also recently been described.\(^{9}\) The vast majority of studied cases in the literature were deep-seated lesions. In this current study evaluating superficial lesions, 4 of 6 LGFMS (67%) showed a rearrangement of FUS. Our observed frequency of FUS rearrangement in superficial LGFMS showed a somewhat lower incidence but is similar with the published results for more deeply seated lesions.

Several molecular studies have identified the 16p11 rearrangement in LGFMS but have also highlighted the

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<th>TABLE 1. FISH Results for FUS Rearrangements in LGFMS</th>
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\(^*\)Mean: 77% positive cells/case, Range: 72–80% positive cells/case.
difficulty in diagnosing these lesions. Matsuyama et al\textsuperscript{14} examined 23 cases originally diagnosed as LGFMS using RT-PCR. After histologic rereview, 7 of the cases were diagnosed as different entities. FUS/CREB3L2 fusion transcripts were detected in 14 of 16 cases of LGFMS (88%).\textsuperscript{14} This group noted that established histologic criteria for reliably distinguishing LGFMS from potential mimics was lacking, requiring expert review and reclassification of a significant number of cases previously diagnosed as LGFMS. Guillou et al\textsuperscript{13} evaluated 63 lesions typical of LGFMS and 66 non-LGFMS-like tumors in the differential diagnosis. RNA of sufficient quality was extracted from 111 of 129 cases (86%) (59 LGFMS and 52 non-LGFMS). Of the 59 LGFMS, 48 (81%) harbored detectable transcripts (45 FUS/CREB3L2 and 3 FUS/CREB3L1). All PCR-positive cases, which were subsequently examined by FISH using a break-apart probe, showed rearrangement of the FUS gene, confirming the PCR results.\textsuperscript{13} Downs-Kelly et al\textsuperscript{12} studied a group of myxoid soft tissue lesions, 10 of which were LGFMS, using an FUS break-apart probe identical to the one utilized in this study. The cases diagnosed as LGFMS showed a lower incidence of FUS rearrangement (7 of 10 cases, 70%) in contrast to previously published studies,\textsuperscript{12} finding similar to the 67% positivity rate found in the present study. These authors also suggested that lower incidence of FUS rearrangement was likely due to the fact that reliable histopathological criteria for the diagnosis of LGFMS remained incompletely defined. Finally, in an earlier study, Panagopoulos et al\textsuperscript{15} utilized RT-PCR and/or FISH to study a spectrum of spindle cell neoplasms to ascertain which tumors may harbor the FUS/CREB3L2 gene rearrangement. This group included 45 low-grade spindle cell sarcomas (none of these initially diagnosed as LGFMS), 2 benign soft tissue tumors, 9 high-grade sarcomas, and 3 LGFMS. Of the 59 tumors analyzed, 12 were positive for the FUS/CREB3L2 translocation. All 12 were diagnosed as LGFMS upon histologic rereview; 3 of these cases were initially correctly diagnosed as LGFMS, whereas the remaining 9 had been diagnosed as a variety of other benign and malignant soft tissue tumors.\textsuperscript{15}

In regard to the FISH-negative cases, it is possible that these tumors represent other entities. However, it seems unlikely in at least 1 of the cases, as it had classic morphology of the HSCTGR variant of LGFMS and was negative for immunophenotypic evidence of other possible diagnoses (eg, nerve sheath tumors; data not shown). Another potential explanation for this is the presence of a variant translocation that involves an insertion or deletion of genetic material, thus altering the break point recognized by the FUS FISH probe. The other negative case did have slightly more nuclear atypia than is usually seen in LGFMS, but high-grade areas have been documented in cases of LGFMS.\textsuperscript{2}

In his initial characterization of LGFMS, Evans\textsuperscript{1} described a neoplasm comprised of contrasting fibrous and myxoid areas, a whorled growth pattern (at least focally), bland benign-appearing spindle cells with low to moderate cellularity, uncommon mitotic figures, and minimal nuclear pleomorphism. Although these histologic criteria are still currently employed, some modifications have been introduced. Guillou et al described histologic criteria (areas of hypercellularity, marked pleomorphism, and epithelioid cytology) that, although not classic, may still be in keeping with the diagnosis of LGFMS.\textsuperscript{13} Ambiguity in the characterization of LGFMS clearly remains, as evidenced by the significant revision of diagnoses as a result of histologic rereview in a number of studies.\textsuperscript{12,14,15}

Our rates of FUS rearrangement in superficial LGFMS are in keeping with those published in the literature for more deeply seated lesions. When applied to suspicious superficial neoplasms, the FUS FISH probe can be a useful ancillary technique in the diagnosis of superficial myxoid lesions. The lack of sensitivity seen in adjunct molecular methods in our study and in the literature may be due to the lack of sensitivity of current histologic diagnostic criteria. Additional studies employing FUS FISH in conjunction with detailed histologic assessment of a large number of cutaneous fibromyxoid lesions are needed to further refine histologic criteria required for accurate diagnosis of superficial LGFMS.

REFERENCES