Original Article
Low-grade fibromyxoid sarcoma: a clinicopathologic and molecular study of 10 genetically confirmed cases

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Abstract: Low-grade fibromyxoid sarcoma (LGFS) is a rare low-grade malignant fibroblastic tumor, harboring a characteristic FUS-CREB3L2 or FUS-CREB3L1 gene fusion. The authors presented 10 genetically confirmed cases in a Chinese population. To the best of our knowledge, the present series consists of the most genetically confirmed cases from a Chinese medical center in English literature. The clinical, histologic, immunohistochemical, and molecular features of all cases are reviewed. The age of the patients (7 females, 3 males) ranged from 4 to 58 years old (median, 26 y; mean, 27 y). Trunk (4/10, 40%) was the most common site. Microscopically, all the cases exhibited an admixture of myxoid nodules and fibrous zones. The tumor cells were deceptively bland and nuclear pleomorphism was observed in focal areas of one case. Immunohistochemically, neoplastic cells were focally reactive for EMA (1/9, 11.1%), and negative for S-100 protein, CD34, smooth muscle actin, and desmin (0/9). Of the 4 cases stained with MUC4, one showed focal expression and others were interpreted as indeterminate. Surgical excision was performed for all patients. Follow-up information was available for 8 cases, and none developed local recurrence or metastasis at last follow-up (mean 31 months). LGFS is a distinctive low-grade malignant tumor. The diagnosis of this tumor might be very challenging and it is mistaken for many benign lesions. A combination of clinical studies, careful morphologic analysis, and a full panel of immunomarkers especially genetic studies is helpful in confirming the diagnosis. This tumor type is associated with favorable prognosis.

Keywords: Low-grade fibromyxoid sarcoma, FUS rearrangement, fluorescence in situ hybridization

Introduction
Low-grade fibromyxoid sarcoma (LGFS) is a distinct entity of malignant fibroblastic soft tissue tumor first described by Evans in 1987 [1-3]. Subsequent studies confirmed Evans’ discovery and depicted the histologic and immunohistochemical features of this tumor, which exhibited innocuous morphology of a proliferation of bland-looking fibroblastic spindle cells, but, paradoxically, could develop distant metastases [4-8].

FUS-CREB3L2 gene fusion chimera, resulting from t(7;16)(q33;p11), was first discovered by Storlazzi et al. in two cases of LGFS [9], which has been now widely accepted as a specific genetic change of LGFS accounting for over 90% of cases, followed by FUS-CREB3L1 and EWSR1-CREB3L1 [10-12]. These characteristic molecular abnormalities are extremely useful in the differential and definite diagnosis for LGFS. Fluorescence in situ hybridization (FISH) and reverse transcription-polymerase chain reaction (RT-PCR) performed on formalin-fixed, paraffin-embedded (FFPE) tissue sections have been widely accepted as highly sensitive and specific diagnostic tools for determining these characteristic molecular changes in suspicious cases [13, 14].

To our knowledge, there have been 26 series consisting of eight or more cases of LGFS in English literatures [4-8, 10, 11, 13-31] and 15 of these studies used molecular techniques to confirm the characteristic molecular changes of their cases [10, 11, 13, 14, 20-29, 31]. Therein, only one study came from China, however, without genetic analysis [15].

To better understand the clinicopathologic spectrum of this rare tumor, we describe a
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Table 1. Clinical information of series of low-grade fibromyxoid sarcoma

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Site</th>
<th>Depth (Deep location)</th>
<th>Size (cm)</th>
<th>Preoperative Duration</th>
<th>Treatment</th>
<th>Follow-up (mo)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4/F</td>
<td></td>
<td>Back</td>
<td>Intramuscular</td>
<td>4</td>
<td>3 mo</td>
<td>Marginal excision (margin-), radRx</td>
<td>36</td>
<td>NED</td>
</tr>
<tr>
<td>2</td>
<td>12/F</td>
<td></td>
<td>Thigh</td>
<td>Intramuscular</td>
<td>8</td>
<td>Unknown</td>
<td>Wide excision (margin-)</td>
<td>43</td>
<td>NED</td>
</tr>
<tr>
<td>3</td>
<td>13/M</td>
<td></td>
<td>Arm</td>
<td>Intramuscular</td>
<td>3</td>
<td>Unknown</td>
<td>Marginal excision (margin-)</td>
<td>5</td>
<td>NED</td>
</tr>
<tr>
<td>4</td>
<td>22/F</td>
<td></td>
<td>Neck</td>
<td>Intramuscular</td>
<td>4.5</td>
<td>6 mo</td>
<td>Marginal excision (margin-)</td>
<td>4</td>
<td>LTF</td>
</tr>
<tr>
<td>5</td>
<td>24/M</td>
<td></td>
<td>Thigh</td>
<td>Intramuscular</td>
<td>17</td>
<td>1 mo</td>
<td>Wide excision (margin-), radRx</td>
<td>10</td>
<td>NED</td>
</tr>
<tr>
<td>6</td>
<td>28/M</td>
<td></td>
<td>Neck</td>
<td>Intramuscular</td>
<td>2</td>
<td>2 y</td>
<td>Marginal excision (margin-)</td>
<td>-</td>
<td>LTF</td>
</tr>
<tr>
<td>7</td>
<td>30/F</td>
<td></td>
<td>Axilla</td>
<td>Intramuscular</td>
<td>9.4</td>
<td>5 mo</td>
<td>Wide excision (margin-)</td>
<td>4</td>
<td>NED</td>
</tr>
<tr>
<td>8</td>
<td>33/F</td>
<td></td>
<td>Wrist</td>
<td>Subaponeurotic</td>
<td>0.7</td>
<td>2 y</td>
<td>Marginal excision (margin-)</td>
<td>61</td>
<td>NED</td>
</tr>
<tr>
<td>9</td>
<td>44/F</td>
<td></td>
<td>Shoulder</td>
<td>Intramuscular</td>
<td>7</td>
<td>2 y</td>
<td>Wide excision (margin-), radRX</td>
<td>34</td>
<td>NED</td>
</tr>
<tr>
<td>10</td>
<td>58/F</td>
<td></td>
<td>Breast</td>
<td>Breast lump</td>
<td>10</td>
<td>30 y</td>
<td>Excision (margin-), radRx</td>
<td>65</td>
<td>AWD</td>
</tr>
</tbody>
</table>

Abbreviation: F, female; M, male; radRx, radiotherapy; NED, no evidence of disease; LTF, lost to follow-up; AWD, alive with disease; "+", positive; "-", negative.

Materials and methods

Study cases and tumor specimens

The present study was approved by the West China Hospital Institutional Review Board. Cases diagnosed as LGFMF with definitive FUS gene rearrangement confirmed by FISH from January 2008 to December 2017 were retrieved from the surgical pathology and consult files of West China Hospital. Finally, 10 cases were included in this study.

Histologic evaluation

Histologic features of all the 10 cases were reviewed by three pathologists with soft tissue tumor pathology expertise (H.Z., H.C. and Z.Z.) and two general surgical pathologists (M.L. and D.S.). The mitotic count was determined by the greatest number of mitotic figures in 10 consecutive high-power fields (1.73 mm² per 10 HPFs) of the most mitotically active areas.

Immunohistochemistry

Standard immunohistochemical staining was performed on routine 4-μm sections of FFPE tissue, according to the manufacturer’s protocols, using the EnVision Plus Detection System (DAKO, Carpinteria, CA) with positive and negative controls. The antibodies used were CD34 (1:100; clonal QBEnd10, Dako), desmin (ready to use; clonal D33, Dako), EMA (1:100; clonal GP1.4, Dako), S-100 protein (1:100; clonal 4C4.9, Dako), smooth muscle actin (1:100; clonal 1A4, Dako), MUC4 (1:300; polyclonal, Biosynthesis Biotechnology) and Ki-67 (1:100; clonal MIB-1, Dako).

Fluorescence in situ hybridization (FISH)

Vysis LSI dual-color break-apart probes (Vysis Inc, Downers Grove, IL, USA) were used to detect the FUS rearrangement on chromosome 16p11 in interphase FISH. FISH analyses were performed according to an established laboratory protocol, as previously described. FISH assays were carried out on 4-μm-thick FFPE tissue sections [32, 33]. The sections were depaaffined twice in xylene for 30 min, washed in 100% ethanol twice for 5 min, treated with 10 mmol/L citric acid for 10 min in humidified microwave and then incubated in 2× sodium saline citrate (2× SSC) for 5 min at 37°C. Tissue sections were digested with 200 μg/mL proteinase K solution (Sigma, St. Louis, MO, USA) at 37°C for 20 min, washed in phosphate-buffered saline (1× PBS) for a brief time, sequentially dehydrated in ethanol (70%, 80%, 100%) and air-dried at room temperature. Tissue sections were denatured at 85°C for 2 min and hybridized overnight at 37°C in a humidified chamber. The slides were then washed in 0.1% NP40/2× SSC at 76°C for 2 min and again washed with the same solution at room temperature for 2 min. The slides of all cases were scored by two investigators (H.Z. and M.C.) independently. A split signal pattern was considered positive for the FUS gene rearrangement if the distance between the green and the red signals was greater than the total diameter of two signals. A tumor was considered as...
translocation-positive if at least 10% of detected tumor cells exhibited FUS rearrangement.

Results

Clinical findings

The clinical information is summarized in Table 1. The patients (7 females, 3 males) were 4 to 58 years old at the time of diagnosis (median, 26 y; mean, 27 y), and 3 of them (3/10, 30%) were less than 18 years old. The most common clinical manifestation was a slowly growing, painless, deep-situated mass found unconsciously of the corresponding location. The tumor involved the trunk (4/10, 40%), neck (2/10, 20%), upper extremities (2/10, 20%), and thigh (2/10, 20%). All the cases were situated in deep location.

Four patients were treated with wide surgical excision and five patients with marginal surgical excision. Only a patient with the breast involved had a positive margin with the residual tumor infiltrating into the pectoral fascia. Three patients were submitted to adjuvant radiotherapy postoperatively. None received chemotherapy. Follow-up information was available for 8 cases, and the interval time varied from 4 to 65 months (median, 34 months; mean, 31 months). All the eight patients were alive without evidence of local recurrence or distant metastasis.

Gross and histologic findings

The majority of the tumors were well-demarcated, which varied from 0.7 to 17 cm (median, 5.8 cm; mean, 6.6 cm) in maximum dimension. On cut surface, most tumors showed a gray-white, firm appearance with variable glistening foci. Cystic formation (2/10, 20%), focal hemorrhage (2/10, 20%), and necrosis (1/10, 10%) were observed.
Microscopically, all the cases demonstrated areas of classic features of LGFMS, which included contrastingly alternating fibrous and myxoid zones with either an abrupt or a gradual transition, a whorled or patternless growth pattern of uniformly spindle-shaped fibroblastic tumor cells arranged within the fibromyxoid background, prominent arcades of curvilinear capillaries, and perivascular hypercellularity (Figure 1). Tumor cells comprised vaguely, pale eosinophilic cytoplasm and spindle to oval shaped nuclei with slightly nuclear atypia or pleomorphism. Cellularity was low to moderate in most cases. Mitotic figures were generally absent to low (0 to 1 mitotic count per 10 HPFs). None of the tumors showed hyalinizing spindle cell tumors with giant rosettes (HSCTR). Some other features also appeared as following: hyalinizing stroma (4/10, 40%), predominant stromal form growth pattern (1/10, 10%), the presence of multinucleated giant cells (1/10, 10%), cyst degeneration (3/10, 30%), hemorrhage (3/10, 30%) and necrosis (2/10, 20%). Also, relatively evident nuclear pleomorphism with increased cellularity (1/10, 10%) (Figure 2) and focal areas resembling sclerosing epithelioid fibrosarcoma (SEF) (1/10, 10%) (Figure 3) were observed.

Immunohistochemical staining and FISH analysis

Immunohistochemically, LGFMS were only focally positive for EMA (1/9, 11.1%), and negative for CD34, desmin, smooth muscle actin, and S-100 protein. Immunohistochemical staining for MUC4 was performed in 4 cases. Among them, one showed focal expression, and others were interpreted as indeterminate. Ki-67 index (9/10) varied from 0 to 5% (median, 2%; mean, 1.8%).

By FISH, all the 10 cases showed FUS rearrangement (Figure 4).

Discussion

In this study, there was a prominent predilection for females (7/10, 70%). In contrast, major-
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Figure 4. FUS gene rearrangement (marked with yellow arrow) was positively detected by FISH analysis.

ity of previous large series showed male predominance or no sex preference [5, 16, 26]. Further study is required to determine whether this phenomenon in the current study is just an incidental finding. Our study demonstrated that LGFMS often developed in young adults with the peak incidence around the third and fourth decade, and a proportion of cases occurred in patients aged less than 18 years, which was similar to historical studies [5, 8, 11, 13, 16, 17]. Comparable with previous series, we found LGFMS occurred most commonly in the deep soft tissues of trunk and proximal extremities. Of interest, although breast was reported as a metastatic site of LGFMS [5], it is an extremely rare location for primary LGFMS, as we encountered one case in our study.

Grossly, the majority of tumors in the present series were well-demarcated, and some of them showed a variety of cystic, hemorrhage and necrotic changes, similar to previous studies [17, 26].

Microscopically, areas of classic features of LGFMS were observed in all cases of the current study, and some other morphologies including unusual ones, such as increased cellularity with evident nuclear pleomorphism and focal areas resembling SEF were also encountered. Histologic evaluation has been considered as an important step for the definite diagnosis of LGFMS. However, both the deceptively bland histologic features and the wide morphologic spectrum of the tumor have posed diagnostic pitfalls for pathologists. In addition, the increasing use of core biopsy on soft tissue pathology resulting in insufficient tumor specimen has also increased diagnostic challenges.

On such occasions, definite diagnosis of LGFMS depends on immunohistochemical analysis and FISH detection for FUS rearrangement after histologic evaluation.

In the current study, neoplastic cells are generally negative for CD34, desmin, S-100 protein, and smooth muscle actin and focally positive for EMA, which was consistent with previous series. MUC4 was regarded as a sensitive and specific marker for LGFMS [31]. However, this marker seems to be not highly sensitive and specific for LGFMS in the present series, which may be resulted from the different antibody clones of the current study and the study of Doyle et al. [31]. In this context, FISH analysis of FUS rearrangement can help to make the definite diagnosis. It is noteworthy that an unusual translocation, EWSR1-CREB3L1, has been recognized in a few cases recently [12, 34]. Therefore, probes detecting this rare rearrangement should be considered when a case highly suspected as LGFMS is FUS translocation-negative.

In the present study, a case exhibiting focal areas indistinguishable from sclerosing epithelioid fibrosarcoma (SEF), which had been addressed as hybrid “LGFMS/SEF” tumor in some studies and gained wide attention [30, 35, 36]. SEF is also an uncommon and more aggressive sarcoma, composed of cords or nests of proliferation of epithelioid cells arranged in heavily sclerotic stroma. Pure SEF and LGFMS have been considered as two different entities mainly owing to their different clinical courses and recurrent molecular changes; that is, the majority of pure SEF cases bear EWSR1-CREB3L1 rather than FUS-CREB3L2. However, the existence of some cases of LGFMS with SEF-like morphology harboring typically FUS rearrangement as well as some clinicopathologic overlap [30, 31, 37], probably reveal the underlying relationship between these two tumors. Of course, a larger series and multiple molecular methods are required in further study.

The differential diagnosis of LGFMS is broad and can be challenging, especially in small biopsy samples. This tumor must be discriminated from other benign, borderline, and malignant fibrous or myxoid lesions, such as desmoid fibromatosis, soft tissue perineurioma, nodular fasciitis, intramuscular myxoma, neu-
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rofibroma, low-grade myxofibrosarcoma, and malignant peripheral nerve sheath tumor (MPNST).

Desmoid fibromatosis may be confused with LGFMS especially when extensive myxoid areas exit. Grossly, desmoid fibromatosis is poorly-demarcated and with a more infiltrative growth pattern compared to LGFMS. The characteristic sweeping fascicular growth pattern and the keloid collagen exhibited in desmoid fibromatosis are usually absent in LGFMS. Immunohistochemically, desmoid fibromatosis exhibits nuclear positivity for β-catenin protein (70%), and negativity for MUC4. FUS rearrangement detection is helpful for difficult cases.

Soft tissue perineurioma is a benign nerve sheath tumor composed of bland spindle-shaped tumor cells which are usually arranged in a storiform whirling or lamellar pattern with a mixture of fibrous and myxoid zones. Perineurioma can mimic LGFMS closely, especially when highly collagenized. But perineurioma usually occurs in superficial locations and histologically lacks prominent vasculature in myxoid areas. In addition, the spindle cells of LGFMS often lack streamer-like cytoplasmic processes, a distinctive histologic feature of perineurioma. Given both tumors can express EMA and claudin-1 variably, molecular tests may be necessary for final diagnosis.

Some areas of LGFMS may mimic nodular fasciitis, but the latter lesion typically consists of characterized plump spindle cells posing a tissue culture-like appearance. Other features, such as extravasation of erythrocytes and cleft-like spaces of nodular fasciitis, are nearly absent in LGFMS. Immunohistochemically, the spindle cells within nodular fasciitis often diffusely express SMA and MSA. Importantly, nodular fasciitis has been shown to harbor a characteristic USP6 gene rearrangement. If necessary, detection for rearrangements of USP6 and FUS is useful to discriminate nodular fasciitis from LGFMS.

Cellular myxoma, can be easily confused with LGFMS. On this occasion, molecular test for FUS translocation can be extremely helpful to obtain definitive recognition.

Neurofibroma often displays a fibromyxoid background resembling LGFMS. But tumor cells in neurofibroma have more slender and wavy nuclei and are consistently positive for S-100 protein.

Low-grade myxofibrosarcoma can be easily confused with LGFMS not only for their similar nomenclature, but also for the histologic features. But, myxofibrosarcoma generally occurs in superficial soft tissues of elderly patients with a peak incidence of fifth to seventh decade of life. Histologically, low-grade myxofibrosarcoma exhibits relatively predominant myxoid substances and a more prominent vascular pattern, and more cellular pleomorphism and nuclear atypia. Also, pseudolipoblasts found in myxofibrosarcoma are nearly absent in LGFMS. For difficult cases, detection for FUS translocation can help.

MPNST can resemble LGFMS by having alternating hypercellular fascicles and hypocellular myxoid zones, whorled growth pattern of spindle tumor cells and focal areas likened to giant rosettes. Unlike LGFMS, MPNST exhibits more cellularity with more cellular pleomorphism and nuclear atypia. Up to 60% of MPNSTs express S-100 protein. Molecular testing for FUS translocation is useful for distinguishing between the two tumors.

Although standard treatment schemes have not been defined at present, surgical excision is the main therapy of LGFMS. Radiotherapy and chemotherapy have showed no significant effect on LGFMS. Therefore, correct preoperative recognition, complete excision with negative margins, and a long-term clinical follow-up are considered as important approaches for LGFMS.

In the current study, all eight patients were alive without any local recurrence or metastasis, showing quite favorable prognosis. Some prior studies also showed low recurrence (9%, 13% and 8%, respectively) and metastases (6%, 0 and 0, respectively) [5, 8, 17]. However, LGFMS was a more progressive lesion in some other studies. Guillou et al. and Evans both presented LGFMS as a malignant tumor with a higher rate of local recurrences (20% and 64%, respectively) and distant metastases (27% and 45%, respectively) [16, 26]. The striking discrepancy of follow-up information among these studies, may be interpreted by the reason of preoperative recognition which can decide the
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treatment whether complete excision or not, surgical excision with positive or negative margins and the length of follow-up [5, 8, 16, 17, 26].

Herein, we presented the clinicopathological and molecular features of 10 genetically confirmed LGFMS. The tumor cells are very bland and therefore the diagnosis of this tumor might be very challenging. A combination of clinical studies, careful morphologic analysis, and a full panel of immunomarkers, especially genetic studies, is helpful in confirming the diagnosis. This tumor type is associated with favorable prognosis. To the best of our knowledge, the present series contains the most genetically confirmed cases from a Chinese medical center in English literature.

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Disclosure of conflict of interest

None.

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