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Primary Retroperitoneal Myxoid Liposarcomas

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Abstract: Myxoid liposarcomas (MLSs) are genetically defined by the presence of *DDIT3* gene fusions and most commonly arise in the extremities of young adults. Whether MLSs develop primarily in the retroperitoneum is controversial, and a recent retrospective study found no molecularly confirmed examples. Because MLSs tend to metastasize to deep soft tissues, purported examples of primary retroperitoneal lesions might represent distant metastasis, most commonly from extremities. In addition, well-differentiated or dedifferentiated liposarcomas, which are characterized by *MDM2* amplifications, may exhibit prominent myxoid changes and mimic MLSs. Here, we document 5 cases of MLSs that originated in the retroperitoneum that were identified through critical clinicopathologic reevaluation. These cases accounted for 2.3% of 214 primary retroperitoneal liposarcomas and 3.2% of 156 MLSs in our database. They occurred in 3 men and 2 women with a median age of 32 years. All tumors were localized to the retroperitoneum at presentation, and no patient developed extra-abdominal recurrences during the clinical course (median, 50 mo). All 5 cases exhibited at least focal classic histologic findings. All harbored *DDIT3* gene rearrangements, and none harbored *MDM2* amplifications according to fluorescence in situ hybridization. This study demonstrates that primary MLSs can occur in the retroperitoneum, albeit rarely, and can be accurately diagnosed through combined clinicopathologic and molecular analysis.

Key Words: liposarcoma, retroperitoneum, diagnosis, fluorescence in situ hybridization

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Myxoid liposarcomas (MLSs) account for 15% to 20% of all liposarcomas[1] and tend to affect young adults, with the incidence peaking in the fourth to fifth decades of life. MLSs typically arise in deep soft tissues of the extremities; uncommon sites include the head and neck, subcutis, and thorax. Histologically, MLSs exhibit a mixture of uniform oval-shaped cells and signet-ring cell lipoblasts on a background comprising myxoid stroma and prominent arborizing capillary vasculature. The round cell component, defined by markedly increased cellularity, is predictive of aggressive behavior when comprising a significant proportion of the tumor volume. MLSs are genetically characterized by the presence of *FUS-DDIT3* (> 90%) or *EWSR1-DDIT3* (< 10%) fusion genes.[2–4]

Whether primary MLSs can develop in the retroperitoneum has recently become a matter of debate. Previously, the retroperitoneum was listed as a relatively common site of MLSs. In 1962, Enzinger and Winslow[5] reported that 25% of MLSs occurred in the retroperitoneum, and more than a third of retroperitoneal liposarcomas were classified as MLSs. However, later published reports described the retroperitoneum as an uncommon site of MLSs,[6,7] and, more recently, primary retroperitoneal MLSs have been considered rare[1] or even nonexistent.[8] This drastic shift in viewpoint stems from several factors. First, clinicopathologic studies established that MLSs have a unique proclivity to metastasize to deep soft tissues and bones,[9–12] and the retroperitoneum represents one of the most common metastatic sites of these tumors.[10] In addition, advances in clinical imaging have facilitated systemic surveys of tumor distribution. As a result, patients who present with metastatic retroperitoneal MLSs and would have previously been diagnosed with primary retroperitoneal MLSs can now be precisely staged by imaging.

Furthermore, molecular genetic evidence has refined the classifications of liposarcomas; as a result, some tumors that were previously classified as MLSs are currently diagnosed as well-differentiated liposarcomas (WDLSs) or dedifferentiated liposarcomas (DDLSs). WDLSs and DDLSs often affect the retroperitoneum and abdominal cavity,[1] and some WDLSs or DDLSs may exhibit relatively uniform spindle cell proliferation on a background comprising abundant myxoid matrix and prominent plexiform capillaries, leading to a significant risk of misclassification as primary retroperitoneal MLSs.[13] Whereas MLSs are genetically defined by *DDIT3* gene rearrangement, WDLSs

and DDLSs are characterized by *MDM2* and *CDK4* gene amplifications and overexpression of the respective protein products,[14,15] and these specific genetic changes are diagnostically useful in histologically ambiguous cases.

To better understand the true incidence and characteristics of primary retroperitoneal MLSs, we retrospectively searched for potential cases of primary retroperitoneal MLS and critically reevaluated their clinical, radiologic, and histologic features. We hereby document 5 cases of primary retroperitoneal MLSs with confirmatory molecular genetic data.

MATERIALS AND METHODS

Patients

We electronically searched the pathology database of the National Cancer Center Hospital in Tokyo for potential cases of primary retroperitoneal MLS accessioned between 1998 and February 2015. Among a total of 219 cases (299 samples) recorded as retroperitoneal liposarcomas, we identified 11 candidate tumors (search terms: ["myxoid liposarcoma" OR "liposarcoma, myxoid type"] AND "retroperitoneum"). The remaining 208 cases were WDLSs/DDLSs (n = 205) or pleomorphic liposarcomas (n = 3), and all had originated from the retroperitoneum. From the 11 candidate tumors, a careful review of the clinical records and pathology materials excluded 6 cases from further analysis for the following reasons: (1) 3 cases had a previous history of MLS arising in the limbs (buttock in 2 cases and thigh in 1 case) and retroperitoneal tumors were considered metastases; (2) 1 patient presented with multiple soft tissue masses, including the retroperitoneal mass, and the primary site could not be confirmed; (3) 1 patient underwent resection of a "recurrent" MLS in the groin 1 year after resection of the retroperitoneal tumor, and the exact order of tumor development could not be verified because of the incomplete imaging studies; and (4) 1 case was excluded because the tumor contained multinucleated floret-like giant cells and spindle cell fascicles, and the diagnosis was revised as WDLS/DDLS with myxoid change. The remaining 5 cases exhibited histology compatible with MLS and met the strict clinical criteria of primary retroperitoneal origin and were therefore further analyzed to determine the *DDIT3* and *MDM2* gene status.

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) analysis was performed on formalin-fixed, paraffin-embedded, 4-μm-thick tumor sections. To examine *DDIT3* rearrangement, we used the Vysis DDIT3 Break Apart FISH Probe Kit (Abbott Molecular, Abbott Park, IL). For *MDM2* amplification status, we used the ZytoLight SPEC MDM2/CEN 12 Dual Color Probe (ZytoVision GmbH, Bremerhaven, Germany) and/or the Vysis LSI MDM2 SpectrumOrange Probe (Abbott Molecular) combined with Vysis CEP 12 (D12Z3) SpectrumGreen Probe (Abbott Molecular). FISH images were captured using the Metafer Slide Scanning Platform (MetaSystems, Altlussheim, Germany), and 100 nonoverlapping tumor cells were examined. For *DDIT3*, tumors in which >20% of the cells showed split signals were considered positive for gene rearrangement. An *MDM2*/control probe ratio of >2.0 in ≥10% of the nuclei was considered positive for *MDM2* amplification.

RESULTS

Clinical Findings

Clinicopathologic data of the 5 patients with primary retroperitoneal MLSs are summarized in Table 1. The patients included 3 men and 2 women, with ages at diagnosis ranging from 30 to 73 years (median, 32 y). All primary tumors were localized to the retroperitoneum, and physical examination did not detect tumors elsewhere. Case 1 underwent computed tomography (CT; neck to thigh) and positron emission tomography (PET) scans (head to thigh), the latter of which indicated increased ^{18}F-fluorodeoxyglucose uptake (maximum standard uptake value, 4.7) only in the retroperitoneum. Case 2 underwent CT and magnetic resonance imaging (MRI) scans from neck to toe and a whole-body PET scan (maximum standard uptake value, 1.3 in the recurrent retroperitoneal lesion). The other 3 cases underwent CT (chest to groin) and MRI scans (abdomen to groin). The maximum tumor diameters ranged from 10 to 36 cm (average, 20 cm). MRI revealed a mixed pattern of hypointensity and hyperintensity on T1-weighted images (WI) and hyperintensity on T2WI (Fig. 1). CT revealed slightly heterogenous, isodense masses. Shell-like mineralization was noted in case 5.

All primary tumors were surgically excised, after neoadjuvant therapies were administered in 2 cases. All but 1 patient developed recurrent disease, and the recurrent sites were anatomically confined to the retroperitoneal or intra-abdominal regions. No patients developed extra-abdominal soft tissue masses during their courses (range, 21 to 115 mo), as supported by physical examination and/or clinical interview. After a median follow-up of 50 months, 2 patients remained disease free for >5 years; the remaining patients either died of the disease or were referred to palliative care units because of advanced disease.

Histologic Findings

All 5 cases exhibited at least focal areas with classic histologic findings of MLS, including proliferating uniform, oval-shaped cells on a myxoid background with a rich plexiform capillary network (Fig. 2). Signet-ring cell lipoblasts were occasionally noted. In addition, prominent hyalinization and a focal area with reduced vascular density were each observed in 1 tumor. Histologically, all recurrent tumors exhibited similar features as the respective primary tumors except for the tumor in case 1, which contained an emergent round cell component at recurrence. Two cases were immunohistochemically examined during the original workups, and both tumors were found to be negative for MDM2 and CDK4.

TABLE 1. Summary of Clinicopathologic Data of MLSs That Originated in the Retroperitoneum

No.	Age	Sex	Size (cm)	Initial Presentation	Preoperative Therapy	Surgical Margins	Recurrent Sites (mo)	Follow-ups (mo)	*DDIT3* FISH	*MDM2* FISH
1	31	M	20	Localized	AI × 3 + RT (SD)	R2	RP + AB (11, 14, 17)	DOD (21)	Rearranged	Not amplified
2	32	M	14	Localized	None	NA	RP (39)	NED (80)	Rearranged	Not amplified
3	30	M	36	Localized	AP × 4 + I × 2 (PR)	R2	AB dissemination (16)	LTF (50)	Rearranged	Not amplified
4	73	F	20	Localized	None	NA	AB + RP (10), AB (46)	LTF (46)	Rearranged	Not amplified
5	34	F	10	Localized	None	R0	None	NED (115)	Rearranged	Not amplified

A indicates doxorubicin; AB, intra-abdominal space; DOD, dead of disease; F, female; I, ifosfamide; LTF, lost to follow-up and referred to palliative care; M, male; NA, data not available; NED, no evidence of disease; P, cisplatin; PR, partial response; R0, no microscopic residual tumor; R2, macroscopic residual tumor; RP, retroperitoneum; RT, radiotherapy; SD, stable disease.

Molecular Cytogenetic Findings

All 5 cases harbored *DDIT3* gene rearrangements (Fig. 3A). None of the cases harbored *MDM2* amplifications (Fig. 3B).

DISCUSSION

In this report, we have documented 5 cases of genetically confirmed MLSs that originated from the retroperitoneum. These cases were identified from among 214 cases of primary retroperitoneal liposarcomas (2.3%) during the review period and represented only 3.2% of 156 myxoid/round cell liposarcoma cases diagnosed during this period. However, the calculated incidences are likely overestimated because of referral bias, as retroperitoneal WDLSs/DDLSs that are untreatable and MLSs in the limbs that were readily managed at local hospitals tend not to be referred to us for pathologic review. Overall, after a critical reevaluation of clinicopathologic parameters, we confirmed the rarity of primary retroperitoneal MLSs.

This rarity likely explains the results of a recent study by de Vreeze et al,[8] who did not identify any genetically confirmed cases of primary retroperitoneal MLS in a smaller cohort of liposarcomas (n = 68). When the authors analyzed 16 tumors originally diagnosed as retroperitoneal MLS, all exhibited MDM2 and CDK4

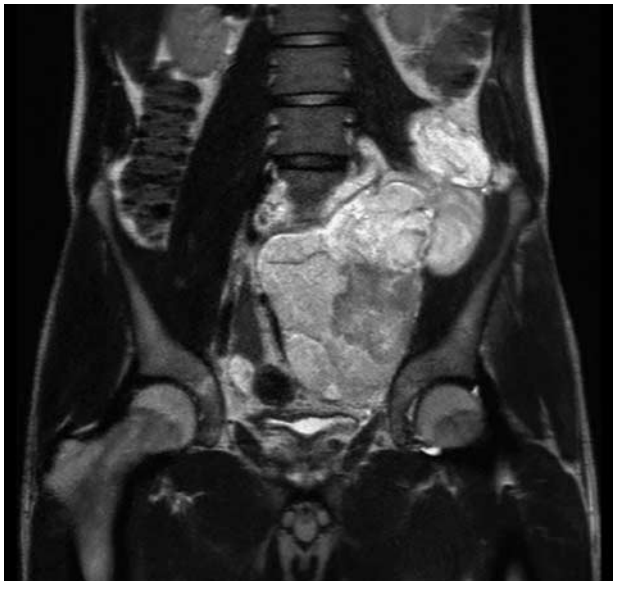

FIGURE 1. T2-weighted magnetic resonance image of a primary retroperitoneal MLS.

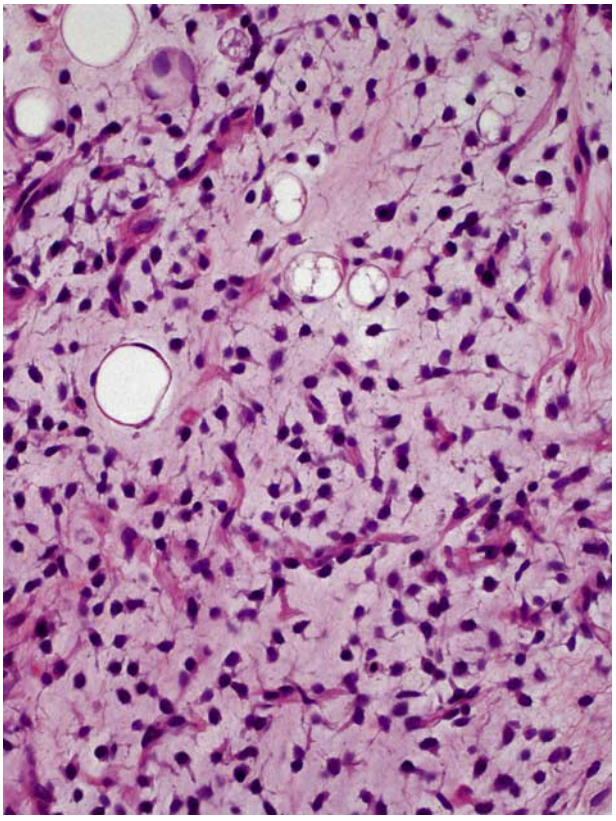

FIGURE 2. Primary retroperitoneal MLS showed a classic histologic appearance, characterized by a mixture of uniform oval cells and signet-ring cell lipoblasts on a background comprising myxoid stroma and plexiform capillary network (hematoxylin and eosin staining).

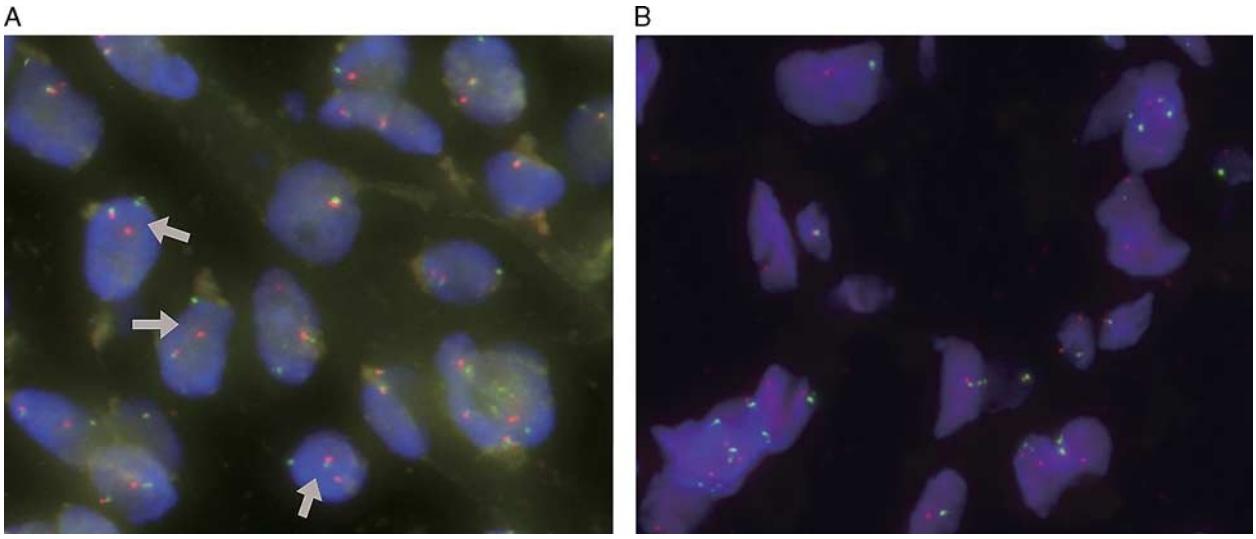

FIGURE 3. A, Primary retroperitoneal MLSs consistently harbored *DDIT3* gene rearrangement (*DDIT3* break-apart FISH assay; arrows indicate splits). B, All primary retroperitoneal MLSs lacked *MDM2* gene amplifications (red signals indicate *MDM2*; green signals indicate *CEP12*).

immunoreactivity or *MDM2* amplification but lacked *FUS-DDIT3* or *EWSR1-DDIT3* fusion genes and were accordingly reclassified as WDLSs or DDLSs. We are aware of 2 potential cases of genetically proven primary retroperitoneal MLS[16] that were described as "localized" to the "retroperitoneum to lower abdomen," although detailed clinical information regarding these cases was not provided. In addition, some large series of MLS cases included those in the retroperitoneum[17–19]; however, those studies lacked either molecular genetic data or clinicoradiologic documentation to confirm a primary retroperitoneal origin. The present study unequivocally demonstrates that MLSs do primarily occur in the retroperitoneum and can be accurately diagnosed through combined clinicopathologic and molecular analysis.

Our study highlights the need for a careful clinical workup before the diagnosis of primary retroperitoneal MLS. Five of the initial 11 candidate cases were excluded because metastatic spread to the retroperitoneum could not be entirely ruled out. Careful clinical interview including an inquiry regarding the remote history is important. According to our review of 3 published series,[9,11,12] 12% (8/66) of MLS patients presented with the first metastases > 5 years after the initial presentation, including 2 cases with a long interval between the initial presentation and metastatic disease (18 and 25 y).[9,12] A thorough physical examination is also mandatory for accurate identification of the primary site. In addition, a variety of imaging modalities are available to rule out possible primary tumors in the extremities, particularly the lower extremities where most MLSs develop.[9–12,16] These modalities include MRI, enhanced CT, and ^{18}F-fluorodeoxyglucose PET/CT, with a caveat that the latter may show low tracer uptake.[12,20] However, extensive imaging studies may not always be economically feasible, and clinical parameters often supplant such assessments in actual practice settings. Among our 5 cases, only case 2 involved a systemic imaging workup, whereas case 1 included MRI and CT scans of the thighs, the most common primary site for MLS. Although the radiologic studies did not cover extremities in the remaining 3 cases, we believe that the primary retroperitoneal origins in all 5 cases were confirmed by the absence of tumors elsewhere over a relatively long follow-up period (median, 50 mo).

As de Vreeze et al rightly noted,[8] it can be difficult to distinguish MLSs from WDLSs/DDLSs with myxoid changes in the retroperitoneum. In our study, 1 case of WDLS/DDLS was initially misinterpreted as MLS. This distinction is of paramount importance for appropriate treatment. WDLSs/DDLSs are usually resistant to radiotherapy and chemotherapy, whereas MLSs are sensitive to these modalities.[21,22] Furthermore, trabectedin, a recently developed agent that interferes with the binding of fusion genes and target promoters,[23] has shown promise against MLS.[24,25] Although the differential diagnosis is ultimately made possible by genetic means, it can be facilitated by the combined use of conventional modalities, including clinical, radiologic, and histologic findings.

Clinically, MLSs typically arise in younger patients, compared with WDLSs/DDLSs.[1] MLSs may affect children, in whom WDLSs/DDLSs are distinctly rare.[1] Radiologically, WDLSs/DDLSs present as multinodular masses that may contain a purely lipomatous component, whereas MLSs exhibit hypointense to isointense signals on T1WI and hyperintense signals on T2WI and occasionally exhibit intermixing with lipomatous areas in a marbled or nebulous textural manner.[26] Intratumoral mineralization might suggest WDLSs/DDLSs, as it is more common in these tumor types.[27] Nonetheless, decisions should not be made solely on these distinctive clinicoradiologic parameters, as exemplified in the present study by case 4, which involved an elderly patient, and case 5, which exhibited shell-like mineralization. Histologic

examination of MLSs generally reveals uniform monomorphic cytomorphology, in contrast to at least focal nuclear pleomorphism observed in WDLSs/DDLSs with myxoid changes. The tumor cells in WDLSs/DDLSs with myxoid changes tend to be spindled, whereas those in MLSs are typically oval with less conspicuous cytoplasms. In addition, plexiform thin-walled vasculature is characteristic of MLSs, whereas the vasculature associated with WDLSs/DDLSs is commonly coarse and curvilinear; however, WDLSs/DDLSs may also show a plexiform and delicate form that is indistinguishable from the pattern noted in MLSs.[8] MDM2 and CDK4 immunohistochemistry may be a practical surrogate for the molecular analysis of *MDM2* amplification.[28]

Another rare liposarcoma variant that should be differentiated from MLS in young patients is the so-called pleomorphic MLS (also known as myxoid pleomorphic liposarcoma).[29] This variant typically occurs in the mediastinum; however, cases involving the retroperitoneal/abdominal regions have been reported. Unlike MLSs, pleomorphic MLSs harbor pleomorphic liposarcoma-like components and lack the *DDIT3* gene fusion.

In summary, we conclude that MLS rarely occurs in the retroperitoneum, and the primary site alone should not be used to rule out a diagnosis of MLS. However, the rarity of such cases demands considerable diagnostic caution in clinical practice settings. Particular attention should be paid to the distinction from WDLSs/DDLSs with myxoid changes and the possibility of retroperitoneal metastasis from extraneous sites.

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