

# Atypical spindle cell lipomatous tumor/atypical pleomorphic lipomatous tumor and atypical mammary-type myofibroblastoma: clinicopathological, immunohistochemical, and multiplex fluorescence *in situ* hybridization analyses

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*To the Editor:* Lipomatous neoplasms with spindle cell components and atypical features are rare soft tissue tumors. Atypical lipomatous tumor (ALT)/well-differentiated liposarcoma (WDLPS) is one of the most common liposarcomas with *MDM2* (12q13–15) gene amplification. However, recently, some studies have suggested that unique lipomatous tumors named “atypical spindle cell lipomatous tumor” (ASLT) and atypical pleomorphic lipomatous tumor (APLT) should be distinguished from WDLPS because they have a better prognosis.<sup>[1,2]</sup> ASLT and APLT are tumors with *RB1* gene deletions and the same chromosomal alterations as classical spindle cell lipoma (SCL)/pleomorphic lipoma.<sup>[2]</sup> The latest edition of the “World Health Organization Classification of Tumors of Soft Tissue and Bone, fifth edition” formally introduced the names of ASLT/APLT<sup>[3]</sup>; these tumors are rare among Chinese patients, and only a few small series of cases have been reported in Asia.

In addition to those for *RB1*, sequence-specific DNA probes targeting 13q14.1–14.2 also include *forkhead box O1* (*FOXO1*). *FOXO1* is a tumor suppressor, and its translocation probe has been used to aid in the diagnosis of alveolar rhabdomyosarcoma. Previous studies have shown that most mammary-type myofibroblastomas (MTMFs) have monoallelic deletions of *FOXO1*. In this study, we applied cohybridization of *FOXO1* with *RB1* in the identification of 13q14 segment deletions in a series of ASLTs/APLTs by multiplex fluorescence *in situ* hybridization (FISH). This is the largest population-based detection of both *RB1* and *FOXO1* in ASLTs/APLTs among the Asian population thus far. We summarized a series of 24 cases from 2009 to 2019 identified through surgical pathology files in one of the largest institutions in China and reported the clinicopathological and immunohisto-

chemical characteristics, cytogenetic alterations, and follow-up information. This study was approved by the Review Board of the Ethics Committee of West China Hospital (No. 2020-439). Informed consent is waived.

The tumors were classified as ASLT ( $n = 21$ ), APLT ( $n = 2$ ), and atypical MTMF ( $n = 1$ ). The clinicopathologic features of the 24 patients are shown in Supplementary Table 1, <http://links.lww.com/CM9/A719>; there were 18 males and six females. The age of the patients ranged from 22 to 68 years (average 47.7 years; median 48 years), and the tumors mainly occurred in older males (75.0%). There were 16 cases located in the subcutaneous region (66.7%) that presented as a slowly growing mass, six cases presented in deep soft tissue, and the remaining two cases were located in the oral and maxillofacial regions. The majority of tumors (15/23, 65.2%) were well circumscribed. The size of the neoplasms ranged from 0.8 to 18.0 cm in largest diameter (mean, 6.5 cm). Tumors that occurred deep within the tissue were larger than those in superficial tissues (average size, 9.9 *vs.* 5.7 cm). No tumors had hemorrhage, necrosis, or cystic changes. Follow-up information was available for 21 patients (87.5%) who had a median follow-up time of 20 months (range 8–64 months, mean 22.9 months). All the patients underwent surgical treatment with complete excision. Four patients received radiotherapy after surgery. No recurrence or metastasis was found in any patient. The histopathologic features are summarized in Supplementary Table 2, <http://links.lww.com/CM9/A719>. All cases were re-examined by two soft tissue tumor pathologists (HYZ and ZZ). The tumors consisted of a relatively clear border with fibrous membranes, but invasive growth was observed in eight cases (8/23, 34.8%). The ASLTs were composed of different proportions of spindle tumor cells and adipocytes

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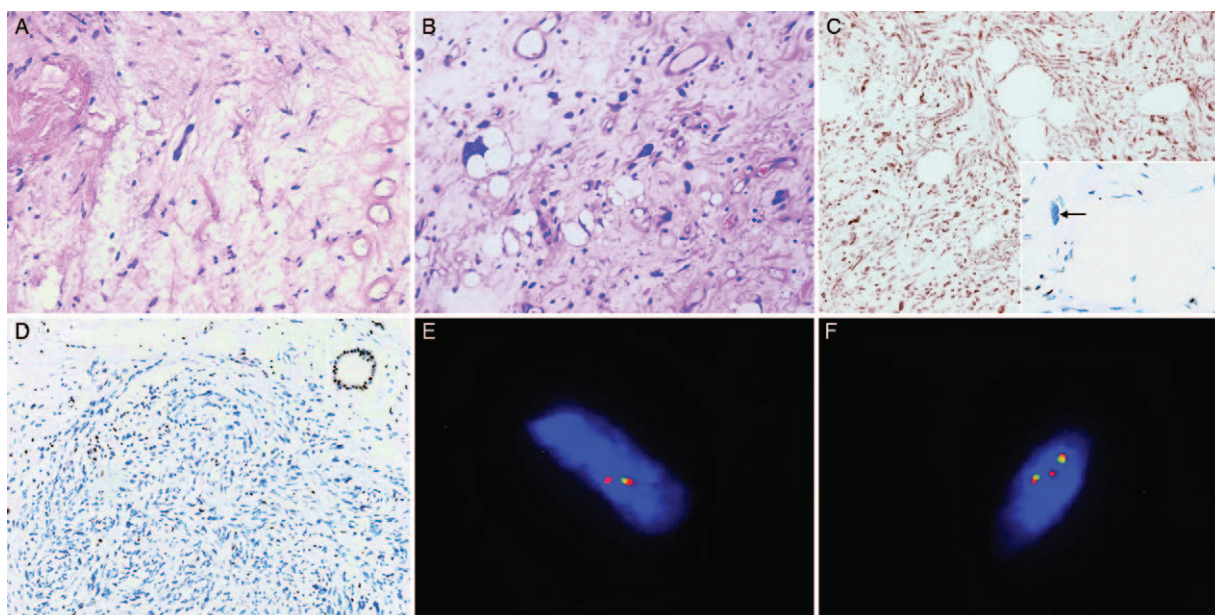
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**Figure 1:** (A) Atypia and hyperchromatic nuclei in ASLT (HE, original magnification  $\times 400$ ). (B) Pleomorphic cells and multivacuolated lipoblasts in APLT (HE, original magnification  $\times 400$ ). (C) Strong reactivity to CD34 and Rb deletion (inserted figure with arrow) in ASLT (EnVision, original magnification  $\times 200$ , inserted figure original magnification  $\times 400$ ). (D) Rb deletion in atypical MTMF: loss of Rb in bizarre tumor cells; upper right: positive expression of Rb in normal mammary gland (EnVision, original magnification  $\times 200$ ). Molecular features of ASLT by FISH analysis: (E) Co-deletion of RB and FOXO1 (1F1O) in ASLT (FISH, original magnification  $\times 1000$ ). (F) Deletion of RB, but not FOXO1 (2F1O) in ASLT (FISH, original magnification  $\times 1000$ ). APLT: Atypical pleomorphic lipomatous tumor; ASLT: Atypical spindle cell lipomatous tumor; F: FOXO1 fused signals; FISH: Fluorescence *in situ* hybridization; FOXO1: Forkhead box O1; HE: Hematoxylin-eosin staining; MTMF: Mammary-type myofibroblastomas; O: RB orange signals.

of different sizes (17/24, 70.8%), distributed in varying amounts of collagen fibers to the myxoid matrix.<sup>[3]</sup> Among our cases, classic ASLT (13/21, 61.9%) was the main subtype, followed by low-fat/fat-free ASLT (8/21, 38.1%). The first pattern showed hypercellularity: abundant spindle cells with scattered hyperchromatic nuclear atypia arranged into storiform or staggered bundle-like stroma [Figure 1A]. This pattern had few adipocytes, similar to the reported “fibrosarcoma-like lipoma”. In this pattern, the differentiated adipocytes appeared more mature, and “florete-like multinucleated cells” could be found in four cases. Some cases presented with various forms of univacuolated lipoblasts (41.7%). Mitoses were rare, averaging no more than (1–5)/50 high power field. The second pattern featured an abundant myxoid background (in cases 1, 3, 5, 6, and 7; accounted for 20.8%): conspicuous stellate-like heterogeneous interstitial cells, thin-walled branched vessels, scattered mast cells, few rope-like collagen fibers, and the absence of adipose tissue; this pattern belonged to the fat-free or low-fat subtypes. The third pattern was the APLT subtype (cases 17 and 21): tumor cells showed obvious pleomorphic morphology with large nuclei and concentrated chromatin in the mucinous stroma, similar to multivacuolated adipoblasts [Figure 1B]. The fourth pattern was MTMF (case 15): this breast tumor was not well circumscribed in the mammary gland. Between mature fat cells, there was an irregular distribution of the spindle cell areas with a remarkable increase in cellular pleomorphism, a deeply acidophilic cytoplasm, and bizarre hyperchromatic nuclei.

The results of the immunohistochemical studies are summarized in Supplementary Table 3, <http://links.lww.com/CM9/A719>. Rb protein (clone 4A4, 1:400, Biocare, Concord, CA, USA) was classified as expression loss

(<10%) or intact (>80%) in tumor cells with nuclear staining. In this series, ASLT/APLT was positive for CD34 (20/24, 83.3%), similar to previous reports<sup>[1–5]</sup> [Figure 1C]. Our results showed that 100% (21/21) of Rb expression was lost [Figure 1C insert], consistent with the study by Creyten *et al*<sup>[3,5]</sup> (50%–70%). Focal reactivity was present in p16, MDM2, and CDK4. These antibodies were not specific, and the differential diagnosis of WDLPS still depended on the detection of *MDM2* amplification. All ASLT/APLT samples were entirely negative for desmin, smooth muscle actin, p53, epithelial membrane antigen, CD99, and Bcl-2. In atypical MTMF, the hyperchromatic spindle cells coexpressed desmin, CD34, and estrogen receptor, suggesting the differentiation of myofibroblastic tumors. Moreover, atypical MTMF was negative for cytokeratine, the *MDM2* gene, and the *CDK4* gene, with no nuclear Rb expression [Figure 1D].

This is one of the largest study to evaluate and validate *RB1* and *FOXO1* hybridization in tumors with atypical 13q14 deficiencies. FISH was performed in 23 cases, excluding one case with low DNA quantity. Among them, 14 cases were analyzed by multiple FISH cohybridization with *FOXO1* and *RB1* probes (both probes from Abbott Molecular, Downers Grove, IL, USA). A case was interpreted as *RB1* or *FOXO1* deleted if only one orange (O) signal (*RB1* probe) or fused (F) orange and green signals (*FOXO1* fusion probe) were detected in > 22% (cut off value) of the nuclei evaluated. A normal pattern of signals (2O for *RB1* gene and 2F for the *FOXO1* gene) was detected in control cases and cells of internal controls (endothelial cells and lymphocytes). Detection of the 13q14 locus revealed deletions of *FOXO1* (19/21, 90.5%), and all cases showed deletion of *RB1* (21/21, 100%). The deletion rate of cohybridized *FOXO1* and *RB1* was 85.7% (12/14), and the positive patterns

included one fused signal and one orange signal [Figure 1E], one fused signal and two orange signals (1F2O), and two fused signals and one orange signal (2F1O) [Figure 1F]. Two or more fused signals and two or more orange signals ( $\geq 2F \geq 2O$ ) were determined to be negative. Cohybridization could increase the sensitivity and specificity of multiplex FISH in detecting chromosomal abnormalities and avoid erroneous interpretations of monosomy as monoallelic deletions. Furthermore, the technological innovations in this series were as follows: first, the deficiency of both *RB1* and *FOXO1* in our study suggested that these genes may be molecular drivers in the pathogenesis of these tumors with atypical 13q14 deficiencies, supporting the hypothesis that these tumors represent variations along a spectrum of different morphological manifestations with genetically related lesions. Second, our study is the first cytogenetic analysis to verify the codeletion status of the *RB1* and *FOXO1* genes in APLT (cases 17 and 21) and atypical MTMF (case 15), demonstrating that APLTs and atypical MTMF share obvious overlapping clinical, morphologic, immunohistochemical, and cytogenetic characteristics with ASLTs. Third, case 16 and case 20 had 2F1O signals by FISH, demonstrating only *RB1* deletions but not *FOXO1* deletions, which suggest that *RB1* deletions are more frequent than *FOXO1* deletions. The deletions of *RB1* and *FOXO1* may suggest the development of these tumors but cannot predict the atypical characteristics. Properly identifying the morphological features is critical to obtaining accurate diagnoses. The differential diagnosis of ASLT/APLT might be challenging. On one hand, it is essential to distinguish between SCL and ASLT. Both tumors have the same immunohistochemical phenotype and genetic changes, but atypical features (such as infiltrative growth, different sizes of adipocytes, various forms of vacuolated lipoblasts, and heterogeneity of atypical spindle cells with hyperchromatic nuclei) are the main diagnostic elements for ASLT. On the other hand, these tumors with 13q14 deficiencies are not at risk of dedifferentiating or metastasizing and should not be overestimated as sarcomas based on the presence of atypical cells. According to the control cases and differential diagnosis, ALT/WDLs showed *MDM2* amplifications and *FUS* rearrangement was observed in low-grade fibromyxoid sarcomas. Evaluations for *DDIT3* may be useful in defining diagnosis of myxoid liposarcoma. Cases of APLT with consistent loss of *RB1* and a lack of significant atypia, pathological mitosis, and tumor necrosis are very different from pleomorphic liposarcomas. *RB1* and/or *FOXO1* deletion by FISH could offer molecular genetic support for the diagnosis of the ASLT/APLT family, especially in the presence of morphology with remarkable myxoid changes and pleomorphic characteristics.

In this study, no recurrence or metastasis was observed in 24 patients, and the name “spindle cell liposarcoma” used in the literature is inappropriate based on the favorable biological behavior. Long-term follow-up evidence showed that ASLT could be treated with local surgical excision to obtain a negative margin. The local recurrence rate was approximately 10% to 15% in a previous study, and the tumor continued to grow partly due to incomplete resection. In our study, there was no difference between the

fat-free subtypes and classic subtypes in terms of clinical or biological behavior or prognosis, and this deserves further long-term follow-up.

In summary, this is the study to evaluate and validate cohybridization of *FOXO1* and *RB1* in tumors with atypical 13q14 deficiencies and one of the largest ASLT/APLT case series in Asia. The presence of adipocytic tumors with atypical spindle cell and/or pleomorphic cell morphology, CD34 expression, and loss of nuclear Rb expression by immunohistochemical study correlated with *FOXO1* or *RB1* deletion by FISH, and these diagnostic clues were highly indicative of ASLT/APLT. Although these tumors have a close cytogenetic relationship with classical SCL, extensive sampling and close observation of spindle cells with hyperchromatic nuclei might avoid misdiagnosis. ASLT/APLT and atypical MTMF are rare neoplasms with *RB1* and/or *FOXO1* deletions and are independent from ALT/WDLPS with *MDM2* amplifications. This newly defined lipomatous tumor family deserves further study.

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### Conflicts of interest

None.

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