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CASE REPORT

Mixed-type liposarcoma: clinicopathological, immunohistochemical, and molecular analysis of a case arising in deep soft tissues of the lower extremity

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Abstract A rare case of mixed-type liposarcoma arising in deep soft tissue of the right thigh of a 45-year-old female patient is reported. The neoplasm was completely excised and was composed of an irregular admixture of areas of atypical lipomatous tumor/well-differentiated liposarcoma of the lipoma-like subtype with areas of myxoid/round cell liposarcoma. An amplification of the *MDM2* and *CDK4* genes respectively in the atypical lipomatous tumor/well-differentiated liposarcoma areas was detected by fluorescence in situ hybridization (FISH) analysis, and translocations of the *CHOP* and *FUS* genes were detected by FISH analysis in the myxoid/round cell liposarcoma areas.

Keywords Liposarcoma · Mixed-type liposarcoma · MDM2 · CDK4 · CHOP · FUS · Immunohistochemistry · Cytogenetics

Introduction

Liposarcoma represents the most common soft tissue sarcoma in adults, accounting for approximately 20% of all sarcomas. Liposarcoma is currently subclassified into four main subtypes, including *atypical lipomatous tumor/well-differentiated liposarcoma* (lipoma-like, sclerosing, inflammatory, and spindle-cell variants), *dedifferentiated liposarcoma*, which represents the morphological form of progression of atypical lipomatous tumor/well-differentiated liposarcoma, *myxoid liposarcoma*, which forms a continuous spectrum with round cell liposarcoma, and rare *pleomorphic liposarcoma*. Cytogenetic and molecular genetic studies contributed substantially to this classification, and specific chromosomal abnormalities have been detected for all types of liposarcoma. Atypical lipomatous tumors/well-differentiated liposarcomas are characterized by supernumery ring and/or giant marker chromosomes containing amplified material of the q13–15 regions of chromosome 12, myxoid liposarcomas show a specific reciprocal chromosome translocation t(12;16)(q13.3;p11.2) with fusion of the *CHOP* and *FUS* genes as the primary chromosomal aberration, and pleomorphic liposarcomas often have multiple, complex structural rearrangements without consistent and specific abnormalities. Very rare cases show a combination of morphological types (mixed-type liposaroma), and we present such a case arising in deep soft tissues of an adult patient.

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Materials and methods

The tissue was fixed in 4% buffered formalin, routinely processed and embedded in paraffin; 4-μm-thick sections were stained with hematoxylin and eosin. In addition, representative sections were stained immunohistochemically by the labeled streptavidin biotin technique using commercially available antibodies; antigen retrieval was used for all antibodies. Stainings for CD68 (clone=PG-M1; dilution=

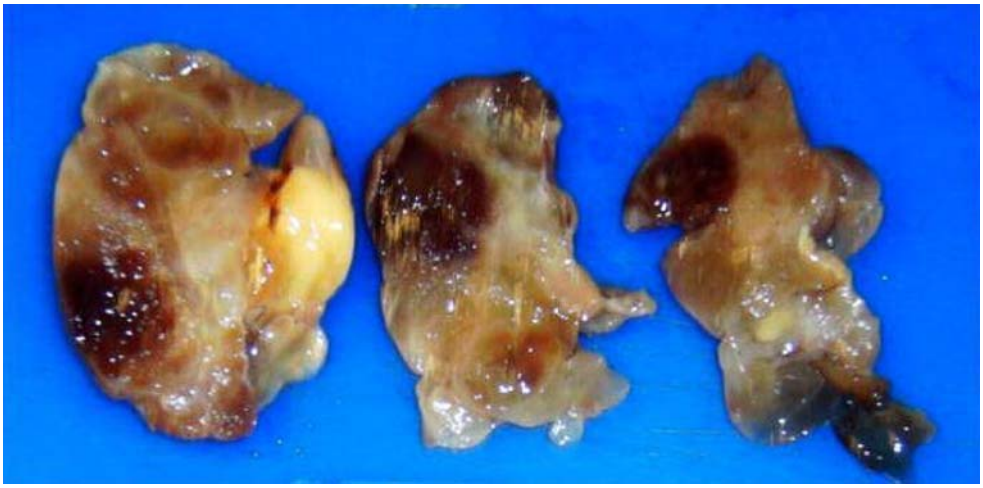

Fig. 1 Grossly, a nodular neoplasm with myxoid, gelatinous as well as yellow, lipomatous areas is seen

1:200; DAKO, Glostrup, Denmark), MDM2 (IF2; 1:200; Invitrogen, Carlsbad, CA, USA), CDK4 (DC9-31; 1:400; Biosource, Nivelles, Belgium), and S-100 protein (polyclonal, 1:4,000; DAKO, Glostrup, Denmark) were available. Appropriate positive and negative controls were used. For the detection of a translocation of the *FUS* and *CHOP* genes, a directly labeled spectrum orange/spectrum green dual color break-apart probe (Abbott, Wiesbaden, Germany) was used for hybridization to a region distal and proximal to the *FUS* and *CHOP* genes, respectively. Amplification of the *CDK4* and *MDM2* genes was detected by hybridization of DIG-labeled bacterial artificial chromosomes (BACs) followed by binding to fluorescein isothiocyanate (FITC) anti-DIG. Fluorescence in situ hybridization was performed on 3-μM sections of formalin-fixed, paraffin-embedded tissue after baking at 65°C for 16 h, deparaffinization with xylene, and dehydration with ethanol. All tissue sections were pretreated with a 30% solution of pretreatment solution and digested with Proteinase K following the instructions of the suppliers (Q-Biogene, Heidelberg, Germany). Digestion times were optimized on a case-by-case basis. After a second dehydration step, the probes were applied to the sections and the covered slides were sealed with rubber cement, heat-denatured, and hybridized at 37°C for 16 h. After stringent washing with 50% formamide in 2× SSC and treating with FITC anti-DIG in the case of indirectly labeled probes, the sections were counterstained with DAPI II in mounting medium (125 ng/ml, Vysis Bergisch Gladbach, Germany) and visualized under a Zeiss Axioplan 2 microscope using a HBO103 lamp and the appropriate filters for the three fluorescence dyes.

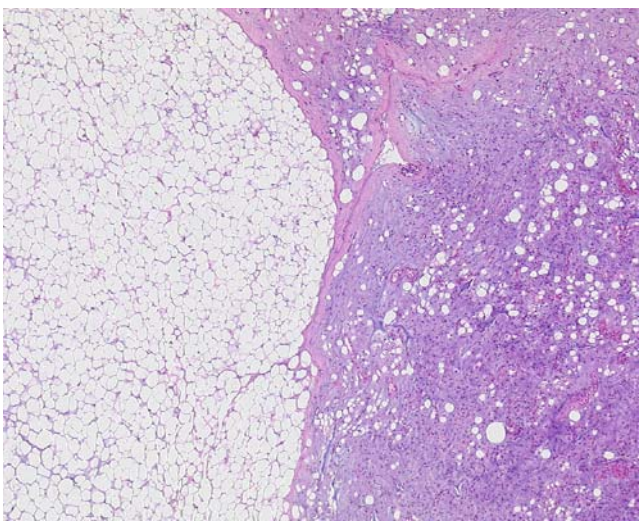

Fig. 2 Low-power view shows lipogenic (*left*) and myxoid tumor areas (*right*)

Results

A 45-year-old female patient complained about an increasing soft tissue swelling in the upper third of the anterior part

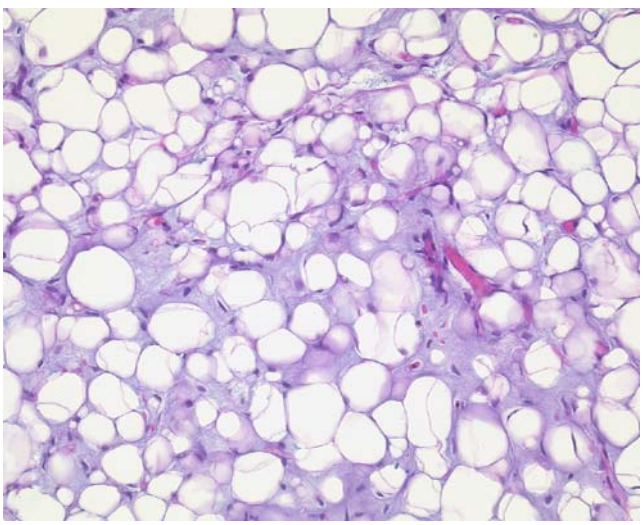

Fig. 3 Lipogenic tumor areas are composed of atypical lipogenic cells showing variations in size and shape. In addition, scattered enlarged tumor cells with enlarged and hyperchromatic nuclei and multivacuolated lipoblasts are seen. The stroma shows focal myxoid changes

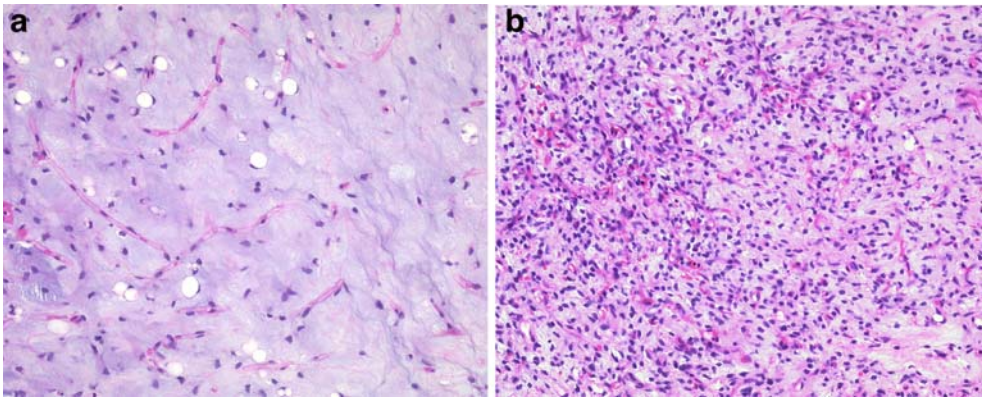

Fig. 4 In myxoid tumor areas, small undifferentiated cells admixed with scattered atypical lipogenic cells and lipoblasts are set in a prominent myxoid stroma with thin-walled and branching blood vessels (**a**). Note focal transition to more cellular tumor areas containing enlarged tumor cells with enlarged, round nuclei (**b**)

of the right thigh within 3 months. Ultrasound investigations showed an intramuscular, lobular, and relatively homogenous lipogenic lesion, and an atypical lipoma was suspected. Intraoperatively, an intramuscular neoplasm measuring 8 cm in largest diameter was found and marginally excised, and after the diagnosis was established a wide tumor excision with tumor-free margins was performed. Grossly, a heterogeneous neoplasm with myxoid, gelatinous areas irregularly associated with lipomatous areas was seen (Fig. 1); no areas of tumor necrosis were evident.

Histologically, two irregularly admixed components were found (Fig. 2). Lipomatous tumor areas did not show lobulation and were composed of mature appearing adipocytic cells showing considerable variations in size and shape. In addition, cells with slightly enlarged and hyperchromatic nuclei as well as scattered lipoblasts in perivascular location were found (Fig. 3). The tumor stroma contained numerous dilated vessels with slightly fibrosed walls and revealed focal myxoid changes. Immunohistochemically, no clear nuclear expression of MDM2 and CDK4, respectively and no increased number of CD68

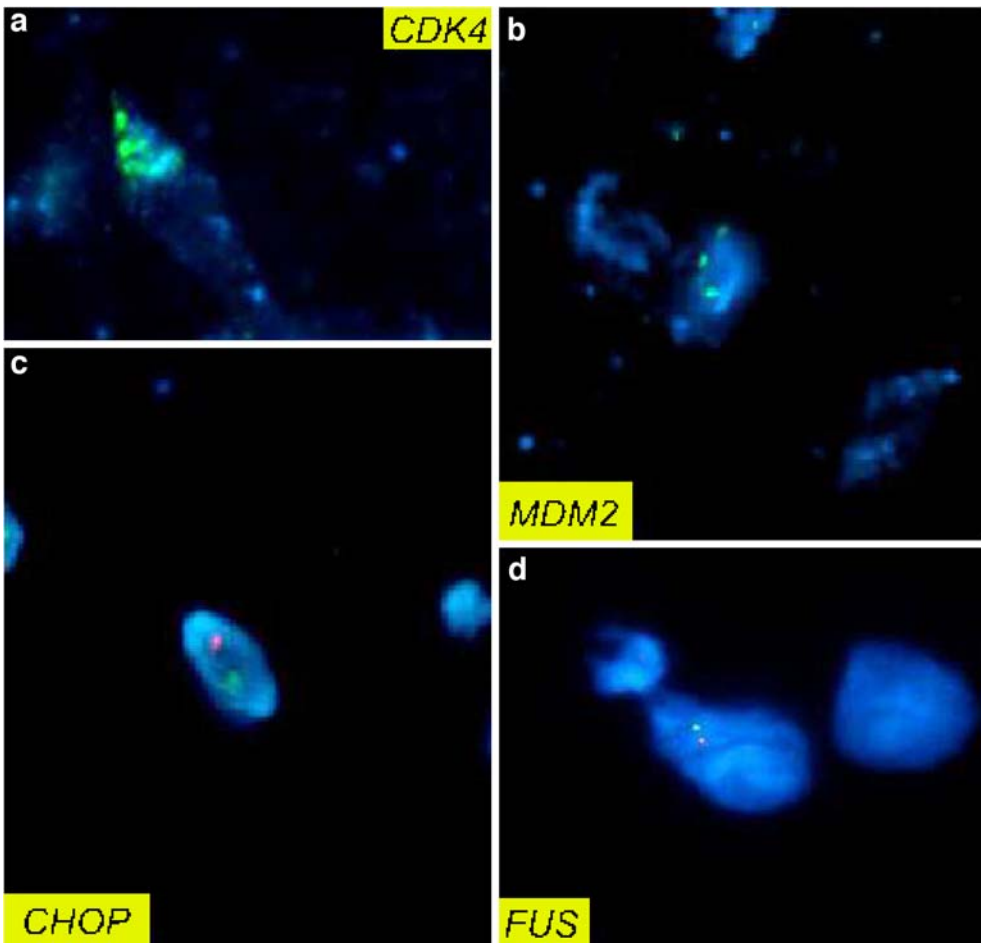

Fig. 5 Amplification of *CDK4* (**a**) and *MDM2* (**b**) detected by hybridization of two FITC-labeled BACs. Translocations of the *CHOP* (**c**) and *FUS* (**d**) genes. Split-apart signals were detected by two break-apart probes

positive histiocytes were present. Molecular analysis by FISH technique showed a *MDM2* amplification in 21 out of 53 analyzed nuclei and a *CDK4* amplification in 14 out of 61 analyzed nuclei (Fig. 5). No *CHOP* and *FUS* gene translocations have been detected in this tumor area by FISH analysis. These findings were consistent with atypical lipomatous tumor/well-differentiated liposarcoma of the lipoma-like subtype.

The myxoid tumor component was composed of small, undifferentiated mesenchymal cells associated with uni- and multivacuolated lipoblasts set in a prominent myxoid stroma with mucin pools and a prominent network of thin-walled and branching capillaries. Focally, more cellular areas containing round to oval tumor cells with enlarged round and hyperchromatic nuclei were noted (Fig. 4). No nuclear expression of MDM2 or CDK4 was detected in this tumor component immunohistochemically. Molecular analysis by FISH technique showed a translocation of the *CHOP* gene in 21 out of 53 analyzed nuclei and a translocation of the *FUS* gene in 23 out of 62 analyzed nuclei (Fig. 5). No *MDM2* and *CDK4* amplifications have been detected by FISH analysis. These findings were consistent with myxoid/round cell liposarcoma, and the final diagnosis of mixed-type liposarcoma, composed of atypical lipomatous tumor/well-differentiated liposarcoma and myxoid/round cell liposarcoma, was made.

Discussion

Mixed-type liposarcoma represents the rarest subtype of liposarcoma with only a few cases reported in the English literature. Mixed-type liposarcoma is defined as a liposarcoma showing either features of combined myxoid/round cell liposarcoma and atypical lipomatous tumor/well-differentiated liposarcoma/dedifferentiated liposarcoma or of myxoid/round cell liposarcoma and pleomorphic liposarcoma. The few reported cases arose predominantly in elderly patients and were seen in retroperitoneal or intra-abdominal locations; more rarely, cases arising in the mediastinum and deep soft tissues of the extremities have been documented [3, 7–9, 12]. The few karyotyped cases showed the presence of ring or giant marker chromosomes as the sole chromosomal abnormality or in association with complex rearrangements [1, 2, 11]. There is only one case of true mixed-type liposarcoma arising in subcutaneous tissue of the thigh in a 29-year-old male patient that metastasized to the supraclavicular region, in which a mixed genotype corresponding to atypical lipomatous tumor/well-differentiated liposarcoma and myxoid liposarcoma has been illustrated in the primary neoplasm as well as in the metastasis [9].

The reported case represents an exceedingly rare example of true mixed-type liposarcoma arising in deep soft tissue of the lower extremity in an adult patient, in which both tumor components were irregularly admixed. The atypical lipomatous tumor/well-differentiated liposarcoma component did not show tumor progression into dedifferentiated liposarcoma, whereas in the myxoid areas, foci of round cell differentiation were found, consistent with progression to grade two of malignancy. The characteristic molecular changes of both tumor components were found and emphasize the presence of polyclonal neoplastic development in rare instances. Interestingly, no nuclear expression of MDM2 and CDK4 was detected immunohistochemically, whereas clear amplification of both genes was seen by FISH technique. This phenomenon most likely reflects a well-differentiated atypical lipomatous tumor with low copy numbers of the amplified genes.

There are a number of cases of so-called mixed-type liposarcoma in the literature, in which no molecular evidence of the two tumor components was given [3–8, 13, 14]. However, at least some of these cases most likely represent examples of atypical lipomatous tumor/well-differentiated liposarcoma or of dedifferentiated liposarcoma with focal prominent myxoid changes, what represents a well-recognized phenomenon especially in long-standing lipogenic neoplasms in retroperitoneal and intraabdominal locations. Very rarely, dedifferentiation has been reported in myxoid liposarcoma, and a close relationship between atypical lipomatous tumor/well-differentiated liposarcoma and myxoid liposarcoma was proposed [10]. Unfortunately, it was impossible to prove true dedifferentiation in myxoid liposarcoma cytogenetically in these three cases, and it can be speculated that these neoplasms represent probably examples of mixed-type liposarcoma showing a combination of myxoid/round cell liposarcoma and dedifferentiated liposarcoma as a form of progressive atypical lipomatous tumor/well-differentiated liposarcoma.

In summary, a rare case of true mixed-type liposarcoma has been reported, emphasizing the broad spectrum of malignant lipogenic neoplasms and the need for careful sampling of large mesenchymal neoplasms.

Conflict of interest statement We declare that we have no conflict of interest

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