Cytogenetic and molecular cytogenetic findings in giant dedifferentiated liposarcoma of the thigh

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Abstract. Non-retroperitoneal dedifferentiated liposarcoma (DDLS) is relatively uncommon and its characterization at the molecular genetic level has been limited. We describe the cytogenetic and molecular cytogenetic findings of giant DDLS arising in the right thigh of an 83-year-old woman. Magnetic resonance imaging revealed a mass composed of two components with heterogeneous signal intensities, suggesting the coexistence of a fatty area and another soft tissue component. A wide resection of the tumor was performed. The resected, grossly heterogeneous mass, measuring 26x18x8 cm, was histopathologically composed of a well-differentiated liposarcomatous component transitioning abruptly into a dedifferentiated one. Cytogenetic analysis exhibited a complex karyotype with several numerical and structural alterations, including ring and giant marker chromosomes. Metaphase-based comparative genomic hybridization analysis showed high-level amplifications of 1q21-q25 and 12q13-q21. Interphase fluorescence *in situ* hybridization analysis revealed *MDM2* and *CDK4* gene amplification in both the well-differentiated and dedifferentiated components. These findings indicate that DDLS of the extremity shares a similar genetic background to retroperitoneal DDLS.

Introduction

Dedifferentiated liposarcoma (DDLS) is a distinct subtype of liposarcoma, showing transition from well-differentiated liposarcoma (WDLS) to non-lipogenic sarcoma of variable histologic grade. It occurs typically in the retroperitoneum of elderly individuals with no gender predilection. The pheno-menon of dedifferentiation in DDLS is essentially considered to be time-dependent. DDLS has a high local recurrence rate but lower metastatic potential than other high-grade sarcomas (1). Due to the histologic complexity of DDLS, many differential diagnoses may be raised on the morphologic aspect alone.

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Cytogenetically, DDLS is characterized by the presence of supernumerary ring and/or giant marker chromosomes (2,3). Fluorescence *in situ* hybridization (FISH) and comparative genomic hybridization (CGH) studies have demonstrated that ring and giant marker chromosomes are composed, exclusively or partly, of amplified 12q13-q15 material, involving *MDM2* and *CDK4* (4-6). We also showed that giant marker chromosomes were composed partly of chromosome 12 material in a human cell line established from a retroperitoneal DDLS (7).

In contrast to retroperitoneal DDLS, cytogenetic and mole-cular biological analyses of non-retroperitoneal DDLS are few (1-3). We describe the cytogenetic and molecular cytogenetic findings of giant DDLS arising in the thigh of an elderly female.

Materials and methods

Case presentation. An 83-year-old Japanese woman was referred to our hospital with a history of a non-painful right thigh mass first noticed 2 months previously. Physical examination revealed an 18x10 cm, firm, non-tender mass in the posteromedial aspect of the right thigh. Magnetic resonance imaging clearly revealed that the mass was composed of two components. One was homo-geneously low signal intensity on T1-weighted sequences with heterogeneously high signal intensity on T2-weighted spectral presaturation with inversion recovery (SPIR) sequences. The other was high signal intensity on T1-weighted sequences with low signal intensity on T2-weighted SPIR sequences, suggesting a lipomatous component (Fig. 1A and B). Contrast-enhanced T1-weighted sequences showed heterogeneous enhancement of the mass (Fig. 1C). Therefore, DDLS or mixed-type lipo-sarcoma was suspected. She underwent an open biopsy and the pathologic diagnosis of WDLS was made. A wide resection of the tumor was performed. Macroscopically, the resected tumor, measuring 26x18x8 cm, was composed of two portions with distinct colors, i.e., yellow and tan-gray. Microscopically, the tumor exhibited biphasic morphology consistent with the macroscopic appearance (Fig. 2A). The yellow component was composed of a proliferation of mature adipocytic cells with hyperchromtic nuclei and multivacuolated lipoblasts, representing WDLS (Fig. 2B). The tan-gray component was composed of a mixture of atypical spindle cells, round cells, and bizarre giant cells, resembling pleomorphic malignant fibrous histiocytoma (MFH) (Fig. 2C). The final pathologic diagnosis was DDLS. At 15 months of follow-up, the patient is alive without any evidence of local recurrence or distant metastasis.
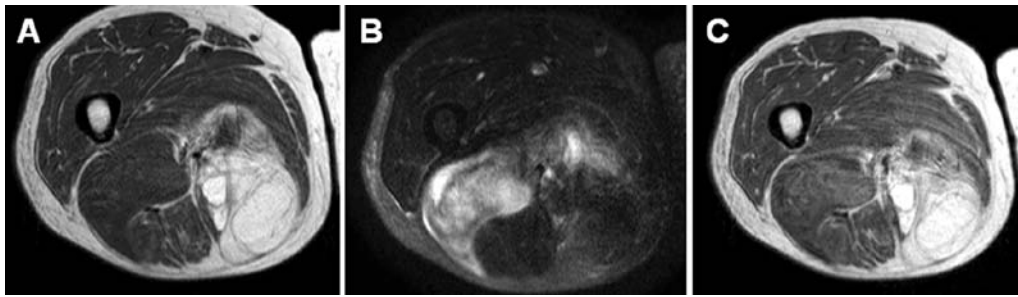
Figure 1. Axial magnetic resonance images of dedifferentiated liposarcoma involving the right thigh. The mass is composed of two components. (A) T1-weighted sequence shows that the medial component has high signal intensity and the lateral component has homogeneously low signal intensity. (B) T2-weighted spectral presaturation with inversion recovery sequence shows that the medial component has low signal intensity and the lateral component has heterogeneously high signal intensity. (C) Contrast-enhanced T1-weighted sequence reveals heterogenous enhancement throughout the mass.

Cytogenetic analysis. Representative fresh tissues were obtained from the surgical resection. Culturing, harvesting, and preparation of slides were performed as described previously (8). Cytogenetic analysis was performed on GTG-banded (Giemsa/ trypsin) metaphases, and karyotypic descriptions were expressed according to the International System for Human Cytogenetic Nomenclature 2009 (9).

Molecular cytogenetic analysis. CGH was performed as described previously (10). Briefly, DNA from the fresh frozen tissue was directly labeled with fluorescein-12-dUTP (Roche Diagnostics, Mannheim, Germany) by nick translation, with the use of a commercial kit (Abbott Molecular, Des Plaines, IL, USA). As a normal reference DNA, we used the Spectrum Red directed-labeled male total human DNA (Abbott Molecular). Subsequently, equal amounts of normal and tumor labeled probes (800 ng) and 20 μg of Cot-1 DNA (Gibco/BRL, Gaithersburg, MD, USA) were coprecipitated with ethanol. The precipitated DNA was dissolved in 10 μl of hybridization buffer and denatured at 75°C for 8 min. Normal metaphase spreads (Abbott Molecular) were denatured for 3 min at 75°C and hybridized with the DNA mixture in a moist chamber for 2 days. Slides were washed according to the protocol supplied by the manufacturer. Chromosomes were counterstained with 4,6-diamino-2-phenylindole (DAPI; Sigma, St. Louis, MO, USA). The location of aberrant CGH signals was analyzed using an image analysis system (Isis, Carl Zeiss Vision, Oberkochen, Germany) based on an integrated high-sensitivity monochrome charge-coupled device camera and automated CGH analysis software (MetaSystems GmbH, Altlussheim, Germany). Three-color images, green (fluorescein-12-dUTP) for the tumor DNA, red (Spectrum Red) for the reference DNA, and blue (DAPI) for the DNA counterstain, were acquired from at least 10 meta-phases. Only metaphases of good quality with strong, uniform hybridization were included in the analysis. Based on the control experiments, 1.2 and 0.8 were used as cutoff levels for gains and losses, respectively. Gains exceeding the 1.5 threshold were termed high-level amplifications. The heterochromatic regions in chromosomes 1, 9, and 16, the p-arms of the acrocentric chromosomes, and Y chromosome were excluded from the analysis because of suppression of hybridization with Cot-1 DNA in these regions.

FISH was performed on paraffin-embedded tissue sections utilizing a commercially available Poseidon™ Repeat-Free™
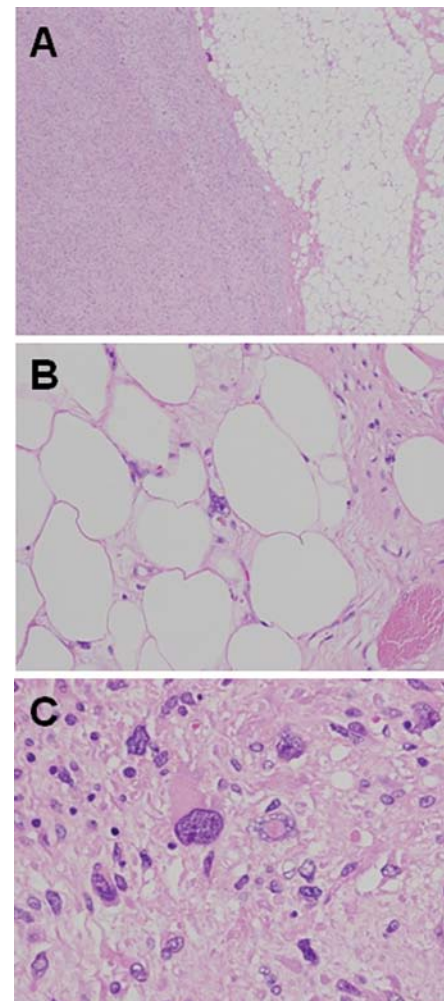

Figure 2. Microscopic findings of dedifferentiated liposarcoma. (A) Abrupt transition between well-differentiated liposarcoma and non-lipogenic area is seen. (B) The yellow component is composed of a proliferation of mature adipocytic cells with hyperchromtic nuclei, representing well-differentiated liposarcoma. (C) The tan-gray component is composed of a mixture of atypical spindle cells, round cells, and bizarre giant cells, resembling pleomorphic malignant fibrous histiocytoma.

MDM2 (12q15) or *CDK4* (12q13)/SE12 control probe (Kreatech Diagnostics, Amsterdam, The Netherlands). Briefly, 4-μm-thick paraffin-embedded tissue sections were deparaf-finized, dehydrated, and incubated with pepsin according to
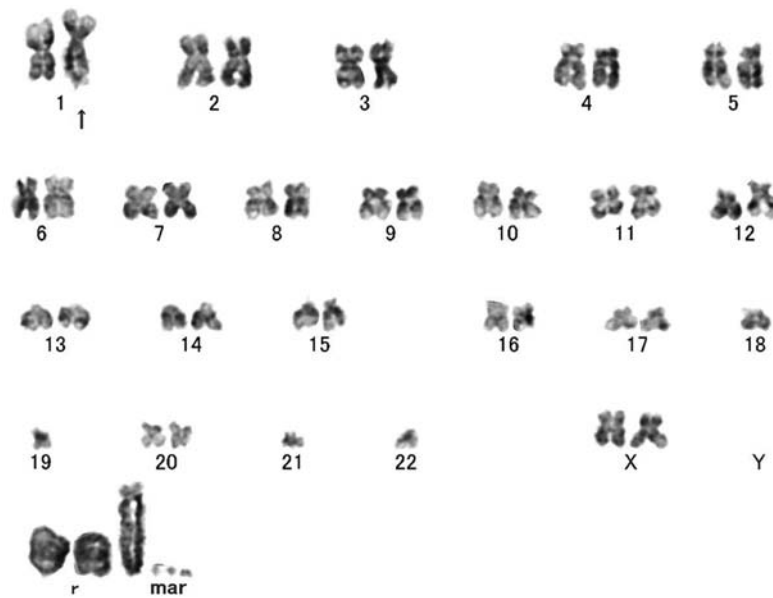
Figure 3. A representative GTG-banded karyotype of dedifferentiated liposarcoma, including ring and giant marker chromosomes. An arrow indicates the structural chromosome aberration.
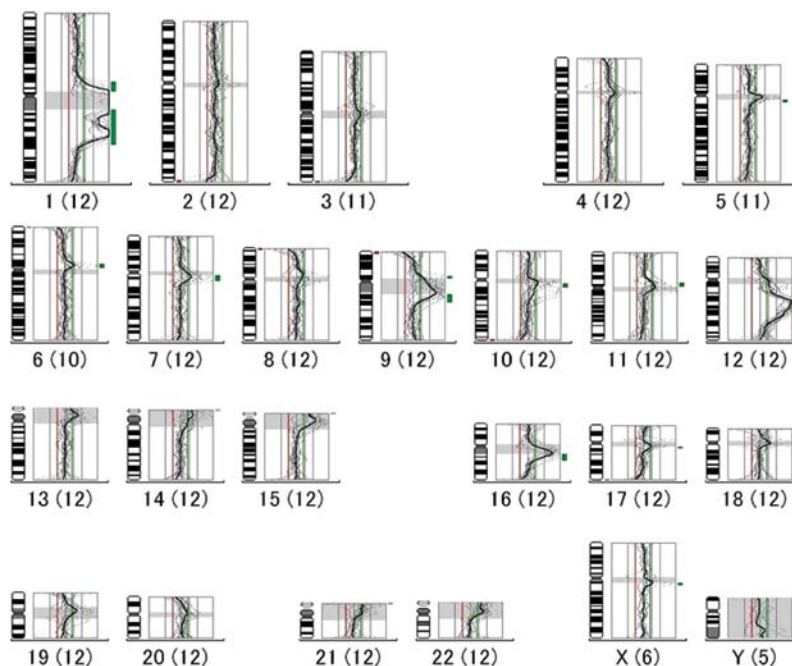

Figure 4. Comparative genomic hybridization profile of dedifferentiated liposarcoma showing high-level amplifications of 1q21-q25 and 12q13-q21. The line in the middle (gray) is the baseline ratio (1.0); the left (red) and right (green) lines indicate ratio values of 0.8 and 1.2, respectively. Bars on the left (red) and right (green) of each frame indicate losses and gains, respectively. The terminology 1 (12) represents 12 aberrations detected on chromosome 1. The same applies to other chromosomes shown in the profile.

the manufacturer's instructions. The probe and slides were co-denatured at 80°C for 5 min and incubated at 37°C overnight in a humidified chamber. Post-hybridization washing was performed following standard procedures. The slides were counterstained with DAPI. A minimum of 100 interphase nuclei with strong, well-delineated signals and distinct nuclear borders were scrutinized. An *MDM2* or *CDK4*/SE12 ratio of ≥2.0 was considered amplified for the *MDM2* or *CDK4* gene, whereas an *MDM2* or *CDK4*/SE12 ratio of <2.0 was considered non-amplified.

Results

Cytogenetic analysis revealed a complex karyotype with several numerical and structural alterations, including ring and giant marker chromosomes (Fig. 3). The composite karyotype was as follows: 44-48,XX,dic(1;?)(q32;?),-13,-18,-19,-21,-22,+1-2r,+1-4mar[cp11].

Metaphase-based CGH analysis showed high-level amplifications of 1q21-q25 and 12q13-q21. Significant loss of DNA sequences was not found. This CGH profile is shown in Fig. 4.
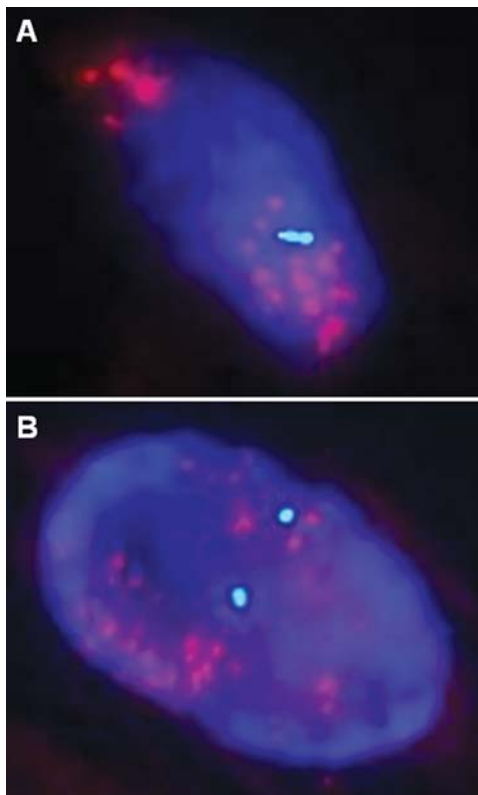
Figure 5. Fluorescence *in situ* hybridization with *CDK4* (red signal)/SE12 (green signal) on paraffin-embedded sections from the well-differentiated liposarcomatous component (A) and the dedifferentiated component (B). High-level amplification of *CDK4* is observed in the cell nuclei.

Interphase FISH analysis showed high-level amplification (>10 copies per cell) of the *MDM2* and *CDK4* genes in both the well-differentiated and dedifferentiated components (Fig. 5).

Discussion

Dedifferentiation occurs most frequently in retroperitoneal liposarcomas (11) and can manifest a variety of histologic patterns, resembling pleomorphic MFH, high-grade myxofibrosarcoma, or adult-type fibrosarcoma. The concept of low-grade dedifferentiation has increasingly been recognized (11). A peculiar neural-like or meningothelial-like whirling pattern has also been reported (12-14). This pattern is often associated with metaplastic bone formation. Interestingly, several authors suggested that this unusual type of DDLS shares many of the cytogenetic and molecular genetic features with more typical DDLS (14,15).

Similar to WDLS, DDLS is characterized by the presence of supernumerary ring and/or giant marker chromosomes containing amplified genomic sequences in the 12q13-q15 region. This amplification includes approximately 150 genes, including several of major potential relevance such as *MDM2*, *CDK4*, *HMGA2*, and *TSPAN31* (formerly known as *SAS*) (16-18). *MDM2* is essential for ubiquitnation and degradation of the tumor suppressor p53. *MDM2* amplification is, therefore, thought to result in reduced levels of p53. *CDK4* encodes a 33-kD protein that is a key factor in the regulation of the G1-S translation of the cell cycle. Accumulation of the *CDK4*-CCDN1 complex leads to phosphorylation of the RB1

protein. It has been proposed that FISH analysis for *MDM2* and *CDK4* amplification is a valuable adjunct in the diagnosis of DDLS (19), especially when the transition from WDLS to DDLS cannot be observed by histology. The cytogenetic and molecular cytogenetic findings of the present case showed the characteristic features of DDLS.

In addition to the 12q13-q15 amplification, high-level amplifications of 1p32 and 6q23 have been identified in DDLS (5,6,17). It is of great interest that co-amplifications of 1p32 and 6q23 have never been observed in WDLS. *JUN* and *ASK1* are up-regulated through amplifications in 1p32 and 6q23, respectively (20,21). *JUN* encodes part of the activator protein transcription factor (AP-1) complex involved in cell proliferation, transformation, and apoptosis and inhibits peroxisome proliferator-activated receptor (PPAR)-γ through C/EBP beta (20,22). *ASK1* encodes a MAP3 kinase involved in the Jun N-terminal kinase (JNK) signaling pathway (21). *ASK1* amplification activates JNK ultimately leading to JUN activation and PPAR-γ inactivation. It has been shown that PPAR-γ plays a key role in adipocytic differentiation (23). Therefore, amplification of *JUN* and *ASK1* may directly block adipocytic differentiation in DDLS. On the other hand, we were not able to detect any gain or amplification in 1p32 and 6q23. Most recently, Tap *et al* (6) reported that 6q amplification occurred primarily in retroperitoneal DDLS. The clinical and biological significance of this observation needs to be evaluated in a large series of DDLS.

High-level amplification of 1q21-q25 was also found in the present case. Extra copies of sequences from this region have been described to occur frequently in soft tissue sarcomas, including DDLS (5,24-26). Nilsson *et al* (27) reported that the *COAS1-3* genes, located at 1q21, were found to be amplified at different levels in adipocytic tumors. The most common localization of extra *COAS* signals in adipocytic tumors was in ring and giant marker chromosomes. Kresse *et al* (28) suggested that *ATF6* and *DUSP12* may be the most likely candidate target genes for the 1q23 amplification in liposarcomas. Of note, Forus *et al* (4) suggested that 1q amplification may be indicative of a more malignant phenotype and ability of metastasis in WDLS.

More recently, Hélias-Rodzewicz *et al* (29) reported amplification of either 3p12 or 11q22 chromosomal bands in a subset of DDLS. *VGLL3* and *YAP1* are up-regulated through amplifications in 3p12 and 11q22, respectively. These two genes encode proteins that are cofactors of the TEAD family of transcription factors (30,31). However, the exact role of these amplifications in the pathogenesis and progression of DDLS remains to be elucidated.

In summary, we describe the cytogenetic and molecular genetic findings of giant DDLS arising in the thigh of an elderly female. DDLS of the extremity appears to have cytogenetic findings and molecular genetic alterations similar to those detected in retroperitoneal DDLS.

Acknowledgements

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