Immunohistochemical, Cytogenetic, and Molecular Cytogenetic Characterization of Both Components of a Dedifferentiated Liposarcoma: Implications for Histogenesis

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Abstract. *Dedifferentiated liposarcoma (DDLS) is a malignant adipocytic tumor showing transition from an atypical lipomatous tumor (ALT)/well-differentiated liposarcoma (WDLS) to a non-lipogenic sarcoma of variable histological grades. We present the immunohistochemical, cytogenetic, and molecular cytogenetic findings of DDLS arising in the right chest wall of a 76-year-old man. Magnetic resonance imaging exhibited a large mass composed of two components with heterogeneous signal intensities, suggesting the coexistence of a fatty area and another soft tissue component. The grossly heterogeneous mass was histologically composed of an ALT/WDLS component transitioning abruptly into a dedifferentiated component. Immunohistochemistry was positive for murine double-minute 2 (MDM2), cyclin-dependent kinase 4 (CDK4), and p16 in both components, although a more strong and diffuse staining was found in the dedifferentiated area. The MIB-1 labeling index was extremely higher in the dedifferentiated area compared to the ALT/WDLS area. Cytogenetic analysis of the ALT/WDLS component revealed the following karyotype: 46,X,-Y,+r. Notably, cytogenetic analysis of the dedifferentiated component revealed a similar but more complex karyotype. Spectral karyotyping demonstrated that the ring chromosome was entirely composed of material from chromosome 12. Interphase fluorescence in situ hybridization analysis revealed amplification of MDM2 and CDK4 in both components. These findings suggest that multiple abnormal clones derived from a single precursor cell would be present in DDLS, with one or more containing supernumerary rings or giant marker chromosomes.*

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Dedifferentiated liposarcoma (DDLS) is a distinct subtype of liposarcoma showing transition from an atypical lipomatous tumor (ALT)/well-differentiated liposarcoma (WDLS) to a non-lipogenic sarcoma. Dedifferentiation occurs in up to 10% of ALT/WDLS (1). However, the underlying mechanism of this process is still unknown.

Similar to ALT/WDLS, DDLS usually has rings or giant marker chromosomes (2). Fluorescence *in situ* hybridization (FISH) and comparative genomic hybridization studies have revealed that these abnormal chromosomes in ALT/WDLS and DDLS are composed, exclusively or partly, of amplified 12q13-15 material, including the murine double-minute 2 (*MDM2*) and cyclin-dependent kinase 4 (*CDK4*) genes (3-5). Amplification of other chromosomal regions, such as 1p32 and 6q23, has also been identified in DDLS (6, 7).

Herein, we describe the imaging, histological, immunohistochemical, cytogenetic, and molecular cytogenetic findings of DDLS arising in the chest wall of an elderly man. In the current study, cytogenetic and molecular cytogenetic analyses were carried-out on both components of DDLS.

Case Report

A 76-year-old man was referred to our Hospital with a 6-month history of a painless mass exhibiting recent increase in size in the right chest wall. Physical examination revealed a 14-cm, elastic-soft, mobile, non-tender mass. Neurovascular examinations were unremarkable. Magnetic resonance imaging clearly revealed that the mass was composed of two components. One had a signal intensity similar to that of subcutaneous fat on both T1- and T2-weighted sequences. The other had iso signal intensity on T1-weighted sequences and slightly high signal intensity on T2-weighted sequences compared to skeletal muscle (Figure 1A and B). Contrast-enhanced fat-suppressed T1-weighted sequences showed significant enhancement of the dedifferentiated component (Figure 1C).

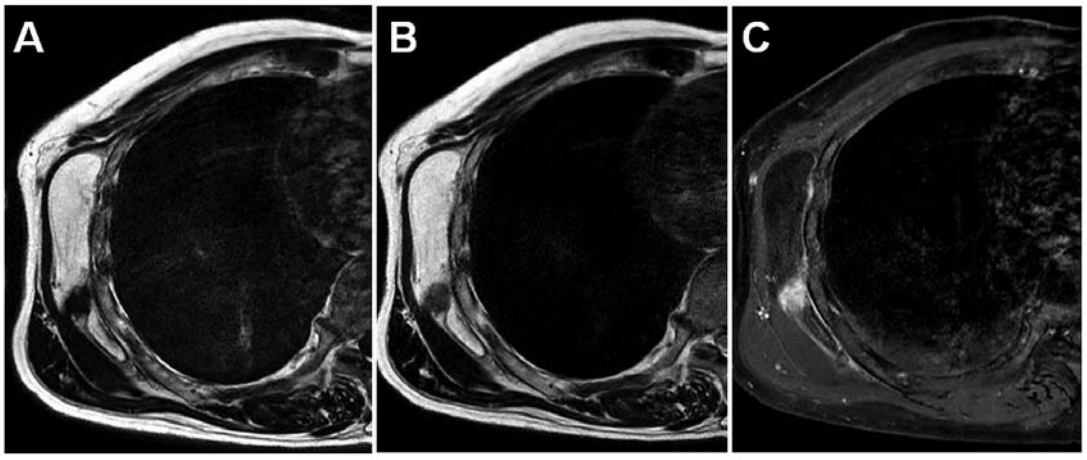

Figure 1. *Axial magnetic resonance images of dedifferentiated liposarcoma involving the right chest wall. The mass was composed of two components. T1-Weighted sequence (A) showed that an atypical lipomatous tumor (ALT)/well-differentiated liposarcoma (WDLS) component had high signal intensity and a dedifferentiated component had low signal intensity. T2-Weighted sequence (B) revealed that the ALT/WDLS component had high signal intensity and the dedifferentiated component had slightly high signal intensity relative to skeletal muscle. Contrast-enhanced fat-suppressed T1-weighted sequence (C) demonstrated significant enhancement of the dedifferentiated component.*

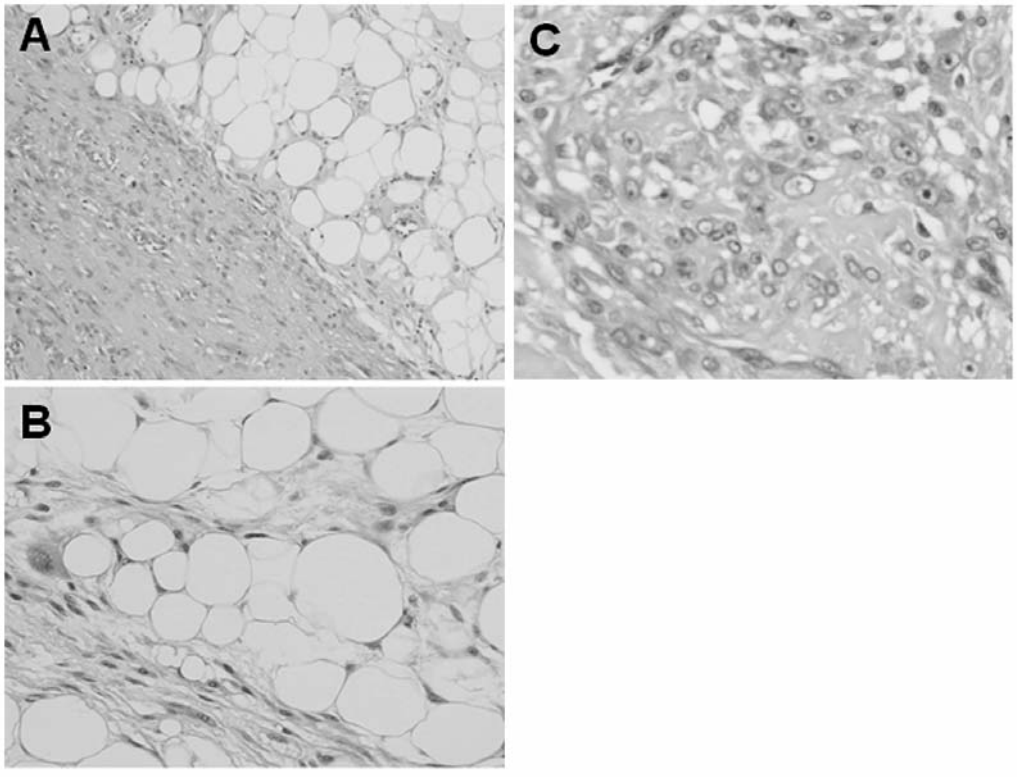

Figure 2. *Histological findings of dedifferentiated liposarcoma. A: Abrupt transition between atypical lipomatous tumor (ALT)/well-differentiated liposarcoma (WDLS) and non-lipogenic area can be seen (hematoxylin and eosin staining, original magnification ×40). B: The ALT/WDLS component was composed of relatively mature adipocytic cells and some hyperchromatic stromal cells (hematoxylin and eosin staining, original magnification ×80). C: The dedifferentiated component was composed of atypical stromal cells including mononuclear cells in a collagenous matrix (hematoxylin and eosin staining, original magnification ×120).*

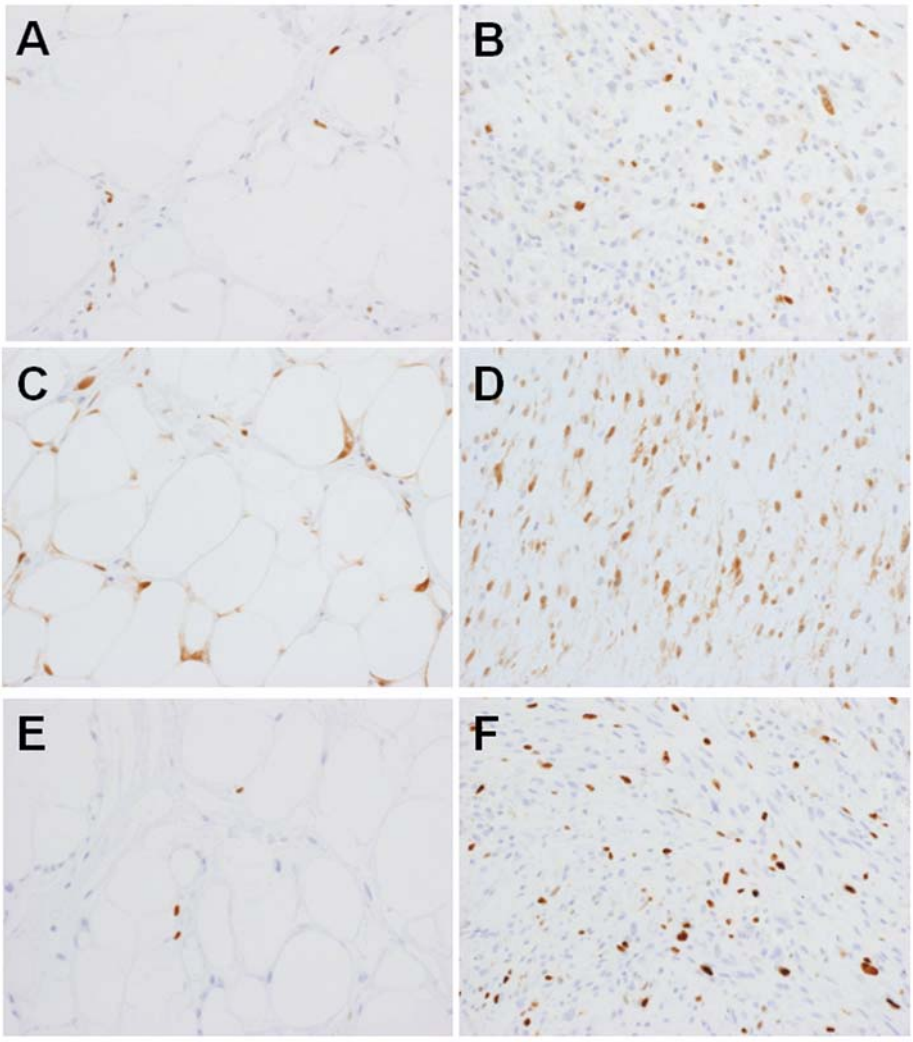

Figure 3. *Immunohistochemical findings of dedifferentiated liposarcoma. The dedifferentiated area (B) shows diffuse nuclear positivity for murine double-minute 2, in contrast to the atypical lipomatous tumor (ALT)/well-differentiated liposarcoma (WDLS) area (A). Nuclear expression of cyclin-dependent kinase 4 is diffusely visible in the dedifferentiated area (D), whereas focal expression can be seen in the ALT/WDLS area (C). The MIB-1 labeling index is much higher in the dedifferentiated area (F) compared to the ALT/WDLS area (E). (A-F, original magnification ×80).*

A sub-extensive resection was performed. Macroscopically, the resected tumor, measuring 14×10×3 cm, was composed of two portions with distinct colors, yellow and tan-gray. Microscopically, the tumor exhibited biphasic morphology consistent with the macroscopic appearance (Figure 2A). The yellow component was composed of relatively mature adipocytic cells with some atypical spindle cells and bizarre giant cells with large hyperchromatic nuclei (Figure 2B). Hyperchromatic stromal cells were also found within the fibrous septa. On the other hand, the tan-gray component was composed of atypical stromal cells including mononuclear and multi-nuclear giant cells (Figure 2C). Abundant collagen was found in the stroma of the dedifferentiated area. By immuno-histochemistry, the ALT/WDLS area displayed focal immunoreactivity for MDM2, CDK4, and p16, whereas the DDLS area exhibited stronger and more diffuse staining (Figure 3A-D). The MIB-1 labeling index was approximately 1-3% in the ALT/WDLS area (Figure 3E), whereas it was 31% in the dedifferentiated area (Figure 3F). Based on these features, the tumor was diagnosed as a DDLS.

Cytogenetic analysis of the ALT/WDLS component revealed the following karyotype: 46,X,-Y,+r (Figure 4). Notably, cytogenetic analysis of the dedifferentiated component revealed a similar but more complex karyotype: 44-46,X,-Y,del(5)(q?),add(9)(p11),add(11)(p11.2),der(11;17)(q10;q10),tas(19;20)(p13;p13),-21,der(21;22) (q10;q10), +r,+mar[cp9] (Figure 5). Spectral karyotyping (SKY)

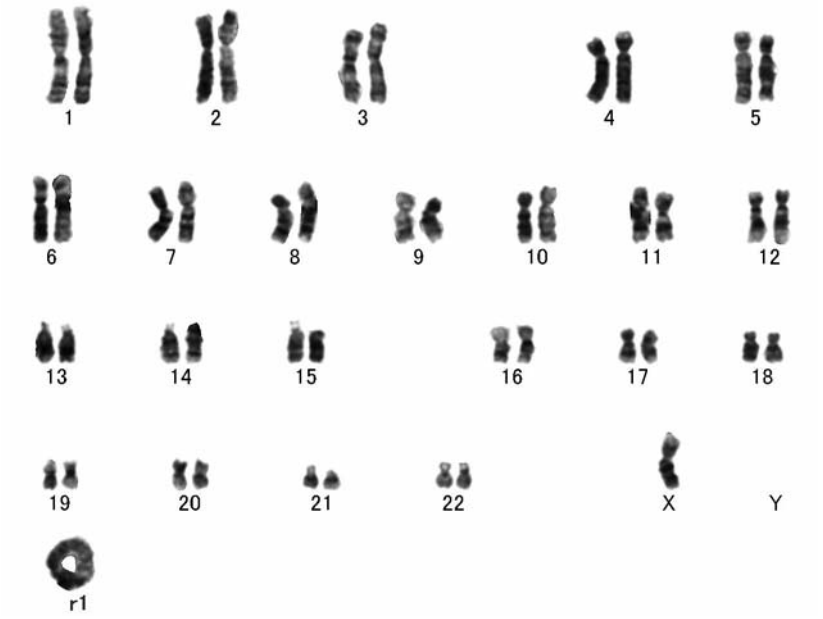

Figure 4. *A representative GTG-banded karyotype of the atypical lipomatous tumor/well-differentiated liposarcoma component displaying a supernumerary ring chromosome (r1).*

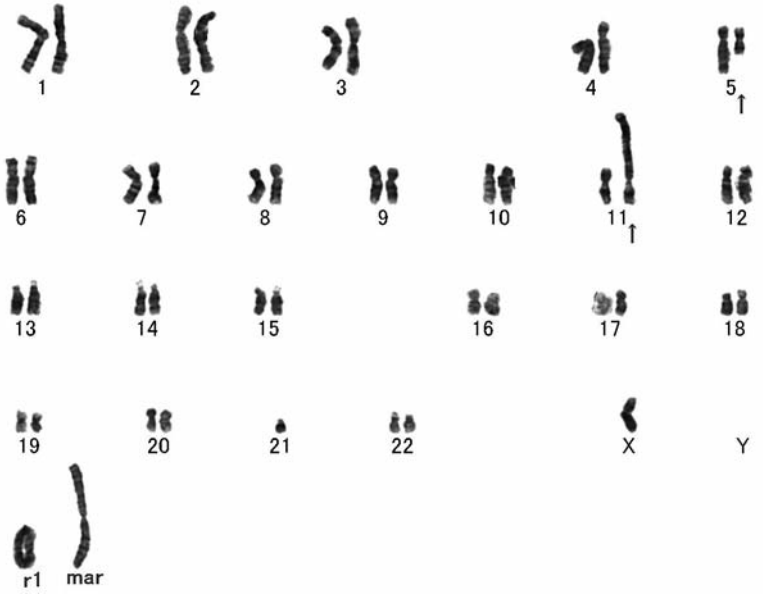

Figure 5. *A representative GTG-banded karyotype of the dedifferentiated component, including ring (r1) and giant marker (mar) chromosomes. Arrows indicate the structural chromosome aberration.*

demonstrated that the ring chromosome was entirely composed of material from chromosome 12 (Figure 6). Interphase FISH analysis showed amplification of *MDM2* and *CDK4* in both components (Figure 7).

Post-operative hematoma occurred and gradually resolved with conservative treatment. At four months' follow-up, the patient was asymptomatic and there was no evidence of local recurrence or distant metastasis.

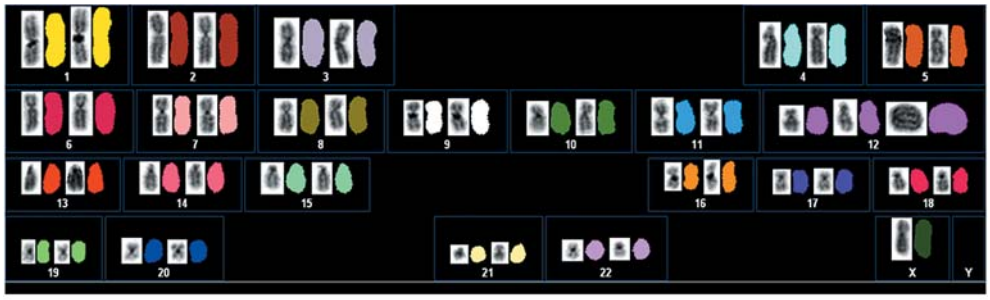

Figure 6. *Spectral karyotyping of the atypical lipomatous tumor/well-differentiated liposarcoma component illustrating the origin of the ring chromosome. Classified image is displayed alongside the reverse 4',6-diamidino-2-phenylindole image.*

Discussion

Comparative conventional cytogenetic studies of both components of DDLS are very few. Most karyotypes are probably derived from the dedifferentiated component (5). In the present study, we compared the cytogenetic and molecular genetic features of both components of DDLS using conventional karyotyping, SKY, and FISH. The current case clearly shows that the tumor cells in both components appear to originate from a single precursor, but there were a substantial number of cytogenetic alterations in the dedifferentiated component.

12q13-15 amplification is thought to play an important role in the initiation of ALT/WLDS (8). Using SKY, we identified that the ring chromosome was exclusively composed of material from chromosome 12, which contains the *MDM2* and *CDK4* genes. Moreover, in agreement with the report of Horvai *et al*., we observed MDM2 and CDK4 protein overexpression and gene amplification of *MDM2* and *CDK4* in both components (6). *MDM2* is an oncogene that promotes degradation of p53 tumor-suppressor protein. *CDK4* is also an oncogene whose product inhibits retinoblastoma 1 (RB1) tumor-suppressor protein. To date, there exist few studies investigating the prognostic significance of these molecular abnormalities in DDLS (9-11). These studies indicated that *MDM2* amplification level is not a significant prognostic factor, whereas *CDK4* amplification level appears to be a promising prognostic biomarker. Recently, Dickson *et al*. reported that treatment with a selective CDK4 inhibitor was associated with a favorable progression-free rate in patients with advanced CDK4-amplified DDLS (12).

The mechanism of dedifferentiation is controversial in DDLS. Segura-Sánchez *et al*. reported that chromosome 12 copy number alterations and co-expression of MDM2, CDK4, and p53 were related to dedifferentiation (13). Takahira *et al*. suggested that loss of heterozygosity of the *RB1* gene located at 13q14 might play a role in dedifferentiation (14). He *et al*. also suggested that aberrant promoter methylation of the *p16* gene located at 9p21 might contribute to dedifferentiation (15). Crago *et al*. demonstrated that 11q23-24 loss was associated with genomic complexity and distinct morphology (8). Several

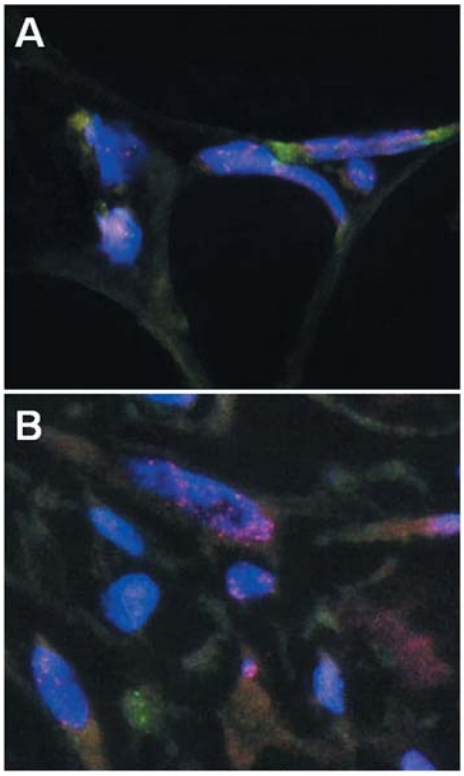

Figure 7. *Fluorescence in situ hybridization with cyclin-dependent kinase 4 (CDK4) (red signal) and chromosome 12 satellite enumeration (green signal) probes on paraffin-embedded sections from the atypical lipomatous tumor/well-differentiated liposarcoma component (A) and the dedifferentiated component (B). CDK4 amplification is observed in both components.*

potential tumor-suppressor genes such as etoposide-induced 2.4 (*EI24*) are located at this chromosome band. Hisaoka *et al*. reported that aberrant expression of an anti-adipogenic factor, calreticulin, was involved in dedifferentiation (16). Recently, several reports suggested an association between dedifferentiation and jun proto-oncogene (*JUN*) amplification and overexpression (17-19). The JUN gene is located at 1p32 and its overexpression can interfere with adipocytic

differentiation. In the current case, we identified the presence of supernumerary ring chromosome containing amplified material from chromosome 12 in both components. This provides strong evidence for a monoclonal origin. However, we also found many different cytogenetic alterations, which occurred after the diversion of the two components. The dedifferentiated component showed structural and/or numerical aberrations in chromosomes 5, 9, 11, 17, 19, 20, 21, and 22. Taken together, these observations suggest that increasingly complex chromosomal abnormalities may develop during tumor progression of DDLS. Among various abnormalities, cell-cycle regulatory molecules p53, p16, and RB1 may also be important in the development of DDLS.

In summary, we described the immunohistochemical, cytogenetic, and molecular cytogenetic findings of DDLS arising in the chest wall of an elderly man. The current case indicates that multiple abnormal clones derived from a single precursor cell would be present in DDLS, with one or more containing supernumerary rings or giant marker chromosomes.

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