# Similarity in genetic alterations between paired well-differentiated and dedifferentiated components of dedifferentiated liposarcoma

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Liposarcoma represents a unique model insofar as some well-differentiated liposarcomas progress to non-lipogenic, so-called 'dedifferentiated,' forms. The well-differentiated and dedifferentiated family of liposarcomas demonstrates amplification of the chromosome subregion 12q13–q15 with resultant amplification of the *MDM2* and *CDK4* genes. However, the specific genetic changes that distinguish between well-differentiated and dedifferentiated liposarcomas are less well understood. To study the genetic changes in dedifferentiated liposarcomas, paired well-differentiated and dedifferentiated components of 29 tumors were analyzed separately by array-based comparative genomic hybridization. A bacterial artificial chromosome array at ∼1-Mb resolution was used. The genetic changes were compared with clinical presentation, grade of the dedifferentiated component and overexpression of *MDM2* and *CDK4*. Most tumors ($n = 21$, 72%) were retroperitoneal, with both components present at initial diagnosis ($n = 25$, 86%). Eight tumors (28%) were classified as low-grade dedifferentiation. In four cases (14%), a well-differentiated liposarcoma preceded the presentation of the dedifferentiated tumor by 1–5 years. 12q13–q15 was amplified in all tumors. Using unsupervised hierarchical clustering of copy-number changes, all but two tumors showed close similarities between well-differentiated and dedifferentiated components, and segregated as pairs. Dedifferentiated components had more total amplifications ($P = 0.008$) and a trend for gain at 19q13.2, but no genetic changes were significant in distinguishing between the two components. High-level amplifications of 1p21–32 ($n = 7$, 24%), 1q21–23 ($n = 9$, 31%), 6q23–24 ($n = 6$, 21%) and 12q24 ($n = 3$, 10%) were common, but none significantly correlated with differentiation. Presentation and grade correlated with the frequency of changes at a number of genetic loci ($P < 0.001$), whereas CDK4 immunostaining showed negative correlation with 12q13.13 amplification. The genotypic similarity, at the limit of the array's resolution, between components implies that most genetic changes precede phenotypic 'progression,' early in tumorigenesis. The relationship between genetic changes and presentation or grade may reflect differences in factors that control genomic instability or the background genotype of the tumor.
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Liposarcoma is one of the most common soft-tissue sarcomas in adults. The most common subtype consists of the well-differentiated–dedifferentiated family of liposarcomas. Well-differentiated liposarcoma is a tumor with a significant recurrence risk and mortality, but the most important risk factor for developing metastasis is the presence of 'dedifferentiation,' a relatively rare event.[1,2] Histologically, dedifferentiated liposarcoma consists of a biphasic tumor with both lipogenic ('well-differentiated') and non-lipogenic ('dedifferentiated') components.[1,2]

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In most instances, the latter component consists of a high-grade sarcoma, such as the so-called 'malignant fibrous histiocytoma.' However, in rare cases, the dedifferentiated area takes the form of a low-grade tumor resembling, for example, desmoid-type fibromatosis.[3] Clinically, the dedifferentiated component can present either synchronously with the well-differentiated liposarcoma or metachronously many years after the initial diagnosis of a well-differentiated liposarcoma.[3–5] Consequently, the dedifferentiated phenotype may represent the progression of a well-differentiated liposarcoma through stepwise accumulation of genetic changes.

The gene copy-number changes in most sarcomas preferentially involve additions rather than deletions, implying amplification of proto-oncogenes rather than loss of tumor-suppressor genes. Cytogenetic studies of liposarcomas have shown characteristic chromosomal rearrangements. More specifically, these tumors frequently show giant marker chromosomes and supernumerary ring chromosomes containing sequences derived from 12q, and less frequently from 1q.[6–9] Amplifications of genes *MDM2*, *HMGA2*, *SAS* and *CDK4*, located in the chromosomal subregion 12q13–q15 have been implicated in liposarcoma tumorigenesis.[10–14] The changes in 12q, especially the amplification of *MDM2* and *CDK4*, have been diagnostically useful in discriminating well-differentiated liposarcomas from benign lipomas and dedifferentiated liposarcomas from other high-grade sarcomas, respectively.[13,15,16] In fact, improved understanding of liposarcoma genetics has supported the reclassification of some tumors from undifferentiated categories to dedifferentiated liposarcoma.[17,18]

However, the specific genetic changes that distinguish between well-differentiated liposarcoma and dedifferentiated liposarcoma are poorly understood. As a group, dedifferentiated liposarcomas show more complex chromosomal aberrations than do well-differentiated liposarcomas.[19] Intriguingly, cDNA microarray analysis shows that the well-differentiated component of dedifferentiated liposarcoma clusters distinctly from purely well-differentiated liposarcoma. The above finding suggests that only a subset of well-differentiated liposarcomas have the capacity for progression.[20] Comparative genomic hybridization (CGH) confirms the presence of 12q13–q15 amplification in dedifferentiated liposarcoma, but in an overall more complex genetic profile, than in well-differentiated liposarcoma. Additional common amplifications include 1q23, 12q24 and either 6q23 or 1p32.[13] Analysis of the 6q23 amplicon shows *MAP3K5* as a candidate gene that, when amplified, may inhibit lipogenic differentiation through *JUN*- and *PPAR-γ*-dependent pathways.[21] Amplification of 1p32 may increase the amount of *JUN* itself. Recently, it has also been shown that overexpression of some AP-1 transcription factors may block adipogenesis *in vitro* and *in vivo*.[22,23] Taken together, these results support

a role of MAP kinase and AP-1 signaling pathways in liposarcoma progression.

Nevertheless, the above model does not completely explain tumor progression for several reasons. First, in the aforementioned studies, up to a third of dedifferentiated liposarcomas did not show any of these amplifications (other than 12q13–q15). Furthermore, the 1q23, 6q23 and 1p32 amplicons are similar in almost all cases in which both components were analyzed separately, suggesting that these genetic changes may already be present in the well-differentiated component. Finally, the majority of cases in the above-mentioned studies consist of only the dedifferentiated area or a mixture of both components of a given tumor. Thus, the relative contributions of the changes at 1q23, 6q23 and 1p32 or other loci in a liposarcoma progression model remain to be determined. A systematic comparison of paired well-differentiated and dedifferentiated areas in a large number of tumors, including synchronous and metachronous types, may allow examination of the genomic divergence between components. Genetic changes unique to one component may, in turn, uncover mechanisms that contribute to progression.

In this study, we compare the genetic changes of paired well-differentiated and dedifferentiated components of 29 well-characterized dedifferentiated liposarcomas using the superior resolution of array CGH. We explore the association between genetic changes and clinical presentation (synchronous or metachronous) or histological grade of the dedifferentiated area. We correlate CDK4 and MDM2 overexpression by immunohistochemistry with the genomic alterations in the tumor.

## Materials and methods

### Case Selection

We initially identified 30 cases of dedifferentiated liposarcomas from the pathology archives at our institution. Of these, 29 resulted in informative array CGH analysis and were further studied. We recently reported more detailed clinicopathologic parameters of 25 of these cases.[24] The diagnosis was based on light microscopic, radiographic and clinical features. The 'gold standard' of diagnosis in every case was the presence of a well-differentiated liposarcoma either adjacent to macroscopic areas of sarcoma without lipogenic differentiation (synchronous) or previously removed from the same anatomical site (metachronous). The grade of the dedifferentiated component was recorded on the basis of previously published criteria.[5,25]

### Microdissection and DNA Extraction

Microdissection and DNA extraction were performed as described previously.[26,27] Briefly, tumor

DNA was extracted from unstained sections obtained from a formalin-fixed paraffin-embedded tissue. Desired areas (well differentiated and dedifferentiated) of the tumors were identified on the basis of hematoxylin and eosin-stained slides. The selected regions were manually dissected into the extraction buffer (10 mM Tris, 1.5 mM MgCl$_2$, 50 mM KCl and 0.5% Tween-20 (Fisher Scientific, Tustin, CA, USA) with 0.4 mg/ml proteinase K (Sigma, St Louis, MO, USA) and concentrated using Microcon YM-30 columns (Amicon Millipore, Bedford, MA, USA). DNA was quantitated as described previously.

### DNA Amplification and Labeling

DNA was amplified and labeled according to published methods.[28] Briefly, template DNA was random primed using the BioPrime DNA labeling system (Invitrogen, Carslbad, CA, USA) according to the manufacturer's directions. Amplified genomic DNA was labeled with cy3-dCTP, and reference genomic male DNA was labeled in the same manner with cy5-dCTP (Amersham Pharmacia, Piscataway, NJ, USA). Unincorporated fluorescent nucleotides were removed using Sephadex G-50 spin columns (Amersham Pharmacia).

### Hybridization

Human scanning arrays consisting of 2464 bacterial artificial chromosome (BAC) clones spanning the genome at ∼1-Mb resolution were used.[29,30] Hybridization and washing were carried out as described previously.[28] Labeled tumor DNA and reference DNA were mixed with Cot-1 DNA (Invitrogen) and hybridized to the array for 48 h at 37 °C in a humidified chamber with slow agitation. After washing, DNA was counterstained with 4′6-Diamidino-2-phenylindole (DAPI) in phosphate-buffered glycerol.

### Image Analysis

The images, including fluorescence in the DAPI, Cy3 and Cy5 ranges were captured using a digitally charged-coupled device-based system. Image data were analyzed using the UCSF Cancer Center's Array Core SPOT and SPROC software.[31]

### Statistical Considerations

All statistical analyses were carried out using the freely available R/Bioconductor software.[32] The array CGH data were corrected for a geometrical dependence of the ratios on the array in some hybridizations, which translates into apparent measurement noise when ratios are plotted according to their positions in the genome using procedures described previously.[33] The data were analyzed using circular binary segmentation to translate noisy intensity measurements into regions of equal copy numbers.[34] Each probe was assigned a segment value referred to as its smoothed value. The scaled median absolute deviation of the difference between the observed and smoothed values was used to estimate the tumor-specific experimental variation. The gain and loss status for each probe was defined using the merged level procedure.[35]

The amplification status for a clone was determined by considering the width of the segment to which that clone belonged as well as a minimum difference between the smoothed value of the clone and the segment means of the neighboring segments. The clone was declared amplified if it belonged to the segment spanning < 22 Mb, and the minimum difference was greater than $e^{-x^3}$, where $x$ is the final smoothed value for the clone.

A clone-wise comparison of variables was made using moderated *t*-statistics.[36] The false discovery rate (FDR) was controlled across all autosomes to obtain adjusted *P*-values, and a cutoff value of 0.05 was used to declare a clone to be significant. Paired *t*-tests were conducted to compare the matched dedifferentiated, well-differentiated components. To test the association of clinical presentation or grade, the effect was controlled as a covariate in a linear model. For analyzing the *CDK4* and *MDM2* interaction in the 12q13–15 region, a multiplicative model was fit for each clone and *P*-values were FDR adjusted for all clones in that region.

The paired Wilcoxon rank sum test was used to analyze the association of dedifferentiated, well-differentiated groups with summary genomic events, such as number of break points, number of chromosomes with break points, number of amplifications, number of chromosomes with amplifications, number of whole chromosome changes and fraction of genome altered. To test the association of summary genomic events with other phenotypes in the dedifferentiated, well-differentiated combined data set, a linear model was used in which the patient effect was controlled. The Wilcoxon rank sum test was used when testing within either the dedifferentiated or well-differentiated subset. A *P*-value cutoff of 0.05 was used to declare significance.

Unsupervised hierarchical clustering of the tumors was performed using smoothed imputed data with outliers present. Agglomerative hierarchical clustering with Pearson's correlation as a similarity measure and the Ward method to minimize the sum of variances was used to produce compact spherical clusters.

### Immunohistochemistry

Immunohistochemical analysis was carried out using previously published techniques[37] on archival, paraffin-embedded sections. Briefly, 4-$\mu$m paraffin-embedded sections were de-paraffinized,

heated in EDTA buffer (Cell Marque, Rocklin, CA, USA), blocked and incubated with a monoclonal antibody to CDK4 (DCS-31, Biosource, Camarillo, CA, USA) at 1:100 dilution or MDM2 (IF2, Zymed, South San Francisco, CA, USA) at 1:100 dilution. Detection for all antibodies used the Envision system (Dako, Carpinteria, CA, USA) according to the manufacturer's directions. Slides were scored as positive if >10% of tumor cells showed specific, strong, nuclear staining. Positive cases were stratified as follows:[38]

  level 1: >10%, but <25% positive cells;
  level 2: > 25%, but <50% positive cells;
  level 3: > 50%, but <90% positive cells;
  level 4: > 90%, positive cells.

## Results

### Clinicopathologic Characteristics

The clinicopathologic information is summarized in Table 1. The study consisted of 29 matched pairs of well-differentiated and dedifferentiated liposarcomas. The clinical and pathological findings of 25 tumors were reported previously.[24] The most common presentation was a retroperitoneal mass ($n = 21$, 72%) with the remaining cases distributed between the extremity, trunk, abdomen and spermatic cord. In 25 cases (86%), the dedifferentiated liposarcoma was present at the time of first diagnosis (synchronous), whereas in the remaining cases ($n = 4$, 14%), a diagnosis of well-differentiated liposarcoma preceded the presentation of the dedifferentiated liposarcoma by 1–5 years (metachronous). Grossly, tumors averaged 18 cm in greatest dimension (range: 5–45 cm). Histologically, all cases showed a component of the well-differentiated liposarcoma ranging from ~10 to 75% of the tumor present on slides. All of the dedifferentiated components were grossly apparent (>2 cm). The most common subtype of the well-differentiated component was lipoma-like ($n = 19$, 66%) although sclerosing and inflammatory types were also identified. Eight cases (28%) were classified as low-grade dedifferentiation on the basis of cellularity and cytomorphologic features more compatible with fibromatosis than with high-grade sarcoma. The remaining cases ($n = 21$, 72%) consisted of high-grade sarcoma. One high-grade component demonstrated meningothelial-like whorls, and one had osteosarcomatous differentiation. The remaining high-grade components were morphologically and immunophenotypically 'undifferentiated' with spindle cell ($n = 10$, 35%), round cell ($n = 2$, 7%) or pleomorphic ($n = 7$, 24%) cytomorphology. A dense acute and chronic inflammatory infiltrate imparting a so-called 'inflammatory MFH' appearance was present in two pleomorphic cases. Regardless of grade, all of the dedifferentiated components showed, at least focally, $\geq$ 5 mitoses per 10 high power (40 ×) fields.

### Genomic Alterations in Liposarcomas

Representative array CGH profiles from tumor pairs are illustrated in Figure 1. Using unsupervised hierarchical clustering of genetic gains and losses, (Figure 2) all but two tumors clustered as well-differentiated–dedifferentiated pairs. More specifically, the well-differentiated component of case 26 clustered with the tumor pair from patient 25 (most closely associated with the dedifferentiated component of this pair), sharing high-level amplification at 2p, gains at 1q, 7q, and 16p and losses at 8p and 12p.

The pattern of amplification at 12q13–15 was remarkably similar for a given tumor pair (Figure 1d). All 29 (100%) dedifferentiated liposarcomas had high-level amplifications in 12q13–15 in at least one component (Figure 1, Table 1). The 12q15 amplicon (BACs CTB-136O14 and CTB-82N15, including *MDM2* at 64.5 Mb) was slightly more common (25/29, 86%) than was the 12q13–14 amplicon (BACs RMC12P001 (*CDK4)* and GS-56N1 (*SAS*) at 56.4 Mb) (23/29, 70%). Moreover, four tumors were discordant with respect to differentiation and amplification of these regions (Table 1). Contiguous amplification of the intervening region was uncommon ($n = 7$, 24%), although gains in this region were sometimes present.

### Differentiation-Specific Genomic Profiles

On the basis of cluster analysis and evaluation of tumor pairs, dedifferentiated areas showed more amplifications than did the matched well-differentiated components ($P = 0.008$). However, no difference in whole chromosome changes or total genomic variability was observed. The only genetic change that showed a trend to specificity for the dedifferentiated component was the gain of 19q13.2, but statistical significance ($P = 0.003$) was lost after adjusting for multiple comparisons. Thus, a hypothetical 'differentiation-specific' genetic change was not identified (Figure 3a). The 19q13.2 region corresponds to BACs RP11-21J15 and CTD-2271H24 (49.7–50.7 Mb), the location of the *FOS-B* gene.

Given previous reports associating amplifications of chromosomal regions 1p, 1q, 6q and 12q24 with dedifferentiated liposarcoma, we analyzed these regions in more detail (Table 1). Additional amplifications that were commonly observed in at least one component of the dedifferentiated liposarcomas included 1p31–32 ($n = 7$, 24%), 1q21–23 ($n = 9$, 31%), 6q23–24 ($n = 7$, 24%) and 12q24 ($n = 3$, 10%) (Table 1, Figures 1 and 2). Coamplification of 1p31–32 and 6q23–24 was a rare event but was detected in one case (Case 16, Table 1). The 1q21–23 and 6q23–24 amplicons were slightly more common in dedifferentiated components. (Table 1 and Figure 2). Again, these genetic changes did not achieve statistical significance with proper correction for multiple comparisons (Figure 3a), and the 6q23–24 and

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**Table 1** Clinicopathologic, immunohistochemical and genomic data of 29 dedifferentiated liposarcomas

| | | | | | | IMMUNOHISTOCHEMISTRY | | | | AMPLIFICATIONS* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Size | CDK4 | | MDM2 | | 12q13.3-12q14 (CDK4) | | 12q15 (MDM2) | | 1p31-32 (JUN) | | 1q21-23 | | 6q23-24 (MAP3K5) | | 12q24 | |
| Case | Site | Grade | Clin | Type | (cm) | WD | DD | WD | DD | WD | DD | WD | DD | WD | DD | WD | DD | WD | DD | WD | DD |
| 1 | RP | Low | Synch | L | 9 | 1 | 1 | 1 | 1 | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | ■ | ■ |
| 2 | RP | High | Metach | L | 24 | 1 | 2 | 1 | 1 | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | ■ | □ | □ |
| 3 | RP | Low | Synch | S | 9 | 0 | 0 | 0 | 1 | □ | □ | □ | ■ | □ | □ | □ | □ | □ | □ | □ | □ |
| 4 | Thigh | High | Synch | L | 29 | 0 | 2 | 1 | 2 | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ |
| 5 | Arm | High | Synch | S | 8 | 0 | 0 | 0 | 1 | □ | □ | □ | ■ | □ | □ | ■ | ■ | □ | □ | □ | □ |
| 6 | SC | High | Synch | L | 10 | 1 | 2 | 1 | 2 | ■ | ■ | ■ | ■ | □ | □ | □ | □ | ■ | ■ | □ | □ |
| 7 | SC | Low | Synch | L | 10 | 2 | 2 | 3 | 3 | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ |
| 8 | RP | High | Synch | L | 13 | 1 | 2 | 1 | 2 | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ |
| 9 | RP | High | Synch | L | 16 | 3 | 3 | 1 | 2 | ■ | □ | ■ | ■ | □ | □ | □ | □ | □ | □ | ■ | ■ |
| 10 | Thigh | Low | Synch | L | 28 | 3 | 3 | 1 | 1 | ■ | ■ | ■ | ■ | □ | □ | ■ | ■ | ■ | □ | ■ | ■ |
| 11 | RP | High | Synch | L | 30 | 2 | 2 | 1 | 2 | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | ■ | □ | □ |
| 12 | RP | High | Metach | L | 42 | 2 | 1 | 2 | 1 | ■ | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ |
| 13 | RP | Low | Synch | L | 6 | 1 | 3 | 1 | 1 | ■ | ■ | ■ | ■ | □ | □ | □ | ■ | □ | □ | □ | □ |
| 14 | RP | High | Synch | S | 29 | 1 | 3 | 1 | 1 | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ |
| 15 | RP | High | Metach | L | 19 | 2 | 4 | 1 | 2 | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ |
| 16 | RP | High | Synch | S | 17 | 3 | 3 | 3 | 3 | ■ | ■ | ■ | ■ | ■ | ■ | □ | □ | ■ | ■ | □ | □ |
| 17 | SC | Low | Synch | S | 5 | 0 | 0 | 1 | 2 | □ | □ | ■ | ■ | □ | □ | □ | ■ | □ | □ | □ | □ |
| 18 | RP | High | Metach | S | 45 | 1 | 3 | 2 | 2 | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ |
| 19 | RP | High | Synch | L | 29 | 2 | 3 | 2 | 2 | ■ | ■ | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ |
| 20 | RP | High | Synch | I | 20 | 2 | 3 | 1 | 3 | ■ | ■ | ■ | ■ | □ | □ | ■ | ■ | □ | □ | □ | □ |
| 21 | RP | High | Synch | L | 20 | 1 | 1 | 2 | 2 | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| 22 | RP | High | Synch | L | 19 | 1 | 3 | 1 | 2 | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ |
| 23 | RP | Low | Synch | S | 10 | 3 | 1 | 2 | 2 | □ | □ | ■ | ■ | □ | □ | ■ | ■ | □ | □ | □ | □ |
| 24 | RP | High | Synch | L | 10 | 0 | 3 | 1 | 1 | □ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ |
| 25 | RP | High | Synch | L | 11 | 2 | 2 | 1 | 1 | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ |
| 26 | RP | High | Synch | L | 9 | 1 | 3 | 1 | 3 | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ |
| 27 | Trunk | Low | Synch | S | 15 | 3 | 3 | 3 | 2 | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| 28 | Thigh | High | Synch | S | 15 | 2 | 2 | 3 | 3 | ■ | ■ | ■ | ■ | ■ | ■ | □ | □ | □ | ■ | □ | □ |
| 29 | RP | High | Synch | L | 18 | 2 | 4 | 2 | 3 | ■ | ■ | ■ | ■ | □ | □ | □ | □ | ■ | ■ | □ | □ |

■, amplification; □, normal copy number; Clin, clinical presentation; Synch, synchronous; Metach, metachronous; RP, retroperitoneum; SC, spermatic cord; L, lipoma-like; S, sclerosing; I, inflammatory; WD, well-differentiated component, DD, dedifferentiated component.
[a]The amplifications correspond to the following BAC clones, respective map positions and genes: 12q13.3–12.14 = RMC12P001, GS-561N1 RP11-39G24 (56.1–57.5 Mb, *CDK4, SAS*); 12q15 = RP11-1847C7, RP11-5J6, CTB-136O14, CTB-82N15 (67 Mb); 1p31–32 = RP11-8F24, CTD-2001C15 (58.9–61 Mb, *JUN*); 1q21–23 = RP11-71L20, RP11-137M19, RP11-97G24, RP11-260G23, CTD-2096I16 (149.6–161.5 Mb); 6q23–24 = CTC-268N4, RP11-32P13, CTD-2015J17 (135.5–137.5 Mb, *MAP3K5*); 12q24 = CTD-2022D21, RP11-3E2, RP11-119J23, RP11-89J2 (102.8–113.9 Mb).

1p31–32 amplicons were each identified in the well-differentiated, but not in the paired dedifferentiated, component of one case (Table 1).

In contrast, specific chromosomal changes associated with grade or clinical presentation. For example, the high-grade dedifferentiated components

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had significantly ($P<0.001$) more genomic gains at 6q14.1 (RP11-217L13, *IRAK1BP1*), 6q23.2–24.1(RP11-139O22 to RP11-15H7, *MYB*, *MAP3K5*), 10q21.1 (RP11-240K9 to RP11-133C15, *PRGK*) and 12q14.1–14.2 and losses at 8p21.3 (RP11-51C1, RP11-117P11 *LPL*) (Figure 3b). Similarly, comparison of synchronous with metachronous dedifferentiated liposarcomas showed that synchronous tumors had significantly more ($P<0.001$) gains at 6q23.2–24.1 (*MYB*, *MAP3K5*), 10q11.2–21.1 (*PRGK*) and 12q14.1–14.2, whereas metachronous tumors more often had losses at 8p21.3 (*LPL*) (Figure 3c).

## CDK4 and MDM2 Status

The results of CDK4 and MDM2 immunohistochemistry are summarized in Table 1, and representative cases are shown in Figure 4. CDK4 was detected in 24 (83%) and 26 (89%) of the well-differentiated and dedifferentiated components, respectively, although the staining score was usually higher in the latter component within a given tumor (Table 1 and Figure 4). MDM2 was detected in 27 (93%) and 29 (100%) of the well-differentiated and dedifferentiated components, respectively. Again, the dedifferentiated component generally showed a higher staining score than did the paired well-differentiated component from the same case (Table 1 and Figure 4). No significant correlation was noted in total genomic variability or number of amplifications and CDK4 or MDM2 status (data not shown). Immunohistochemistry for CDK4 and MDM2 correlated with the amplification of 12q13.3–14 ($P<0.001$) and 12q15 ($P=0.015$), respectively, although the association was not exclusive (Table 1). More specifically, two cases were positive for CDK4 and MDM2 by immunohistochemistry without high-level amplification of 12q13.3–14 and 12q15, respectively. The above cases had gains of the respective regions of chromosome 12 (Figure 2).

Finally, we analyzed the interaction effect of the CDK4 and MDM2 immunophenotypes on the genome. In the well-differentiated components, this analysis showed a significant negative correlation effect of CDK4 immunostaining with 12q13.13 amplification. That is, MDM2+ CDK4– tumors had over sixfold more amplification of 12q13.13 (RP11-132H4) than did MDM2+ and CDK4+ tumors ($P=0.002$). This BAC maps ~2.7 and 15 Mbp centromeric to the *CDK4* and *MDM2* gene loci, respectively, near the gene for transcription factor *ATF7*.
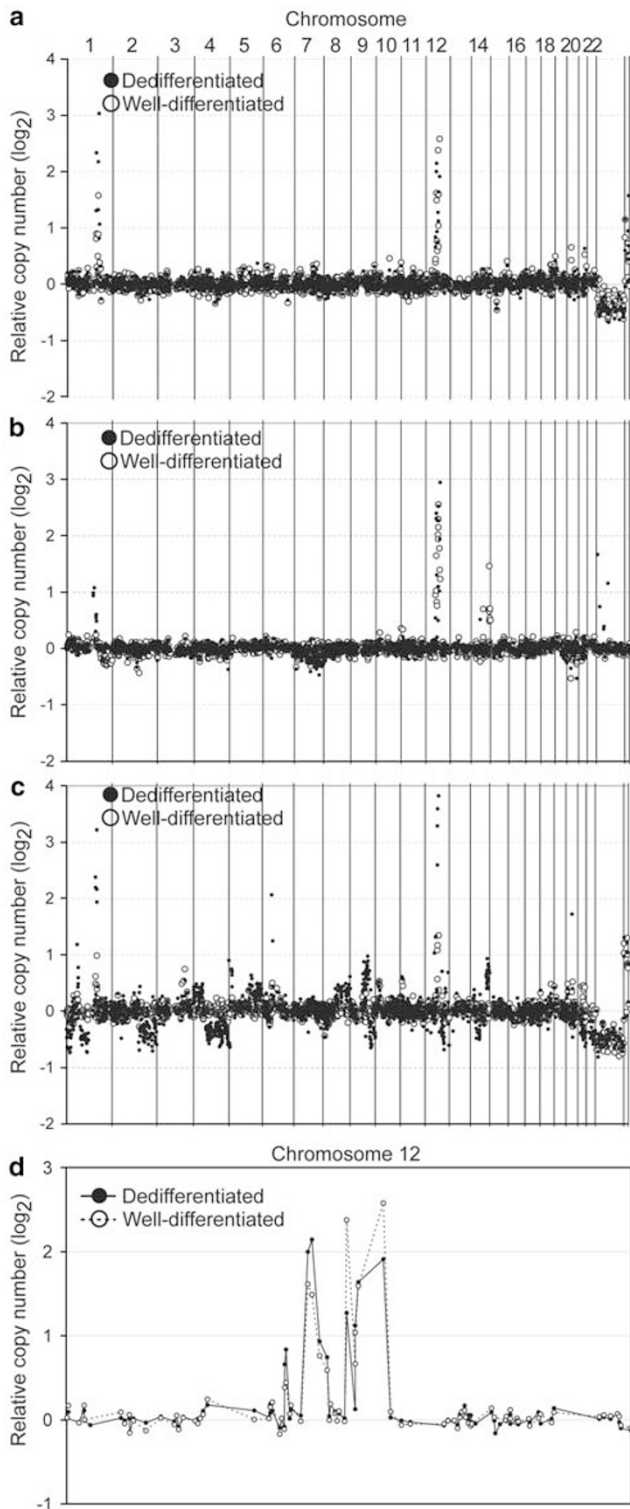


**Figure 1** Array CGH profiles from representative pairs of dedifferentiated liposarcoma components. Copy-number changes relative to normal DNA are shown for each clone. Clones are ordered from chromosomes 1 to 22 and within each chromosome based on their map position. The X and Y chromosomes are not informative in these studies as the control DNA is not gender matched. (**a**) Whole genome profile of case 22 shows essentially identical genotype between components. (**b**) Whole genome profile of case 14 shows increased copy number of 1q21–23 only in the dedifferentiated component and 14q32 only in the well-differentiated component. (**c**) Whole genome profile of case 24 which shows the increased copy number of 1p31–32 and 1q21—23, as well as numerous other gains and losses specific to the dedifferentiated component. (**d**) Chromosome 12 profile of case 22 shows similarity at 12q13—15, which consists of two amplicons.
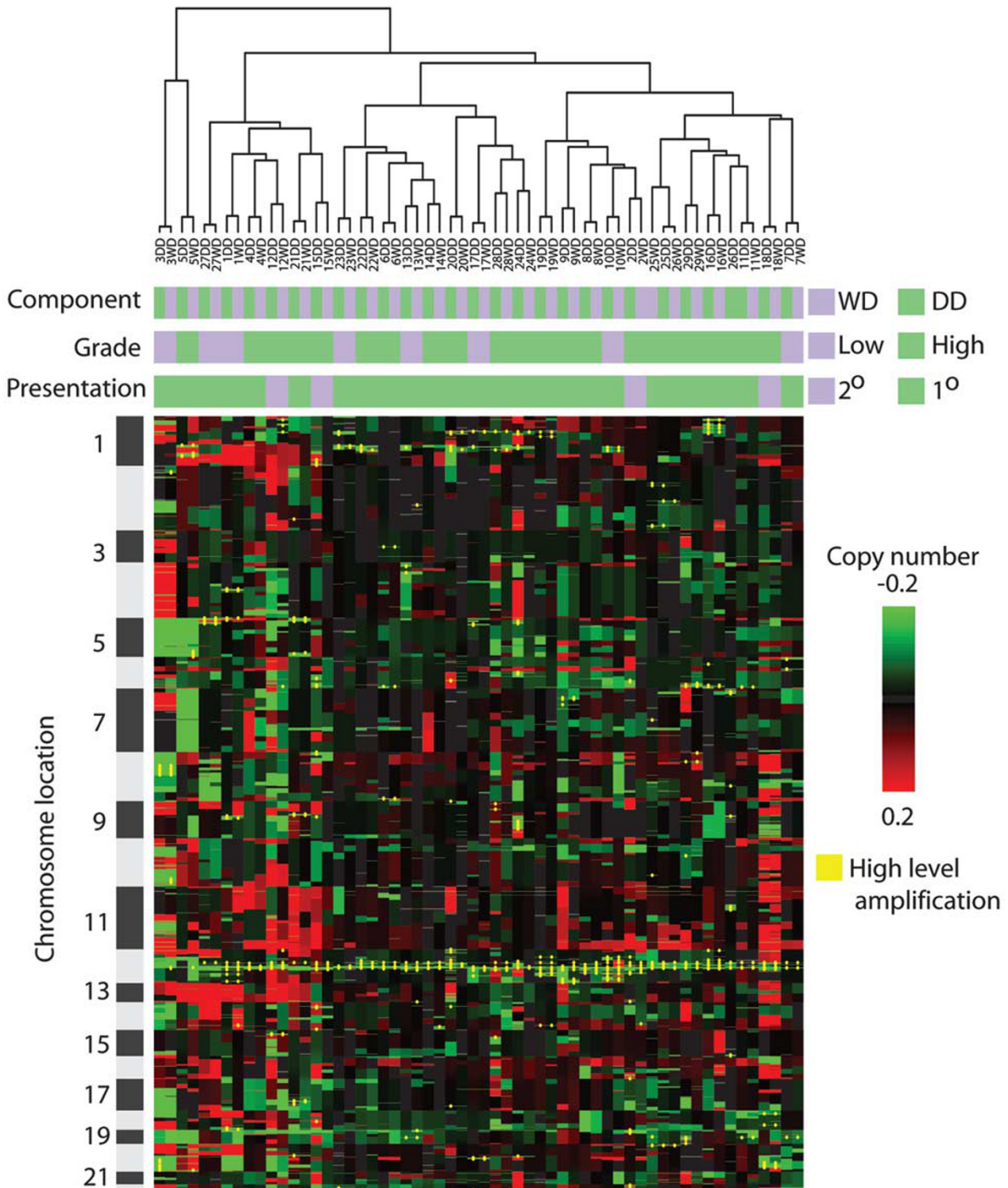
**Figure 2** Unsupervised hierarchical cluster analysis of genome copy number of 29 dedifferentiated liposarcoma pairs. The liposarcomas are distributed along the horizontal axis and genome is along the vertical axis. Chromosomes 1–22 are represented by the black and gray bars, respectively, at left and patient numbers are along the top. Three major clusters based on genetic complexity (complex, simple and intermediate) are arbitrarily ordered from left to right. Increased copy number is indicated in green, decreased copy number in red and high-level amplifications in yellow. The upper color bars indicate clinicopathologic properties of the tumor. Color codes are at the right of the figure: for differentiation status, green indicates dedifferentiated and blue indicates well-differentiated; for grade green indicates high and blue indicates low; for presentation green indicates synchronous and blue indicates metachronous. WD = well-differentiated component; DD = dedifferentiated component; 1° = primary; 2° = secondary.
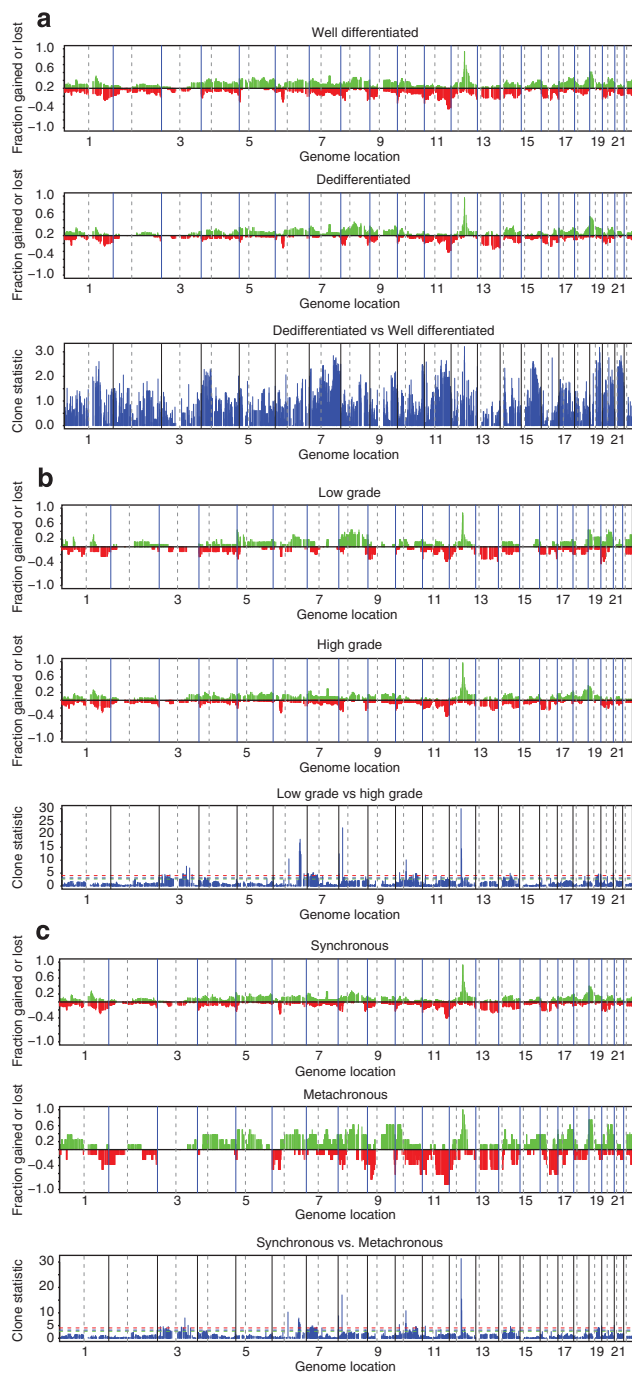
**Figure 3** Recurrent abnormalities in both components of 29 dedifferentiated liposarcoma pairs. Plots comparing the frequency of copy number increases (above the *X* axis) and decreases (below the *X* axis) of specific chromosomal regions on the basis of clinicopathologic parameters. Vertical lines indicate chromosome boundaries, and vertical dashed lines indicate centromeres. In each panel, the top two plots represent the fraction of cases with gains or losses as a function of genome location. The third plot shows the clone statistics (see 'Materials and methods' section). The green, blue and red horizontal dotted lines represent the threshold for *P* = 0.1, 0.05 and 0.01, respectively (moderated *t*-test). (**a**) Well-differentiated compared with dedifferentiated components. (**b**) Low-grade dedifferentiated compared with high-grade dedifferentiated components. (**c**) Synchronous compared with metachronous clinical presentation.

## Discussion

The progression of liposarcoma from a well-differentiated to a dedifferentiated phenotype raises the possibility of specific genetic changes that mediate such progression. In this study, we tested the hypothesis that specific, reproducible genetic changes are responsible for the dedifferentiated phenotype. Using unsupervised hierarchical cluster analysis, paired well-differentiated and dedifferentiated liposarcomas segregated together in almost all cases. The results show that although dedifferentiated liposarcomas are a genetically complex group of tumors with many reproducible changes, no genetic change uniformly distinguishes the well-differentiated *vs* dedifferentiated components in a statistically significant manner. A trend for gain of 19q13.2 was observed in dedifferentiated components, but was only statistically significant if uncorrected for multiple comparisons. Nevertheless, the latter finding is intriguing because this region includes the *FOS-B* gene a member of the AP-1 family of transcription factors, which heterodimerizes with c-JUN to regulate transcription and may inhibit adipogenesis.[23,39] Thus, the finding of gains of the *FOS-B* gene is at least consistent with the importance of upregulated AP-1 signaling in the dedifferentiated phenotype.

These data support a strong clonal relationship between components of dedifferentiated liposarcoma, including metachronous tumors separated by as much as 5 years. In previous reports of expression array analyses, the well-differentiated components of dedifferentiated liposarcomas clustered distinctly from purely well-differentiated liposarcomas.[20] Other studies have suggested that purely well-differentiated liposarcomas are genetically simpler and have distinct profiles from the well-differentiated components of dedifferentiated liposarcomas.[13] Taken together, these findings imply that genetic changes mediating dedifferentiation may precede phenotypic changes. This conclusion is important because it indicates that the background genotypes of some apparently 'well-differentiated' liposarcomas already harbor the changes required for progression. In turn, the genetic changes that separate purely well-differentiated from dedifferentiated liposarcomas may be prognostically useful in predicting progression. Metachronous tumors are rare, and prospective studies may be necessary to confirm this hypothesis. Finally, we acknowledge that although the BAC-based array used in this study offers superior resolution to chromosomal CGH, at ~1-Mb resolution, the BAC array is limited compared with some oligonucleotide-based platforms.[40] The BAC array was chosen in this study because of consistent results when studying DNA from archival formalin-fixed paraffin-embedded tissue. However, these data do not rule out that small chromosomal changes, below the level of detection of the BAC array, or allelic imbalances,
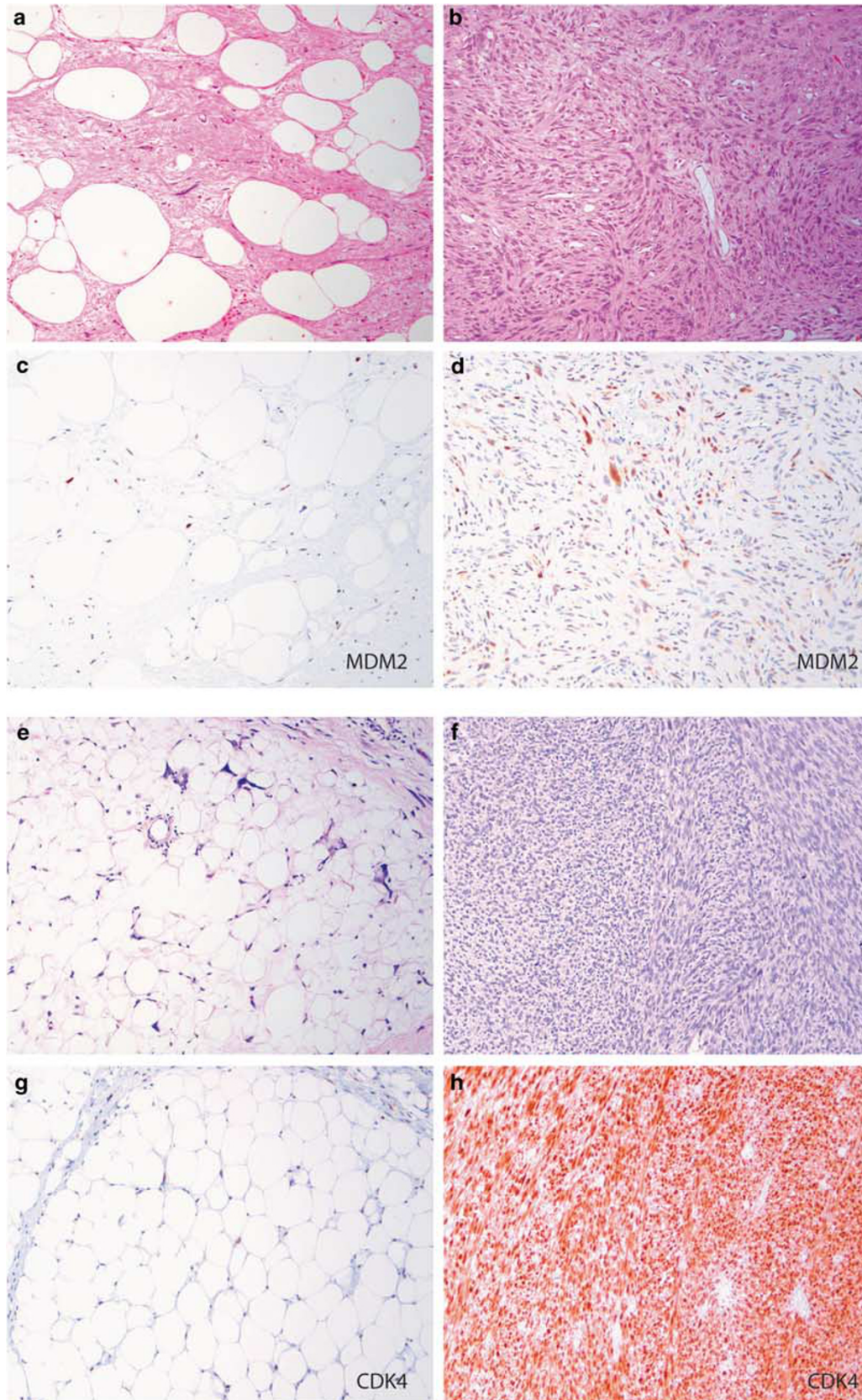
**Figure 4** Immunohistochemical staining for CDK4 and MDM2 in dedifferentiated liposarcomas. Paired well-differentiated and dedifferentiated tumors are in the left and right columns, respectively. (**a** and **b**) Hematoxylin and eosin (H&E) of case 8. (**c** and **d**) MDM2 case 8. (**e** and **f**) H&E case 15. (**g** and **h**) CDK4 case 15.

may distinguish well-differentiated and dedifferentiated components of liposarcoma.

A relationship between the amplification of specific loci (1p31–32, 1q21–23, 6q23–24 and 12q24) and the differentiation status of liposarcoma has been implicated in previous reports.[13,21,41,42] Some of these studies have also suggested that the 1p32 and 6q23 amplicons are mutually exclusive. That is, dedifferentiated liposarcomas demonstrate one, but not both, amplicons resulting in the overexpression of either *JUN* or *MAP3K5* (which map to 1p32 and 6q23, respectively).[13] Intriguingly, in a separate study, gene expression profiling comparing well-differentiated and dedifferentiated liposarcomas did not show an upregulation of either *JUN* or *MAP3K5* in the top 50 differentially expressed genes.[20] In the present study, amplification at each of these regions was relatively common, but was present in both the well-differentiated and dedifferentiated components. Interestingly, the 1p31–32 and 6q23–24 amplicons were occasionally identified only in the well-differentiated components. Only one tumor showed coamplification of 1p32 and 6q23 supporting the fact that, in the majority of tumors, amplification of these regions is mutually exclusive. Taken together, these results suggest that the above-mentioned amplicons are neither necessary nor sufficient to account for the phenotypic difference between well-differentiated and dedifferentiated components. However, the present findings are still consistent with a role of genes in the AP-1 signaling pathway (specifically, *MAP3K5* and *JUN*) in dedifferentiated liposarcoma tumorigenesis. These data suggest that gene amplification may be one of multiple mechanisms (possibly also including point mutation, transcriptional regulation or protein stability) that modulate the AP-1 signaling pathway in liposarcoma.

Our data confirm that 12q13–q15 amplification is a very common event in both components of dedifferentiated liposarcoma.[6–9] The copy-number profile of the 12q13–q15 amplicon did not differ significantly between paired components, implying that quantitative or qualitative changes in this genomic segment do not contribute to the dedifferentiated phenotype. The array CGH results confirm recent findings that the amplification of 12q13–15 actually consists of two amplicons.[14] More specifically, the 12q13.3–12q14 amplicon, which includes *CDK4* and *SAS*, is discontinuous with the 12q15 amplicon (the location of *MDM2).* Contiguous amplification of the intervening region was a rare event in this study. Our results also support previous findings that *MDM2* is more commonly amplified than is *CDK4* in dedifferentiated liposarcomas.[14,41,43] Nevertheless, *MDM2* amplification is not universal as it was absent in at least one component of four cases. The very close correlation between CDK4 or MDM2 immunostaining and the 12q13.3–12q14 or 12q15 amplicons, respectively, is in keeping with the established diagnostic utility of these immunostains.[15] A small number of cases showed positive immunostaining for either MDM2 or CDK4 without amplification of the corresponding gene, suggesting that other mechanisms that alter expression may be involved.[44] The statistical analysis of the interaction effect of MDM2 or CDK4 immunostaining on 12q alterations showed an unexpected result, namely that MDM2+, CDK4− tumors had significantly high-level amplifications of a region centromeric to both *MDM2* and *CDK4*. The BAC in question maps near the *ATF7* gene, suggesting that this transcription factor and *CDK4* may have redundant functions in promoting liposarcoma tumorigenesis.

Amplification of 1q21–23 has been identified in both well-differentiated and dedifferentiated liposarcomas.[13] This is a relatively large segment; therefore, the exact genes involved are not known although *COAS1-3* genes have been implicated in the past.[45] In this study, the pattern of amplification within 1q21–23 varied between tumors. However, the most commonly amplified BAC within this region (CTD-2096I16 at 161.3–161.5 Mb) encompasses the *RGS4* and *RGS5* genes, ~19 Mb telomeric to the *COAS3* gene. *RGS4* and *RGS5* genes encode regulators of G-protein-coupled receptors. These RGS proteins negatively regulate G-protein-coupled receptor signaling which, in turn, mediates numerous metabolic and developmental processes.[46] Obviously, the exact role and importance of these genes in liposarcoma will require additional studies.

This study identified genetic gains at a number of loci including *PRGK*, *MYB*, *MAP3K5* and loss of *LPL* (the gene encoding lipoprotein lipase) that were significantly more common in high-grade and metachronous tumors as compared with low-grade and synchronous tumors, respectively. To our knowledge, these data are the first to specifically describe that the grade and presentation of the dedifferentiated component correlate with specific genetic changes. Little is known about the clinical significance of the grade of dedifferentiation, although some studies suggest that outcome is independent of tumor grade.[3,47] However, this observation is based on a relatively small number of patients, and as most cases occur in the retroperitoneum, local tumor effects, rather than metastasis, often influence outcome. The identification of specific regions of the genome that correlate with grade may provide insights into mechanisms of liposarcoma biology. Similarly, the changes specific to metachronous tumors may stem from differences in tumor initiation, factors that control genomic instability or the background genotype of the well-differentiated component. Insofar as metachronous tumors represent documented clinical progression, over time, of a well-differentiated liposarcoma into a non-lipogenic sarcoma, prospective studies of phenotypically well-differentiated liposarcomas may be the ideal model system to further study genes that influence tumor progression.

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## Disclosure/conflict of interest

The authors declare no conflict of interest.

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