Copy Number Losses Define Subgroups of Dedifferentiated Liposarcoma with Poor Prognosis and Genomic Instability

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Abstract

Purpose: Molecular events underlying progression of well-differentiated liposarcoma (WDLS) to dedifferentiated liposarcoma (DDLS) are poorly defined. This study sought to identify copy number alterations (CNA) associated with dedifferentiation of WDLS, with DDLS morphology, and with patient outcomes.

Experimental Design: Fifty-five WDLS and 52 DDLS were analyzed using Agilent 244K comparative genomic hybridization and Affymetrix U133A expression arrays. CNAs were identified by RAE analysis. Thirty-nine of the DDLS specimens were categorized morphologically by a single pathologist.

Results: Nine regions of CNA were identified as recurrent in DDLS but not WDLS; 79% of DDLS had at least one of these CNAs. Loss of the chromosome segment 11q23–24, the most common event, was observed only in DDLS that morphologically resembled the genomically complex sarcomas, undifferentiated pleomorphic sarcoma and myxofibrosarcoma. 11q23–24 loss was itself associated with increased genomic complexity in DDLS. Loss of 19q13, but not 11q23–24, was associated with poor prognosis. Median disease-specific survival was shorter for patients with 19q13 loss (27 months) than for patients with diploid 19q13 (>90 months; $P < 0.0025$), and 19q13 loss was associated with local recurrence (HR, 2.86; $P = 0.013$). Common copy number losses were associated with transcriptional downregulation of potential tumor suppressors and adipogenesis-related genes (e.g., *EI24* and *CEBPA*).

Conclusions: Dedifferentiation of WDLS is associated with recurrent CNAs in 79% of tumors. In DDLS, loss of 11q23–24 is associated with genomic complexity and distinct morphology whereas loss of 19q13 predicts poor prognosis. CNAs in liposarcoma improve risk stratification for patients and will help identify potential tumor suppressors driving liposarcoma progression. *Clin Cancer Res; 18(5); 1334–40. ©2012 AACR.*

Introduction

Liposarcoma is the most common type of soft tissue sarcoma, accounting for approximately 20% of the tumors in adults. Liposarcoma shows histologic and genetic variability, and has been subdivided into 3 main biologic groups encompassing 5 subtypes. These are (i) well-differentiated/dedifferentiated liposarcoma (WDLS/DDLS), (ii) myxoid/round cell liposarcoma, and (iii) pleomorphic liposarcoma. WDLS contains regions of mature adipose tissue, interspersed with lipoblasts and atypical stromal cells. DDLS always occurs in the context of a WDLS and represents progression to high-grade disease. Histologically, DDLS lacks mature adipose cells and can have variable microscopic appearance (1).

Although WDLS and DDLS are often surgically resectable, retroperitoneal tumors frequently recur, and 60% of patients eventually die of disease. Dedifferentiation is a poor prognostic feature, as DDLS has metastatic potential and a more aggressive local disease course; DDLS is 6 times as likely to recur as WDLS (2–4). Radiation and chemotherapy have a limited ability to control inoperable or metastatic disease. Therefore, it is essential that we improve our understanding of the molecular events underlying liposarcoma progression so as to develop novel targeted therapies.

Changes in genome structure resulting in copy number alterations (CNA) occur commonly in human cancer and result in aberrant expression of putative oncogenes and tumor suppressors (5). Among WDLS and DDLS, more than 90% of tumors have amplification of the chromosome segment 12q13–15 (1, 6, 7). The 12q13–15 amplicon carries supernumerary copies of the oncogenes *CDK4* and

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Translational Relevance

Dedifferentiation of a well-differentiated liposarcoma (WDLS) is associated with the acquisition of metastatic potential and high rates of local recurrence. The genetic events that drive progression from WDLS to dedifferentiated liposarcoma (DDLS) remain undiscovered. Using comparative genomic hybridization, common, specific progression-associated copy number alterations (CNA) were identified in 79% of DDLS but were rarely found in WDLS. These recurrent CNAs associate with tumor morphology, patient outcomes, and expression of potential tumor suppressors/oncogenes. Our findings form the basis of a model describing liposarcoma progression and dedifferentiation. This model enables clinicians to better stratify patients with DDLS for clinical trials and will improve outcome prediction for the individual patient.

MDM2 (8, 9). Expression of *CDK4* and *MDM2* is essential for survival of liposarcoma cells *in vitro*, and therefore, the 12q13–15 amplicon is thought to play a role in initiation of liposarcomagenesis. DDLS is genomically complex, however, and additional CNAs often affect multiple chromosomes (1). One such CNA affects 1p32 and is associated with *JUN* amplification, an event that may contribute to dedifferentiation, though it is not sufficient to induce progression (10, 11).

In this study, we aim to improve our understanding of the mechanisms underlying progression thorough systematic analysis of CNAs associated with dedifferentiation. A direct, unbiased comparison of CNAs in DDLS versus WDLS has not previously been carried out. Using array comparative genomic hybridization (CGH), we identify specific CNAs that are common in DDLS but not in WDLS. We relate chromosome losses to (i) patient prognosis, (ii) microscopic appearance of DDLS, (iii) genomic complexity, and (iv) expression of potential tumor suppressors. In doing so, we provide a framework for understanding progression in a representative cohort of WD/DDLS.

Materials and Methods

Patient population and sample acquisition

Fifty-five WDLS and 52 DDLS samples were collected from patients treated surgically from 2002 to 2009. Informed consent was obtained per a protocol approved by the Institutional Review Board. Cryomolds were prepared from tumor samples, and a dedicated sarcoma pathologist examined hematoxylin and eosin (H&E) sections from both cryomold surfaces to assess liposarcoma subtype.

DNA/RNA isolation and array preparation

For nucleic acid extraction, we selected cryomolds containing a single subtype with more than 75% cellularity. Cryomolds were macrodissected to remove normal, fibrotic, inflammatory, and necrotic tissue.

Genomic DNA and RNA were prepared as previously described by RNeasy Lipid Tissue Mini and DNeasy Blood and Tissue kits (QIAGEN; refs. 6, 12). cDNA was synthesized in the presence of oligo(dT)$_{24}$-T7 from Genset, then cRNA was prepared using biotinylated UTP and CTP prior to hybridization to HG U133A arrays (Affymetrix; ref. 12). Genomic DNA was analyzed with Agilent 244K ($n = 44$ WDLS, $n = 40$ DDLS) or 1M ($n = 11$ WDLS, $n = 12$ DDLS) oligonucleotide arrays according to manufacturer's specifications. Competitive hybridization was conducted in the presence of a DNA reference set [Human Genomic DNA from blood (buffy coat), Roche Applied Science].

GC-normalization and RAE analysis of array CGH samples

Analysis of array CGH data used a customized normalization method. Signals were expected (i) to be highly correlated between CGH probes targeting adjacent regions in the genome and (ii) to show significant correlation with GC content. As no software was readily available to address these unique aspects of CGH data, novel algorithms for normalization were developed; complete methods and code are available at http://cbio.mskcc.org/CGCC.

To identify CNAs, the normalized data set was processed using a custom pipeline, which conducts the standard circular binary segmentation from the R/bioconductor DNAcopy library and then processes all samples with the RAE algorithm (6, 13). The gene-based and region of interest (ROI) copy number calls of -2, -1, 0, 1, or 2 (deletion, loss, copy neutral, gain, amplification; as assessed by RAE) were computed on 3 samples cohorts (DDLS only, WDLS only, and all samples). Copy number changes for each gene and ROI were converted from the 5-level RAE scale to a 3-level scale: loss, neutral, or gain (-1, 0, and 1). The Fisher exact test was used to compare copy number between DDLS and WDLS for each ROI. In many cases, ROIs adjacent along the genome had nearly identical copy number calls; in such cases, ROIs were further collapsed into a single event for subsequent analysis. The ANOVA F test was used to identify genes that were differentially expressed between copy number classes (i.e., -1, 0, or 1) and whose sign of change matched the copy number event. Genes of interest were defined as those having at least 2-fold change in expression for DDLS with a given CNA versus DDLS without the event or versus WDLS. Genomic complexity was calculated for each sample as the percentage of the genome altered (as scored by RAE).

Histology and survival analyses

In analyses of both histology and survival, CNAs were considered by chromosome arm. A given arm was scored as having a specific CNA if the 3 most common ROIs identified on that arm were all observed in the tumor.

Paraffin blocks from the original surgical specimens were rereviewed; presence of necrosis and tumor grade (regions of low-grade tumor, high-grade, or both) were documented. Tumors with greater than 5 mitoses per 10 high-power fields were characterized as high grade. The percentage of each

lesion consisting of DDLS histology was recorded. Size of the primary tumor was obtained from a prospectively collected database of patients with sarcoma treated surgically at our institution. DDLS were characterized as resembling undifferentiated pleomorphic sarcoma (UPS, also referred to as malignant fibrous histiocytoma or MFH), myxofibrosarcoma, spindle cell sarcoma not otherwise specified (resembling adult fibrosarcoma), small blue round cell tumors, or solitary fibrous tumor. Areas of divergent morphology (osteosarcoma or rhabdomyosarcoma) and inflammatory infiltrate were noted. Groups defined by individual histologic characteristics or morphology were tested for differences in CNAs using the Fisher exact test and tested for differences in the percentage of the genome altered using the Kruskal–Wallis test for nonparametric data. In a subset of analyses, UPS and myxofibrosarcoma were considered as one morphologic subgroup as these tumors can represent a spectrum of disease and no consensus has been reached on definitions of the 2 histologies.

Patient outcomes were prospectively collected from the time of surgical resection and were retrospectively reviewed. Local recurrence–free survival (LRFS), distant recurrence–free survival (DRFS), and disease-specific survival (DSS) were calculated from the date of surgical resection. Effect of CNA and clinical characteristics on DSS was evaluated using the Cox regression model and Kaplan–Meier analyses. For analysis of LRFS and DRFS, patients were stratified by the presence of individual CNAs and analyzed by the competing risk survival analysis methods (the Gray test); the competing event was death (14). For multivariate analyses, competing risk regression models were used (15).

Results

Common chromosomal gains and losses characterize the transition from WDLS to DDLS

CNAs were analyzed in 55 WDLS and 52 DDLS samples (Table 1; Supplementary Tables S1 and S2). RAE analysis identified CNAs on 11 chromosomes in WDLS (Fig. 1A; Supplementary Table S3). These included the classic 12q13–15 amplification encompassing *CDK4*, *MDM2*, and *HMGA2*. These genes were amplified in 95%, 87%, and 76% of WDLS, respectively. Only one WDLS had no 12q amplification. Less common CNAs in WDLS included gains at 1q21–25 and 7q34 and copy number losses at 1q25–44, 8q24, 9p24, 10q26, 11p13–15, 13q14, 13q31, 18q23, and 22q11–13. CNAs observed in specimens of WDLS regions within tumors classified histologically as DDLS (27% of WDLS specimens) were compared with those observed in samples from tumors classified histologically as WDLS. CNAs in these subsets were not significantly different.

As a group, DDLS tumors had more complex genomic alterations than did WDLS. CNAs affected on average 5.7% of the genome in WDLS (SD: ±7.0%; range: 0.1% to 36%) versus 21% (SD: ±16%; range: 1% to 62%; $P < 0.001$) in DDLS (Fig. 1B; Supplementary Fig. S1). In DDLS, CNAs were identified on 21 chromosomes (Supplementary Table S4). To assess which CNAs may be

important in progression of WDLS to DDLS, the 2 tumor types were directly compared. CNAs with at least 20% higher frequency in DDLS versus WDLS and found to be significantly different between the 2 groups [false discovery rate (FDR) < 0.001] were defined as progression-associated CNAs. Nine such CNAs were observed: losses centered at 3p14–21, 3q29, 9p22–24, 10p15, 11q23–24, 17q21, and 19q13 and gains at 17p11 and 20q11 (Table 2, Supplementary Table S5). Seventy-nine percent of DDLS tumors had at least one progression-associated CNA. In 9 cases, WDLS and DDLS samples from the same tumor or serial resections from the same patient were available for analysis (Supplementary Table S1); 5 of the DDLS but none of the WDLS in this subset had at least one of these CNAs. Of the progression-associated CNA, 11q23–24 loss was the most commonly observed. This loss was noted in 42% of DDLS compared with 4% of WDLS (FDR = 0.0002). The previously observed amplification of 1p32, which includes the *JUN* locus, was observed in 15 DDLS (28%) but also in 5 WDLS (9%; FDR = 0.03) so did not meet our criteria for a progression-associated CNA.
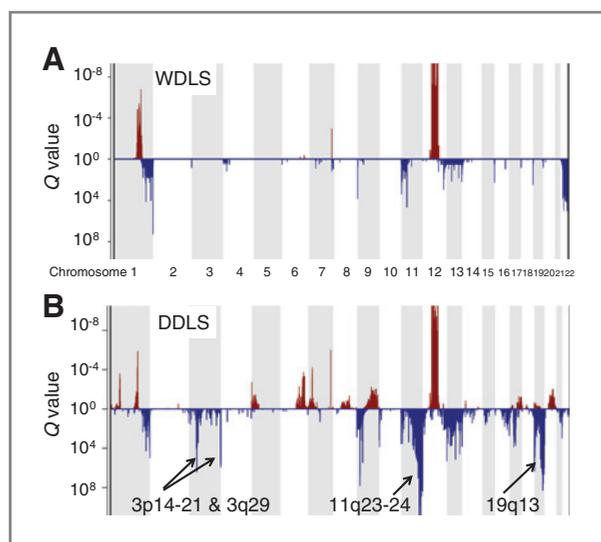
Table 1. Patient and tumor characteristics

	Sample type	
	WDLS **(n = 55)**	**DDLS** **(n = 52)**
Age, median (range)	58 (22–86)	60 (41–90)
Gender, n (%)		
Male	28 (51)	32 (62)
Female	27 (49)	20 (38)
Tumor histology[a], n (%)		
Well differentiated	40 (73)	3 (5.8)
Dedifferentiated	15 (27)	49 (94)
Primary vs. recurrent, n (%)		
Primary	44 (80)	36 (69)
First recurrence	9 (16)	12 (23)
Second or later recurrence	2 (3.6)	4 (7.7)
Tumor location, n (%)		
Retroperitoneal/ intra-abdominal	46 (84)	49 (94)
Chest	2 (3.6)	3 (5.8)
Extremity	7 (13)	0 (0)
Surgical margin, n (%)		
R0	24 (44)	19 (37)
R1	27 (49)	28 (54)
R2	4 (7.3)	5 (9.6)
Primary tumor size, n (%)		
Unknown	3 (5.5)	1 (1.9)
5–10 cm	6 (11)	3 (5.8)
>10 cm	46 (84)	48 (92)

[a]Tumor defined as DDLS if DDLS comprises more than 20% of tumor volume.

Figure 1. Statistically significant genomic alterations in WDLS (A) and DDLS (B). Plots indicate the FDR (Q value, y-axis), with red peaks representing amplifications and blue representing deletions. The most common CNAs associated with dedifferentiation were on 11q23–24, 19q13, 3p14–21, and 3q29.

CNAs correlate with tumor histology and genomic instability

The microscopic appearance of DDLS exhibited significant heterogeneity. Thirty-nine DDLS samples were available for pathologic rereview. The morphology most commonly appeared similar to UPS, myxofibrosarcoma, spindle cell sarcoma not otherwise specified (similar to adult fibrosarcoma), or small blue round cell tumors (Supplementary Fig. S2). One tumor appeared similar to solitary fibrous tumor and one had areas morphologically reminiscent of desmoid-type fibromatosis. Prominent inflammatory components and divergent morphologies (osteosarcoma, rhabdomyosarcoma) were each observed in 4 tumors. In 23 tumors (59%), the DDLS component had a variegated morphology (Supplementary Table S6).

Given the variability of both CNAs and morphology observed in DDLS, we hypothesized that histologic morphology may reflect the presence of specific CNAs. No CNAs were significantly associated with primary tumor size, tumor grade, or presence of necrosis in the specimen (as documented in Supplementary Table S6), but 17p11 gain in DDLS was associated with an inflammatory UPS-like morphology ($P = 0.028$). In addition, progression-associated CNAs affecting 11q23–24, 9p22–24, 10p15, 17p11, 17q21, and 20q11 were identified only in DDLS with regions appearing similar to UPS and myxofibrosarcoma, not in DDLS that had only spindle cell not otherwise specified, small blue round cell, or solitary fibrous tumor-like morphologies. This association was significant in the case of 11q23–24 loss (Table 3; Supplementary Table S6; $P < 0.02$).

UPS and myxofibrosarcoma are genomically complex tumors (1), and analysis of DDLS that contained a component resembling UPS or myxofibrosarcoma showed that these tumors tended to have CNAs in a higher percentage of the genome (23% ± 16%) than did other subtypes (11% ± 5.6%) though this finding did not reach statistical significance ($P = 0.062$; Supplementary Fig. S3A). Loss of 11q23–24, which, as noted earlier, was found only in this subset of DDLS, was also associated with increased genomic complexity; CNAs affected 29% ± 15% versus 15% ± 14% of the genome in DDLS with versus without 11q23–24 loss ($P = 0.002$; Supplementary Fig. S3B). Loss of 11q23–24 was specifically associated with other progression-associated CNAs on 9p22–24, 10p15, and 17q21 (all $P < 0.03$).

Table 2. CNAs associated with progression to DDLS

Copy number alteration	DDLS, n (%)	WDLS, n (%)	FDR
3p14–21 loss	17 (32)	1 (2)	0.0006
3q29 loss	16 (31)	0 (0)	0.0002
9p22–24 loss	14 (27)	2 (4)	0.0006
10p15 loss	12 (23)	0 (0)	0.0006
11q23–24 loss	22 (42)	2 (4)	0.0002
17p11 gain	13 (25)	0 (0)	0.0003
17q21 loss	14 (27)	2 (4)	0.0006
19q13 loss	17 (32)	0 (0)	0.0003
20q11 gain	14 (27)	0 (0)	0.001

Table 3. DDLS morphology ($n = 39$)

Morphology	n (%)	Tumors with chromosome 11q loss (n)
UPS only	6 (15)	3
Mixed UPS + other morphology	7 (18)	4
Myxofibrosarcoma only	5 (13)	1
Mixed myxofibrosarcoma + other morphology	10 (26)	6
Spindle cell sarcoma, NOS only[a]	3 (8)	0
Solitary fibrous tumor only	1 (3)	0
Mixed small blue round cell + other morphology	3 (8)	0
Divergent only	1 (3)	0
Mixed divergent + other morphology	3 (8)	2[b]

Abbreviation: NOS, not otherwise specified.
[a]Resembling adult fibrosarcoma.
[b]Both with myxofibrosarcoma component.

CNAs at 3p14–21, 3q29, and 19q13 were not associated with any histologic correlate or with genomic complexity (Supplementary Table S6).

Survival analysis

We examined survival following R0 or R1 resection of primary DDLS or first local recurrence of DDLS. Median follow-up for this cohort of 40 patients was 2.3 years. Clinical variables (margin, gender, age, and presentation status) did not associate with DSS; however, loss of 19q13 was associated with significant reduction in DSS (HR, 7.36; $P = 0.0025$; Table 4; Fig. 2). Loss of 19q13 was also associated with reduced LRFS (HR, 2.86), as were 9p22–24 loss (HR, 2.35), 17q21 loss (HR, 2.75), R1 resection, and young age. On multivariate analysis of LRFS, loss of 19q13 and young age were independently predictive of shorter LRFS (HR, 2.99 and 0.36, respectively; both $P = 0.01$; Supplementary Table S7). Because of the limited number of events in this cohort, only 3 clinicopathologic characteristics could be evaluated in a single analysis. When multivariate analyses including 19q13 loss and 17q21 or 9p22–24 loss were conducted, the effect of 19q13 loss remained significant (HR, 2.62; $P = 0.025$ and HR, 2.74; $P = 0.019$, respectively).

For DRFS, R1 resection and 3q29 loss were associated with better outcomes. Strikingly, no patient with loss of 3q29 was found to develop distal metastases ($n = 16$).

Possible tumor suppressors on chromosomes 11 and 19

To identify possible tumor suppressors affected by progression-associated CNAs in DDLS, we integrated CNA and gene expression data for 36 DDLS and 33 WDLS samples. Four hundred and thirty genes located within a CNA had a substantial expression change associated with dedifferentiation and/or with presence of the CNA (Supplementary Table S8). The 11q23–24 loss spanned multiple genes that were underexpressed in DDLS compared with WDLS and are, therefore, potential tumor suppressors. Three of these (*ZBTB16*, *PPP2R1B*, and *EI24*) are known tumor suppressors in other cancers. Among DDLS samples, a CNA affecting *EI24* was specifically associated with reduced *EI24* expression; *EI24* expression tended to be lower in DDLS tumors with 11q23–24 loss versus those with diploid 11q23–24 (Supplementary Fig. S4A).

The transition from WDLS to DDLS is characterized by downregulation of the normal adipogenesis program, and several genes that regulate this process were identified in regions of copy number loss. *ASAM* (*CLMP*, which encodes an adhesion protein implicated in adipocyte maturation) was the gene most commonly lost on 11q23–24, though loss of gene expression was not clearly associated with dedifferentiation or CNA. *PLIN2* (perilipin 2, on 9p22), *LIPE* (hormone-sensitive lipase, on 19q13), and *CEBPA* (19q13) are also located within regions of chromosome loss and were underexpressed in DDLS compared with WDLS. The magnitude of *CEBPA* downregulation tended to be greater in tumors with *CEBPA* loss than in those without *CEBPA* loss (Supplementary Fig. S4B). *DLAT* and *ACAD8*, both genes involved in fatty acid metabolism, are located within the 11q23–24 region of loss and were downregulated in concordance with this CNA (i.e., more significantly in tumors with 11q23 loss versus those without the event; Supplementary Table S8).

Table 4. Factors associated with patient outcome

| | Univariate analysis | | | | | |
| | LRFS | | DRFS | | DSS | |
Characteristic	HR	*P*	HR	*P*	HR	*P*
Surgical margin (R1 vs. R0)	**2.92**	**0.035**	**0.12**	**0.043**	1.47	0.503
Gender (male vs. female)	0.46	0.084	0.53	0.40	1.36	0.592
Age (>60 vs. ≤60 y)	**0.32**	**0.011**	0.66	0.59	0.98	0.502
Presentation status (primary vs. local recurrence)	1.4	0.43	1.13	0.87	0.97	0.968
CNAs						
3p14–21 loss	1.94	0.16	0.33	0.30	1.76	0.32
3q29 loss	1.57	0.32	**0.00**	**<0.01**	1.39	0.56
9p22–24 loss	**2.35**	**0.037**	1.28	0.76	0.77	0.70
10p15 loss	1.05	0.92	0.48	0.48	1.55	0.47
11q23–24 loss	1.84	0.16	1.80	0.43	2.69	0.085
17p11 gain	2.08	0.13	1.06	0.94	1.19	0.77
17q21 loss	**2.75**	**0.021**	1.65	0.49	1.04	0.94
19q13 loss	**2.86**	**0.013**	1.51	0.57	**7.36**	**0.0025**
20q11 gain	0.97	0.96	1.40	0.68	0.60	0.51

NOTE: Bold represents significant findings ($P < 0.05$).

Figure 2. A, recurrence-free survival (including both local and distant recurrence). B, DSS among 40 patients with DDLS stratified on the basis of 19q13 copy number.
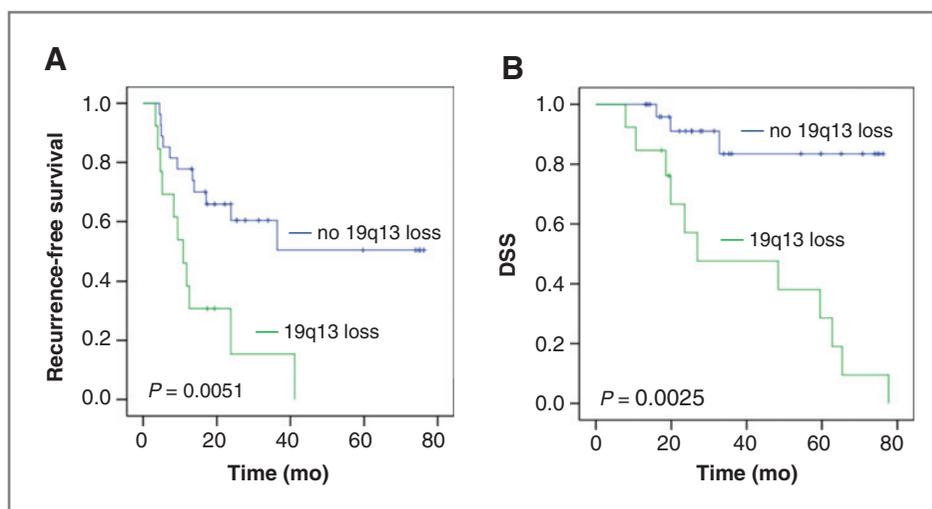
Discussion

While 12q13–15 amplification is associated with the initiation of WDLS, the molecular events that characterize progression to the DDLS histology were previously uncharacterized. In this study, we have assembled the largest data set describing genomic characteristics of WDLS and DDLS. Using array CGH, we showed increased genomic complexity in DDLS versus WDLS and identified 9 common progression-associated CNAs that are nearly specific to DDLS (vs. WDLS). Our results correlated one of these events, 11q23–24 loss, with variations in the genomic complexity and in the morphologic phenotype of DDLS, while showing that 19q13 loss is associated with LRFS and DSS. Each of these represents a novel finding in the description of liposarcomagenesis.

The most common progression-associated CNA involved variable lengths of 11q (centered at 11q23–24). Similar losses have been associated with melanoma and neuroblastoma, though interestingly gains, not losses, on 11q correlate with glioma progression (16–18). In DDLS, 11q23–24 loss was associated with greater genomic complexity. Tumors with 11q23–24 loss, compared with those without this loss, have higher percentages of their genomes altered by CNAs and higher frequencies of other progression-associated CNAs. The genomic complexity associated with 11q23–24 was of particular interest in the context of our examination of tumor histology. The UPS and myxofibrosarcoma subtypes of soft tissue sarcoma are genomically complex. In DDLS, tumors that had components resembling UPS and myxofibrosarcoma tended to have increased genomic complexity compared with those that did not. Moreover, 11q23–24 loss was identified only in DDLS with UPS or myxofibrosarcoma-like components.

Chromosome region 11q23–24 carries several genes that were downregulated during dedifferentiation (e.g., *ZBTB16*, *PPP2R1B*, and *EI24*) and a subset involved in fatty acid metabolism (e.g., *DLAT*, *ACAD8*). It remains unclear, however, whether the loss of 11q23–24 is a result of genomic instability, or if a gene on the chromosome modulates

genomic stability. The 11q23 gene *H2AX* is thought to play a role in regulation of genomic stability in neuroblastoma (17), but we were unable to detect downregulation of *H2AX* in DDLS with the 11q23–24 loss either by U133A array or quantitative real time PCR (not shown). Many of the genes on 11q23–24 whose expression is downregulated in conjunction with loss of copy number are poorly characterized and could modulate chromosome integrity.

Loss of 19q13, although associated with progression of WDLS, was not associated with an overall increase in genomic complexity, with the other progression-associated CNAs, or with DDLS histology. However, 19q13 loss was closely associated with patient outcomes. In this relatively homogenous group of tumors (in terms of size, site, and margin status), outcome could not be clearly stratified on the basis of clinical factors, but loss of 19q13 was associated with both LRFS and DSS. Though 3q29, 9p22–24, and 17q21 loss were associated with LRFS or DRFS, these events did not seem to alter DSS; this finding was specific for 19q13 loss.

Tumors with 19q13 loss (and concomitant *CEBPA* loss) tended to have lower expression of *CEBPA*, a gene recently identified as a tumor suppressor in DDLS (Wu and colleagues; manuscript submitted). Lower expression of *CEBPA* is also associated with worse outcomes among patients with liposarcoma of any subtype, and this gene contributes to a multigene predictor designed to identify patients with high-risk liposarcoma (19). The association we observed between *CEBPA* loss and *CEBPA* levels suggests that expression of this gene may be a marker of 19q13 loss or, given its tumor suppressor effects in DDLS *in vitro*, may partially mediate the survival effects seen in this subgroup of patients.

Taken together, our results suggest a model in which 12q13–15 amplification causes initiation of liposarcomagenesis and formation of WDLS (Supplementary Fig. S5). Tumors that dedifferentiate develop any of several divergent morphologies in response to varied genomic events. Loss of 11q23–24, either in response to an unknown event or as an

independent event, is associated with tumors that are chromosomally complex and are morphologically similar to myxofibrosarcomas or UPS. For tumors without 11q23–24 loss (tumors of other morphologies and a subset of myxofibrosarcoma-like and UPS-like lesions), progression to DDLS is caused by unknown events. Loss of 19q13 is associated with an aggressive phenotype, perhaps as a result of reduced expression of *CEBPA* and is independent of morphology.

This model can act as a scaffold for our understanding of liposarcomagenesis. Data about tumors that do not have common CNAs can easily be integrated into this outline, and genomic and histologic subtype data can be used as the basis for correlative studies in upcoming clinical trials. The tumor subgroups defined by this model may respond very differently to therapies, whether novel or traditional. For example, 11q23–24 carries the gene *EI24*, implicated in regulation of p53 and representing a potential tumor suppressor in DDLS, and 11q23–24 loss is associated with resistance to etoposide-based chemotherapies in other diseases (20, 21). Therefore, 11q23–24 copy number (and *EI24* expression) may identify patients more or less likely to respond to etoposide-containing combination therapies.

Similarly, high-risk patients stratified on the basis of 19q13 loss may be more likely to benefit from neoadjuvant regimens or combination therapies at the time of disease recurrence.

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