Clinical Cancer Research

Genomic Evolutionary Patterns of Leiomyosarcoma and Liposarcoma

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Abstract

Purpose: Leiomyosarcoma and liposarcoma are common subtypes of soft tissue sarcoma (STS). Patients with metastatic leiomyosarcoma or dedifferentiated liposarcoma (DDLPS) typically have worse outcomes compared with localized leiomyosarcoma or well-differentiated liposarcoma (WDLPS). A better understanding of genetic changes between primary/metastatic leiomyosarcoma and between WDLPS/DDLPS may provide insight into their genetic evolution.

Experimental Design: We interrogated whole-exome sequencing (WES) from "trios" of normal tissue, primary tumor, and metastatic tumor from individual patients with leiomyosarcoma ($n = 9$), and trios of normal tissue, well-differentiated tumor, and dedifferentiated tumor from individual patients with liposarcoma ($n = 19$). Specifically, we performed mutational, copy number, and tumor evolution analyses on these cohorts and compared patterns among leiomyosarcoma and liposarcoma trios.

Results: Leiomyosarcoma cases harbored shared drivers through a typical parent/child relationship where the metastatic tumor was derived from the primary tumor. In contrast, while all liposarcoma cases shared the characteristic focal chromosome 12 amplicon, most paired liposarcoma cases did not share additional mutations, suggesting a divergent evolutionary pattern from a common precursor. No highly recurrent genomic alterations from WES were identified that could be implicated as driving the progression of disease in either sarcoma subtype.

Conclusions: From a genomic perspective, leiomyosarcoma metastases contain genetic alterations that are also found in primary tumors. WDLPS and DDLPS, however, appear to divergently evolve from a common precursor harboring 12q amplification, rather than as a transformation to a higher-grade tumor. Further efforts to identify specific drivers of these distinct evolutionary patterns may inform future translational and clinical research in STS.

Introduction

Sarcomas represent a heterogeneous group of malignant tumors, with over 50 histologic subtypes identified in the most recent World Health Organization Classification of Tumors of Soft Tissue and Bone (1). Leiomyosarcoma and liposarcoma are two of the most common histologies, accounting for approximately 30% and 25% of all soft tissue sarcomas, respectively.

Leiomyosarcoma typically expresses markers of smooth muscle differentiation and can occur at almost any soft tissue anatomic site as a primary tumor. Leiomyosarcoma has a notable risk of developing distant metastasis (up to 50% depending on the site and grade of the primary tumor), most frequently to distant sites via hematogenous spread, with local recurrences being less common. Once metastatic, leiomyosarcoma is incurable with current therapeutic approaches (2).

The most frequent form of liposarcoma is represented by a disease spectrum comprising well-differentiated liposarcoma (WDLPS), a nonmetastasizing, indolent, but locally recurrent disease with adipocytic differentiation; and dedifferentiated liposarcoma (DDLPS), an aggressive, rapidly growing sarcoma with a significant risk of locoregional recurrence, as well as a 15%–20% risk of distant metastatic spread (3). Both the well-differentiated and the dedifferentiated components of liposarcoma can present as the sole component of a tumor or they can coexist within the same tumor.

For both leiomyosarcoma and liposarcoma, the genomic drivers of oncogenic transformation have been explored, and these include recurrent *TP53* mutations in leiomyosarcoma and elements of the focal chromosome 12q amplification (including *CDK4* and *MDM2*) in both WDLPS and DDLPS (4). However, for

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Translational Relevance

This study identifies heterogeneity of genomic evolution in leiomyosarcoma and liposarcoma. Whereas leiomyosarcomas demonstrate a typical pattern of progression to metastatic disease, liposarcomas exhibit early divergence between well- and dedifferentiated foci. These findings provide insights into the clinical evolution of leiomyosarcoma and liposarcoma, and more generally, progression patterns in cancers.

both leiomyosarcoma and liposarcoma, genomic analysis of patient-matched tumors following progression from localized/indolent to metastatic/aggressive tumors has not yet been described. For liposarcoma, prior studies have implicated additional copy number gains in *ASK1/MAP3K5* and *JUN* in the transition to DDLPS (5, 6), although these studies were not performed in samples obtained from the same patient and/or tumor.

We hypothesized that the genomic evolutionary patterns of localized to metastatic leiomyosarcoma and well-differentiated to dedifferentiated liposarcoma might be distinct and reflect unique biological processes, as manifested by their differing clinical behavior and patterns of spread. To address this hypothesis, we performed genomic analyses using whole-exome sequencing (WES) on paired samples from individual patients with early localized and late metastatic leiomyosarcoma tumors ($N = 9$), as well as paired specimens of WDLPS and DDLPS from within individual patients ($N = 19$) to determine the phylogenetic relationship underlying histopathologic differences and disease progression in these tumors.

Materials and Methods

Patient selection

Patients with leiomyosarcoma and liposarcoma were identified through retrospective clinical review (A.J. Wagner, S. George, and S. Sioletic). All patients provided written informed consent for research review of medical records and analysis of pathology specimens; including molecular profiling of tumors and germline (Dana-Farber Cancer Institute Institutional Review Board approved protocol no. 05-434). Studies were conducted in accordance with recognized ethical guidelines.

Pathology review

All samples underwent detailed pathology review to confirm diagnosis by an expert sarcoma surgical pathologist in a single center (B. Quade, M.R. Nucci, S. Sioletic, and J.L. Hornick), including delineation of WDLPS and DDLPS features within a given sample.

Molecular profiling

WES. For WES, 150 bp insert libraries were prepared by Covaris sonication (Covaris), followed by SPRI size-selection (Agencourt AMPure XP beads) and ligation to molecular barcoded adaptors for multiplexed analysis. Exome hybrid capture was performed using Agilent SureSelect All Exon v2.0 hybrid capture kit as described previously (7).

Whole-genome sequencing. Library construction was performed as described previously (7) with some slight modifications. Initial genomic DNA input into shearing was reduced from 3 μg to 80 ng in 50 μL of solution. Genomic DNA was then acoustically sheared using the Covaris E210 to a mean fragment length of 385 bp. In addition, all enzymatic steps were performed using the Kapa Library preparation kit, while Illumina TruSeq PCR free adapters, containing unique 8 base index sequences embedded within the adapter, were used in adapter ligation. Using the Kapa hifi library amplification kit, eight cycles of PCR amplifications were performed using a 3-minute extension time during each cycle. Following PCR, an AMPure bead cleanup was performed, after which these libraries were subsequently quantified using PicoGreen.

Somatic mutation analysis and filtering. Somatic single-nucleotide variations and small insertions or deletions were identified using MuTect (ref. 8; v2.4) and Indelocator (http://www.broadinstitute.org/cancer/cga/indelocator), respectively, and were annotated using Oncotator (v1.0.0.0rc27; http://www.broadinstitute.org/cancer/cga/oncotator). Oxidative DNA damages were removed as described previously (9). The cross-individual score was determined using the ContEst algorithm (10). ContEst scores of >4% were excluded. Mean target coverage and contamination scores can be found in Supplementary Tables S1–S4.

Copy-number analysis. Somatic copy-number alterations were called from the WES data by using the ReCapSeg tool (http://gatkforums.broadinstitute.org/gatk/categories/recapseg-documentation; additional details are available at https://www.biorxiv.org/content/10.1101/566505v1). Also, allele-specific analysis was performed using Allelic-Capseg as described previously (11, 12).

Phylogenetic analysis. Cancer cell fractions (CCF) for each mutation and purity estimates for each tumor sample were discovered using the previously published ABSOLUTE algorithm (13, 14) A CCF value of 0.7 would suggest that the mutation in question is present in 70% of all cancer cells. Phylogenetic trees were constructed using the Phylogic algorithm. Phylogic clusters mutations with similar CCF values; this will estimate the number of (sub) clones. Phylogic then uses the number of (sub)clones to infer evolutionary trees. The length of each branch is determined by the number of mutations added between each (sub)clone. CCF values for all mutations and indels can be found in Supplementary Tables S5 and S6.

Deep assessment of liposarcoma sample quality. To account for alternate explanations of the observed divergent mutations in paired WDLPS and DDLPS samples, we performed the following additional procedures. First, we explored the potential for tumor-in-normal contamination and applied deTin algorithm (15); the highest contamination estimate was 2%, which is not likely to impact mutation calling (Supplementary Table S2). Next, we accounted for whether formalin-fixed, paraffin-embedded (FFPE) degradation had reduced the quality of our FFPE samples. The difference between two read pair orientations was attributed to the FFPE-specific error rate and converted to a Phred-based quality score (FFPE_Q). FFPE_Q values less than 30 reflect the component of sequencing errors arising from FFPE artifacts, whereas FFPE_Q values exceeding 30 indicate a low contribution of FFPE artifact to the total sequencing error rate in a given DNA sample (Supplementary Table S3). However, only 10 of 38 of our samples

did not have FFPE_Q scores of >30. Finally, because low tumor purity could also impact mutation detection power, we assessed tumor purity through ABSOLUTE (ref. 13; Supplementary Table S2) and determined that all tumor samples had purity estimates between 24% and 70%, with a median of 46%. A purity of 24% means that we may miss some subclonal mutations, but it is enough purity to be able to detect clonal events.

Results

Tumor evolution from localized primary to metastatic leiomyosarcoma

To establish the baseline genomic status of leiomyosarcoma across the exome, WES was performed on 28 pairs of primary leiomyosarcoma tumor and normal blood (Table 1). The germline mean target coverage was 135× (115×–161×) and tumor mean target coverage was 139× (115×–178×; Supplementary Table S1). *TP53* alterations (these could be point mutations, heterozygous or homozygous deletions, or indels) were present in 17 of 28 (61%) primary leiomyosarcoma (Fig. 1A), consistent with previous studies (16). As previously described, other less common somatic alterations also were noted in *RB1*, *PTEN*, and *PIK3CA* (Fig. 1A). Focal chromosome 17 amplifications, including *MAP2K4*, were also observed in 16 of 28 (57%) primary leiomyosarcoma (Fig. 1B), consistent with prior studies (1, 16). Thus, other than *TP53* and focal chromosome 17 gain, no additional recurrent somatic mutations were observed across the exome in this leiomyosarcoma cohort. Genomic abnormalities in uterine ($N = 16$) versus nonuterine leiomyosarcoma ($N = 12$) were generally similar in these patients sampled, and the unique histologic subset of myxoid uterine leiomyosarcoma ($N = 7$) did not appear to have a distinct genomic pattern.

Building upon the baseline leiomyosarcoma genomic data, whole exomes of matched "trios" of normal blood, primary tumor, and available metastatic tumor samples from 9 of the 28 patients were sequenced to enable phylogenetic analyses between primary and metastatic leiomyosarcoma arising within each individual. A representative phylogenetic tree from the leiomyosarcoma cohort (patient 4; Fig. 1C) revealed truncal somatic alterations, including clonal mutations in *TP53* that were shared between the primary and metastatic tumors. The phylogenies of the eight other leiomyosarcoma patient sample trios showed similar patterns (Supplementary Fig. S1). Across the nine leiomyosarcoma primary metastasis-matched cases, 83.4% (332/398) of clonal mutations in the primary were also observed in the metastasis of the same individual (Fig. 1D), including all putative driver mutations (e.g., *TP53*), consistent with genomic tumor evolution models for other cancer types (17, 18). Mutations in genes exclusive to the metastases were not recurrent and were unique to the individual tumors (Supplementary Table S2), and some involved putative tumor suppressor genes/oncogenes, including *TP53*, *CREBBP*, *PTEN*, *FGFR1*, and *PLAG1*. Therefore, leiomyosarcoma exhibits a typical parent/child evolutionary relationship with shared clonal drivers and additional clonal mutations between matched primary and metastatic tumors, without clearly identifiable recurrent changes that correlate with development of metastases.

Characterization of WDLPS and DDLPS somatic genomics

To examine the genomic differences between WDLPS and DDLPS, and to compare with leiomyosarcoma patient tumors, 19 tumors that harbored both WDLPS and DDLPS components were identified through pathology review (Table 1). DNA was extracted from the regions of WDLPS and DDLPS and then subjected to WES (Fig. 2A–C; Supplementary Table S3). The germline mean target coverage was 149× (105×–183×) and tumor mean target coverage was 151× (90×–187×) in these cases (Supplementary Table S4).

In one representative case (patient 11), genomic analysis across the whole exome of WDLPS and DDLPS from a single tumor from 1 patient revealed a high degree of overlap of copy number changes in chromosome 12 (Fig. 2D; Supplementary Fig. S3). It also showed a short truncal component of the phylogenetic tree with only one shared nonsynonymous point mutation of uncertain functional significance (*CDK4* P40S; Fig. 2E). To further examine this finding, WES was subsequently performed on a trio of specimens including WDLPS, DDLPS, and normal tissue (mean coverage ∼19×) from this patient. The tumors, but not the normal sample, had nearly complete overlap between breakpoints in the chromosome 12 region that included *CDK4* and *MDM2* (Supplementary Fig. S2) indicative of an initial genome-wide rearrangement event in a precursor cell, consistent with reported models of neochromosome formation in liposarcoma (19). However, beyond the amplification and rearrangements involving chromosome 12, the WDLPS and DDLPS samples shared only one other rearrangement (Fig. 2F–H), again suggesting genomic divergence from an early precursor event.

Expanding the investigation across the entire liposarcoma cohort, focal chromosome 12 amplifications were observed in all WDLPS and DDLPS samples (Fig. 3A and B). There was significant intrapatient correlation between chromosome 12 copy ratios ($r^2 = 0.87$; $P < 0.001$; Pearson; Fig. 2D; Supplementary Fig. 3), and all cases manifested similar divergent mutation-based phylogenies with short phylogenic trunks and a paucity of overlap in shared mutations (Supplementary Fig. S4). In fact, only 14.8% (92/620) of clonal mutations were shared between WDLPS and DDLPS within the same tumor (Fig. 3D), sharply contrasting with the genomic evolutionary model inferred from the comparative study of primary versus metastatic leiomyosarcoma tumors

Table 1. Clinical characteristics of the leiomyosarcoma and liposarcoma cohorts

Histotype	Location/histology	Number of patients	Female (%)	Median age, years (range)
Leiomyosarcoma	Uterine/myxoid	7	100	58 (44–77)
	Uterine/high-grade spindle cell	8	100	64.5 (54–73)
	Nonuterine/high-grade spindle cell	13	85	51.9 (31.2–63.8)
Liposarcoma	Retroperitoneal	15	53	59.3 (35.3–78.5)
	Inguinal	2	0	55.6
	Extremity	2	100	58.8

NOTE: Baseline clinical characteristics of the patient cohorts are described in this table, including histologic subtyping when available.

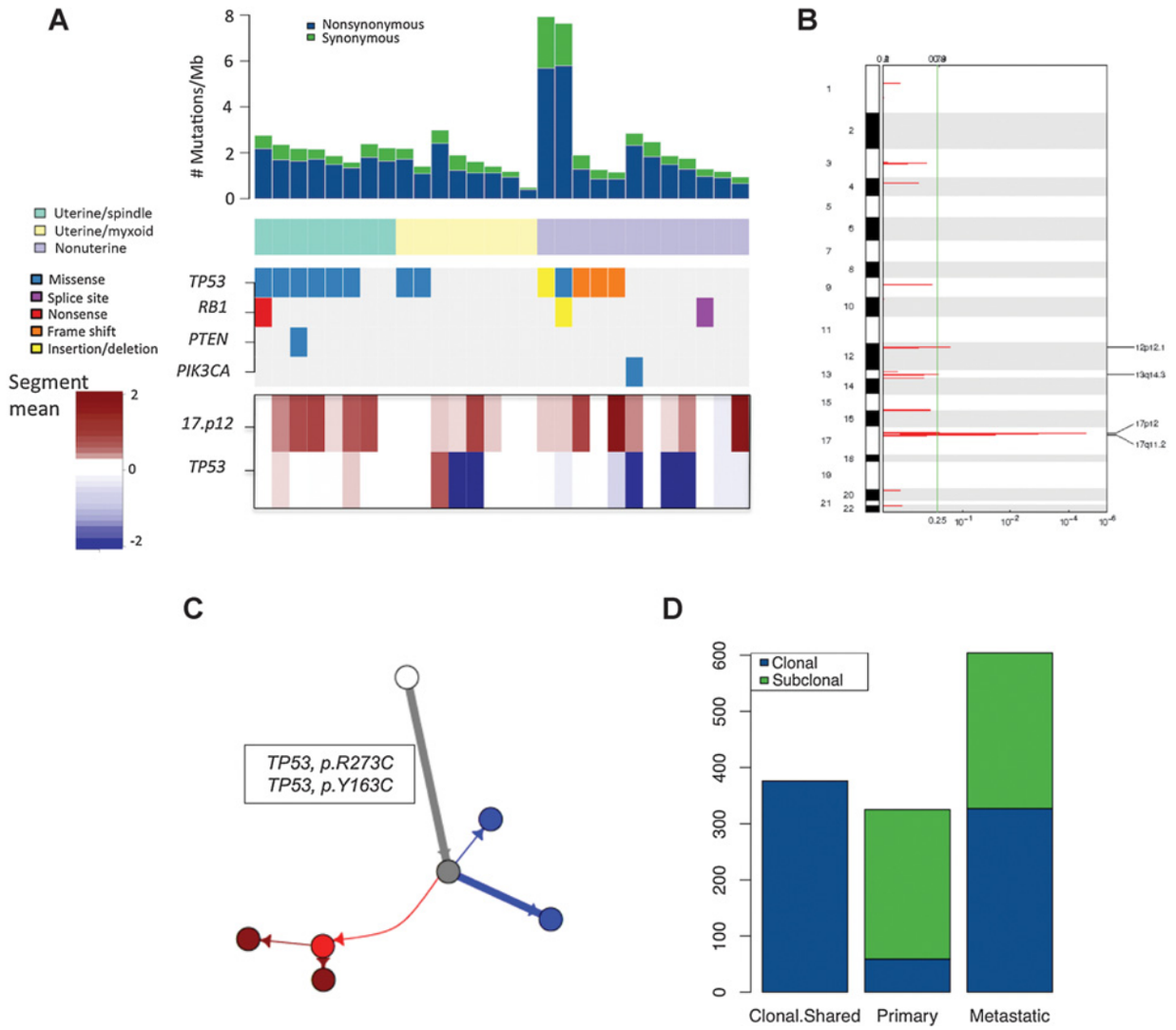

Figure 1.

Genomic overview of matched primary (localized) and metastatic leiomyosarcomas. **A,** Overview of mutations observed in the 28 primary localized leiomyosarcoma samples, each from a unique patient. Each column represents a sample/patient. Mutation rates in the tumor samples are shown in the top barplot with reference to synonymous and nonsynonymous mutation rates. Leiomyosarcoma cases were classified into three subtypes of uterine-myxoid, uterine-spindle cell, and nonuterine. Mutations in established biologically relevant genes in leiomyosarcoma (*TP53, RB1, PTEN*, and *PIK3CA*) are shown. *TP53* mutations were the most frequent somatic alterations observed. Similarly, the bottom track shows amplifications in chromosome 17p12. **B,** Copy number alteration significance in primary leiomyosarcoma samples. Copy number alteration significance through GISTIC (24) in primary leiomyosarcoma samples demonstrate chromosome 17p12 is the only region that is significantly amplified (FDR < 0.0001). **C,** Phylogenetic tree of the primary and metastatic sample of patient 4. The white circle represents the germline sample, the gray circle shows the inferred parent clone of the tumor samples before there was divergence, the red circles represent the metastatic (sub)clones, and the blue circles represent the primary (sub)clones. The length of the branches shows the proportion of mutations accumulated between each clone. **D,** Barplot of the frequency of mutations demonstrates how many of the mutations were categorized as shared between primary and metastatic (shared), exclusive to the primary samples, or exclusive to the metastatic samples. The mutations have been also distinguished on the basis of clonality. A considerable percentage of clonal somatic alterations are shared between the primary and metastatic samples.

(Fig. 1D). Mutational divergence was observed across the cohorts even before correcting for tumor purity, filtering for formalin fixation/paraffin embedding artifact, and performing tumor-in-normal deconvolution analyses to investigate potential alternative explanations for these discordant matched WDLPS/DDLPS mutation sets.

While no highly recurrent genomic driver distinguishing WDLPS and DDLPS was observed, somatic *CDK4* point mutations were detected at low frequency in four of 19 (21%) DDLPS tumors (Fig. 3E), and in only one case was the *CDK4* mutation also observed in the matched WDLPS component (patient 11). None of these patients had received CDK4 inhibitors prior to resection of their tumors. The observed *CDK4* mutations have not previously been described (20) and are of unknown functional significance. Broadly, WDLPS and DDLPS exhibit nearly private clonal mutations indicative of early divergence from a common

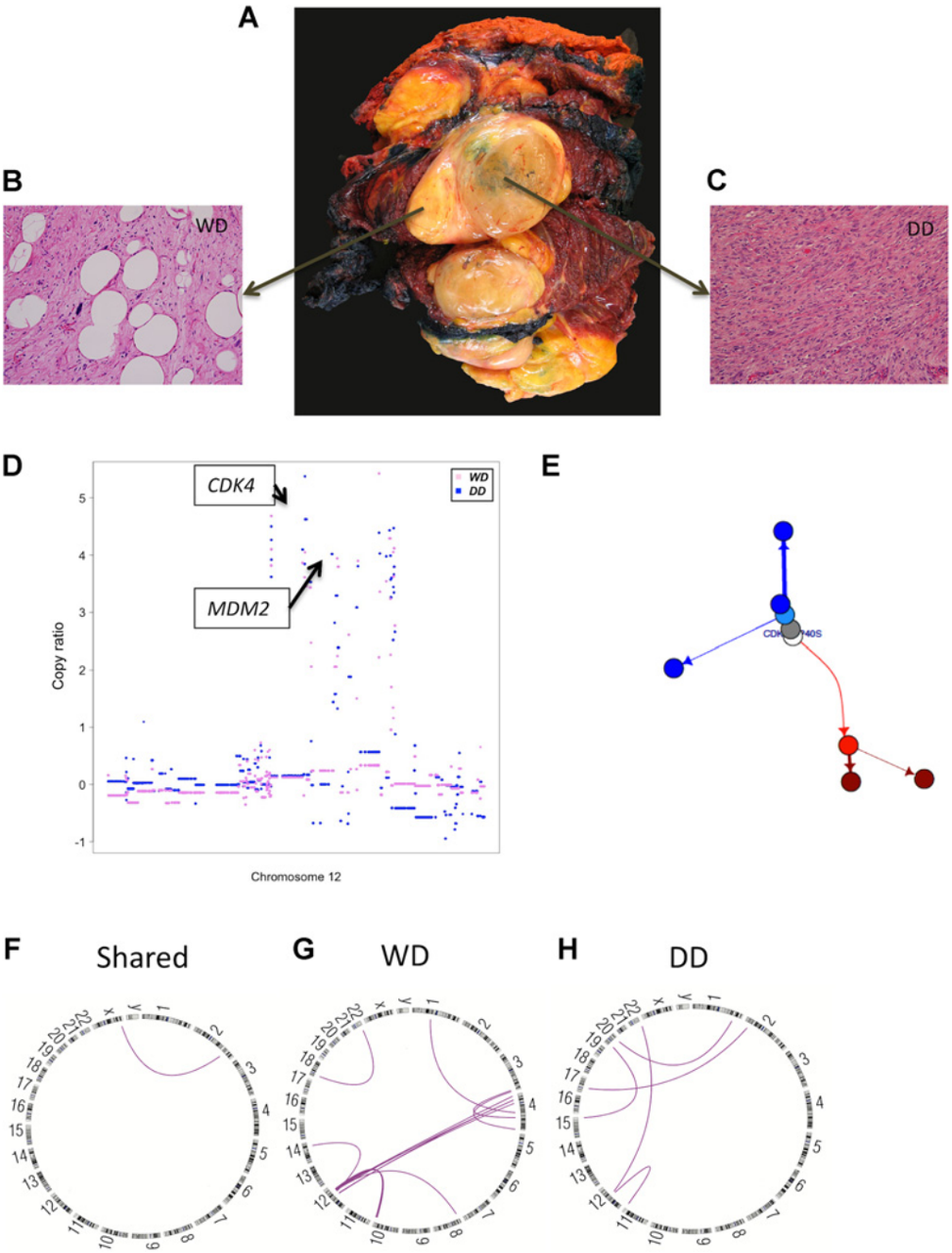

Figure 2.

Patient-matched WDLPS and DDLPS showing a distinct genomic pattern of tumor evolution. **A,** Gross resection specimen of a representative liposarcoma sample highlights well-differentiated (WD) and dedifferentiated (DD) components of a mixed liposarcoma. Histology images of WDLPS (**B**) and DDLPS (**C**) from patient 11 are shown. **D,** Overlap of copy number changes in well-differentiated and DDLPS in chromosome 12 of patient 11 demonstrates high concordance between segmentations at those loci. Breakpoints occur in the same locations in both samples. *CDK4* and *MDM2* are highlighted. **E,** Phylogenetic tree of the well-differentiated and DDLPS sample of patient 11. The white circle represents the germline sample, the gray circle shows the inferred parent clone of the tumor samples before there was divergence, the red circles represent the dedifferentiated (sub)clones, and the blue circles represent the well-differentiated (sub)clones. The length of the branches shows the proportion of mutations accumulated between each clone. **F,** Rearrangements shared between the well-differentiated and DDLPS samples in patient 11. **G,** Rearrangements unique to WDLPS. **H,** Rearrangements unique to DDLPS.

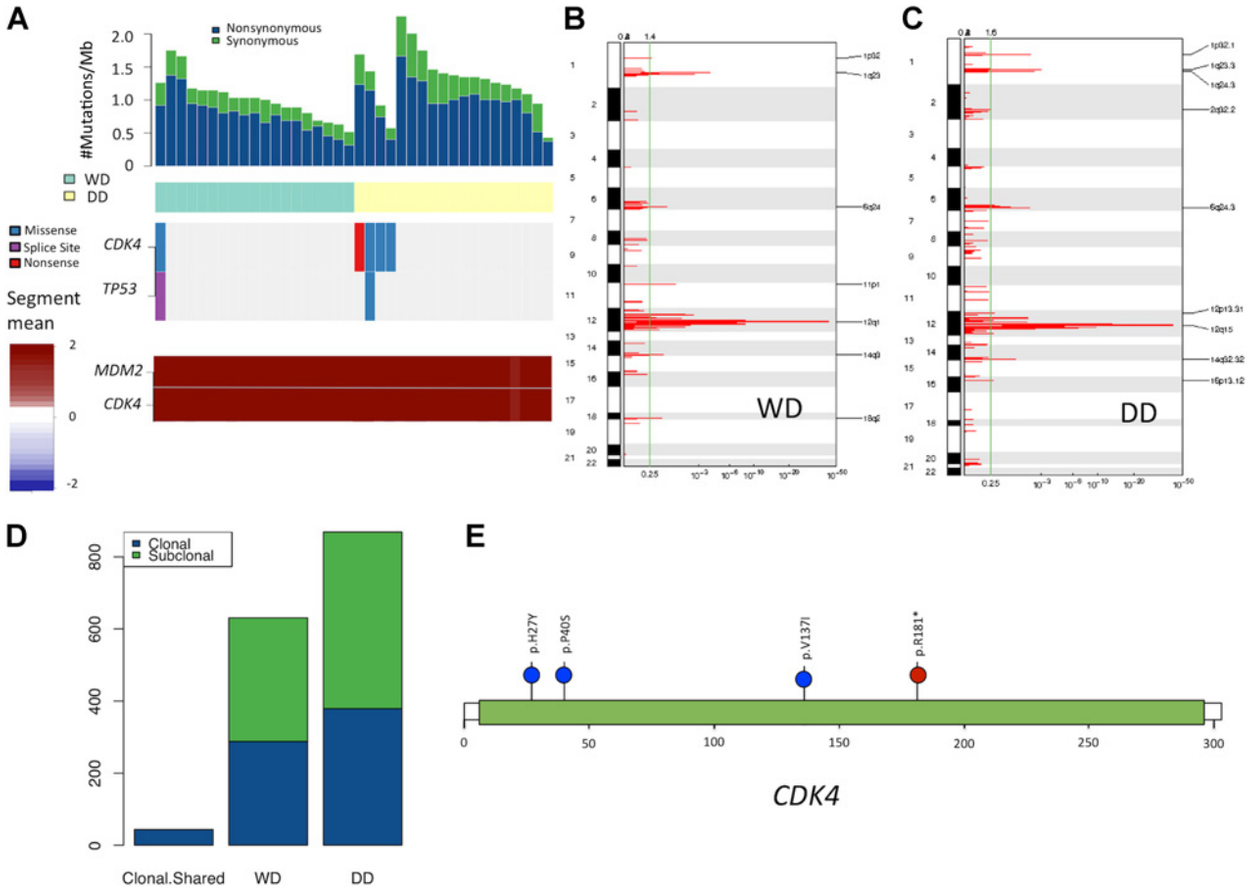

Figure 3.

Genomic overview of the 19 matched well-differentiated (WD) and dedifferentiated (DD) liposarcoma samples. **A,** An overview of mutations in the 19 well-differentiated and DDLPS samples highlights mutation rate (top), established biologically relevant genes in liposarcoma (middle), and focal amplifications involving *MDM2*/*CDK4* (bottom). GISTIC copy number analysis demonstrates focal amplification in chromosome 12 in well-differentiated (**B**) and dedifferentiated (**C**) liposarcoma samples. **D,** Overview of the frequencies of subclonal and clonal mutations that are either shared between primary and metastatic samples from the same individual or that are exclusive to either cohort. **E,** Rare (∼1%) point mutations are shared by the well-differentiated and DDLPS samples from the same patient.

12q-amplified precursor, rather than transition from WDLPS to DDLPS within a given tumor.

Discussion

Genomic evaluation of tumors longitudinally and in a multiregional fashion has provided molecular insights into tumor evolution patterns across a variety of malignancies (13, 18). While genomic characterizations of leiomyosarcoma and liposarcoma have previously been performed (1, 4, 16, 21), a comprehensive assessment of changes between early and advanced tumor states in individual patients has not been described and may identify molecular features related to this progression.

We have demonstrated that primary and metastatic leiomyosarcoma from individual patients share a significant number of mutations, providing genomic evidence that a subclone of the primary tumor shed cells that disseminated, and thus the metastasis is presumed to be the progeny of the primary tumor. Although additional mutations were seen in the metastases that were not present in the primary tumor, no clearly recurring genomic alteration in an individual gene defined the metastatic event across samples from multiple patients. The presence of shared clonal mutations in both the primary and metastatic samples indicate that therapeutic development geared toward these alterations, if possible, may be an effective strategy to impact all sites of tumor within a patient.

In contrast, the study of different elements coexisting in liposarcoma tumors suggests an evolution that differs from the leiomyosarcoma pattern. While all analyzed components of tumors shared highly concordant chromosome 12 amplification, this event appears to have occurred early in oncogenesis and was followed by divergent clonal evolution, rather than a straightforward serial transition from WDLPS to DDLPS, which instead would have been reflected by subsets of mutations shared in both regions of the tumor, as illustrated in the primary-to-metastasis examples of leiomyosarcoma. This observation challenges the previously established view that dedifferentiated liposarcomas arise in a transformation event from well-

differentiated liposarcomas. Rather, our data demonstrate the novel observation that these tumors arise from a shared precursor cell and diverge early through mechanisms not observable in the sequenced exome.

Notably, in neither leiomyosarcoma nor liposarcoma were we able to identify a recurrent alteration exclusive to the aggressive or advanced tumors when compared with the patient-matched primary or well-differentiated tumors. It is possible that the driving lesions for these clinical phenotypes occur in noncoding regions or are the product of epigenetic changes. For example, miRNAs were found to be differentially expressed in subtypes of liposarcoma and may contribute to clinical behavior (22). In addition, *HDAC1* mutations were reported at a low frequency in liposarcoma (4). Similar alterations were not found in any of the 19 cases that we analyzed in this cohort, which may be the result of limited sample size. The role of chromatin modification or chromosomal instability (e.g. chromoplexy) in the development or maintenance of liposarcoma remains unknown; however, given the relative paucity of mutations in the exome, further evaluation of mechanisms other than genomic sequence alterations is warranted.

Of note, in 21% of tumors we identified novel mutations in *CDK4*, a gene known to be highly amplified in most liposarcomas. The functional significance of the amino acid substitutions observed in our study is unknown, and their prevalence should be explored in a larger cohort of samples. Similarly, an increase in *CDK4* copy number potentially could contribute to the development of DDLPS; however, the absolute *CDK4* copy number was qualitatively similar in the whole-genome patient trio, and the correlation between WDLPS and DDLPS lesions in chromosome 12 was strong (see Supplementary Data).The ability to determine the amount of focality (e.g., 25 copies vs. 50 copies) from bulk WES data is confounded by many technical (purity and sequencing depth) and biological (e.g., spatial heterogeneity) effects that make it not reliably quantifiable and limits the ability to assess changes in *CDK4* copy number from this dataset.

CDK4 inhibitors are FDA approved for treatment of metastatic breast carcinoma and have been studied in patients with advanced WDLPS/DDLPS (23). In liposarcoma the radiographic response rate to the CDK4 inhibitor palbociclib is low, although minor prolongation of disease control compared with historical values was reported. The *CDK4* mutational status of tumors from patients participating in these studies was not reported and thus it is unclear whether there is any correlation between the *CDK4* genotype and response or resistance to inhibitors.

Finally, this study highlights the utility of serial patient-matched tumor profiling to inform tumor evolution trends, especially as applied to uncommon tumor types with unique clinical features. In this context, patient-matched sample "trio" analyses enabled the observation of tumor evolution patterns that sharply contrast among two common mesenchymal tumors. These results may have implications for understanding the origins of liposarcoma and open possibilities for other factors that influence transition to either WDLPS or DDLPS within an individual (e.g., epigenetic). Furthermore, given the phylogenetic relationship between primary and metastatic leiomyosarcoma, this study highlights the need to sample metastatic sites in this context as the genomic profile may be related but not identical to the primary tumors, although the primary drivers of metastasis in these diseases remains to be defined.

Disclosure of Potential Conflicts of Interest

S. George has immediate family members who hold ownership interest (including patents) in Allergan and Abbot Labs, is a consultant/advisory board member for Deciphera, Blueprint Medicines, Eli Lilly, AstraZeneca, Bayer, Research to Practice, and Huron Consulting, and reports receiving other remuneration from Bayer, UpToDate, Wolters Kluwer, NCCN, WebMD, E Squared Communications, and MORE Health. G. Getz reports receiving commercial research grants from IBM and Pharmacyclics. J.L. Hornick is a consultant/advisory board member for Eli Lilly and Epizyme. G.D. Demetri is an employee of Blueprint Medicines and Merrimack Pharmaceuticals, reports receiving commercial research grants from Janssen, PharmaMar, Epizyme, Daiichi-Sankyo, and Abbvie, and is a consultant/advisory board member for Alexandria, Bayer, Caris Life Sciences, Daiichi-Sankyo, EMD Serono, Epizyme, G1 Therapeutics, Mirati Therapeutics, Pfizer, Physicians Education Resource, Polaris Pharma, Sanofi, WIRB Copernicus Group, and Ziopharm. L.A. Garraway is an employee of Eli Lilly and Company, a previous employee of Howard Hughes Medical Institute and Foundation Medicine, reports receiving commercial research grants from Novartis and Astellas, and holds ownership interest (including patents) in Tango Therapeutics. E.M. Van Allen reports receiving commercial research grants from Novartis and Bristol-Myers Squibb, holds ownership interest (including patents) in Syapse, Genome Medical, Tango Therapeutics, and Microsoft, and is a consultant/advisory board member for Genome Medical, Tango Therapeutics, Dynamo, Invitae, and Illumina. A.J. Wagner reports receiving commercial research grants to his institution from Eli Lilly, Plexxikon, Daiichi-Sankyo, AADi Bioscience, and Karyopharm and is a consultant/advisory board member for Eli Lilly, Daiichi-Sankyo, Novartis, Nanosphere, and Five Prime Therapeutics. No potential conflicts of interest were disclosed by the other authors.

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