Genomic characterization of sarcomatoid transformation in clear cell renal cell carcinoma

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The presence of sarcomatoid features in clear cell renal cell carcinoma (ccRCC) confers a poor prognosis and is of unknown pathogenesis. We performed exome sequencing of matched normal-carcinomatous-sarcomatoid specimens from 21 subjects. Two tumors had hypermutation consistent with mismatch repair deficiency. In the remainder, sarcomatoid and carcinomatous elements shared 42% of somatic single-nucleotide variants (SSNVs). Sarcomatoid elements had a higher overall SSNV burden (mean 90 vs. 63 SSNVs, $P = 4.0 \times 10^{-4}$), increased frequency of nonsynonymous SSNVs in Pan-Cancer genes (mean 1.4 vs. 0.26, $P = 0.002$), and increased frequency of loss of heterozygosity (LOH) across the genome (median 913 vs. 460 Mb in LOH, $P < 0.05$), with significant recurrent LOH on chromosomes 1p, 9, 10, 14, 17p, 18, and 22. The most frequent SSNVs shared by carcinomatous and sarcomatoid elements were in known ccRCC genes including von Hippel–Lindau tumor suppressor (*VHL*), polybromo 1 (*PBRM1*), SET domain containing 2 (*SETD2*), phosphatase and tensin homolog (*PTEN*). Most interestingly, sarcomatoid elements acquired biallelic tumor protein p53 (*TP53*) mutations in 32% of tumors ($P = 5.47 \times 10^{-17}$); *TP53* mutations were absent in carcinomatous elements in nonhypermutated tumors and rare in previously studied ccRCCs. Mutations in known cancer drivers AT-rich interaction domain 1A (*ARID1A*) and BRCA1 associated protein 1 (*BAP1*) were significantly mutated in sarcomatoid elements and were mutually exclusive with *TP53* and each other. These findings provide evidence that sarcomatoid elements arise from dedifferentiation of carcinomatous ccRCCs and implicate specific genes in this process. These findings have implications for the treatment of patients with these poor-prognosis cancers.

kidney cancer | sarcomatoid | transformation | p53 | dedifferentiation

Sarcomatoid transformation is common in various epithelial malignancies, featuring further loss of differentiation and acquisition of characteristics typical of a sarcoma. In renal cell carcinoma (RCC), sarcomatoid features are observed in 5% of tumors. However, among individuals with stage IV disease, it occurs in 15% (1, 2). Although once believed to represent a distinct subtype of RCC, it is now considered a specific histologic feature (3). Whereas sarcomatoid features are found in all forms of kidney cancer, >65% of cases are found with clear cell RCC (ccRCC) (4, 5). When sarcomatoid features are present, renal tumors generally are large (median size 10 cm), invasive (20%), and/or metastatic (50%) at presentation (4, 5). Whereas all such tumors are considered to be Fuhrman grade IV (6), their prognosis is significantly worse compared with other high-grade tumors (7). These tumors, when metastatic, have among the poorest survival of all genitourinary malignancies, with a median survival of only 6 mo (1, 2, 4). Even with resected localized disease, nearly 75% recur and have a median survival of <2 y (4, 8). The response to systemic therapy is poor, with rare durable responses occurring with any therapeutic strategy (9–12).

Although there have been major breakthroughs in the understanding of kidney cancer, progress in the characterization of the genetic events associated with sarcomatoid kidney tumors has been limited (9). Various theories have been proposed regarding the origins of sarcomatoid features in renal tumors. Given that they virtually always occur in conjunction with typical epithelial RCC elements, the terminology of a "mixed malignancy" appeared a half century ago (13). Proposals have included independent occurrences of tumor types in close proximity, as has been observed in various genitourinary malignancies (14), and the influence of tumor microenvironment (15). The current prevailing theory is that sarcomatoid features represents a subclonal dedifferentiation or transformation from an incident carcinomatous component (16). However, the current theory is based on limited evidence. Evidence of common cell of origin is suggested by the shared patterns of X chromosome inactivation (17). Although there is limited evidence that the epithelial component transforms into the sarcomatoid element, groups have considered sarcomatoid features to result from a final common dedifferentiation pathway in RCC

Significance

Parts of clear cell renal cell carcinomas (ccRCCs) sometimes have histologic features characteristic of a sarcoma. So-called sarcomatoid tumors are more aggressive, difficult to treat, and associated with a poor prognosis. Their pathogenesis has been uncertain. Through separate exome sequencing of carcinomatous and sarcomatoid components, we show that these components share many somatic mutations, including many in genes characteristic of ccRCC. Sarcomatoid elements had significantly more new somatic mutations, particularly in cancer driver genes, than carcinomatous components. In particular, tumor protein p53, AT-rich interaction domain 1A, and BRCA1 associated protein 1 had sarcomatoid-specific homozygous mutation in 10 tumors and were all mutually exclusive, implicating these genes in sarcomatoid degeneration.

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(16). This hypothesis is based on the sarcomatoid component more frequently metastasizing, possessing higher tumor grade, increased proliferative index, and frequent reduced expression of epithelial adhesion molecules such as E-cadherin (18–20). Because some tumors demonstrate increased expression of N-cadherin, it has been suggested that epithelial-mesenchymal transformation (EMT) may be involved in the development of sarcomatoid elements (21). However, what could be driving this process has escaped elucidation.

To address the molecular basis for sarcomatoid elements in RCC, we performed whole exome sequencing of distinct regions of clear cell and sarcomatoid morphology from the same tumors.

Materials and Methods

Patient and Specimen Acquisition. From 1989 to 2010, all patients undergoing nephrectomy for presumed renal cancer at the University of California, Los Angeles (UCLA) had clinical information entered into an approved database. ccRCC tumors featuring sarcomatoid transformation were reviewed by a genitourinary (GU) pathologist (J.W.S.). The clinical data, tumor characteristics, and survival have been described (1, 4, 20). A second GU pathologist (M.J.M.) reviewed representative slides to confirm the presence of distinct morphologic regions that represented (*i*) ccRCC and (*ii*) a definitive region with sarcomatoid transformation. All living patients studied provided written informed consent for participation in this research. A waiver of consent was approved to study anonymized samples from deceased patients. The research protocol was approved by the UCLA and Yale Human Investigation Committees. All experiments were conducted according to the principles expressed in the Declaration of Helsinki.

DNA Extraction, Exome Capture, and Exome Sequencing. Twenty-nine tumors had available formalin-fixed, paraffin-embedded blocks with adjacent normal kidney for genomic control. The blocks were reviewed by a third independent GU pathologist (A.J.A.), who confirmed the histology and identified distinct regions of normal kidney, clear cell, and sarcomatoid histology. From each of these regions, 1-mm punches were obtained and DNA extracted by using a described protocol (22). Exome capture was performed by using Nimblegen 2.1M Human Exome Array followed by 74 base paired-end DNA sequencing on the Illumina HiSeq instrument. Tumor regions were sequenced to greater depth of coverage than normal tissue to account for admixture of tumor and normal cells. High quality sequences were obtained for 21 matched sets of normal, carcinomatous, and sarcomatoid elements.

Sequence Analysis and Comparisons. Sequences were aligned to the hg19 reference genome by using Burrow–Wheeler Aligner-MEM (BWA-MEM) (23). Somatic single nucleotide variant (SNV) calling was performed by using Mutect (24) and indel calling was performed by using Indelocator (https://www.broadinstitute.org/cancer/cga/indelocator). Additional somatic mutation calls were acquired by using a reported pipeline (25). Variant calls with less than a total of eight independent reads in any of the three sequenced samples from each tumor were discarded. Variants previously identified as germ-line variants in 1000 Genomes Project (26); National Heart, Lung, and Blood Institute Exome Sequencing Project; and the Yale University exome database were excluded. All somatic mutation calls were manually verified by visual inspection. Somatic mutations identified by Mutect in one component (e.g., the sarcomatoid but not carcinomatous elements) were called specific for that component when the variant was not called in the other component, had $P \leq 0.05$ for two-tailed Fisher's exact test of the difference in the distribution of reference and nonreference reads between the sarcomatoid and carcinomatous components, and MAF <10% or nonreference read count <3 in the component lacking the variant. Empirically, component-specific variants had median counts of 14 independent variant reads in the component in which they were called and 0 variant reads in the component in which they were absent. All other somatic mutations were called in both components or were not excluded and were classified as shared. Phylogenetic trees were constructed for each sample based on the component distributions of somatic mutations. In gene burden analysis, the significance threshold for gene mutations was $P = 0.05$ for known cancer driver genes (Pan-Cancer gene set; ref. 27) and $P = 2.5 \times 10^{-6}$ for all other genes to account for consideration of ∼20,000 different genes. Chromosomal segments with loss of heterozygosity (LOH) were identified from departure of the minor allele frequencies of heterozygous SNPs in tumor samples from the frequencies seen in matched normal samples (25). Chromosomal arms with elevated rates of LOH specific to sarcomatoid components were identified by using a binomial distribution with a false discovery rate < 0.25. Tumor purities were estimated by using the difference in allele frequencies between tumor and normal components in regions of LOH.

Results in these tumors were compared with sequencing results seen in 424 ccRCCs in The Cancer Genome Atlas (TCGA) ccRCC (28). Local pathology reports of TCGA samples were reviewed to identify samples with sarcomatoid features. Data from cBioPortal was used to determine the frequency of specific mutations, overall mutational burden, and concomitant LOH in these samples (29).

Results

Exome Sequencing of Carcinomatous and Sarcomatoid Elements of ccRCCs. The clinical and pathologic features of the 21 patients with ccRCC with sarcomatoid transformation are shown in Table S1. Cancer-specific survival was poor, with a 1- and 2-y survival of 38 and 30%, respectively (Fig. S1). Similar to other cohorts, tumors were large (median 10 cm), were frequently associated with metastases (66.6%), and frequently showed local invasion (80.9% T3/T4; Table S1).

Carcinomatous and sarcomatoid elements were separately dissected from each tumor. Whole exome sequencing was separately performed on matched DNA samples comprising normal tissue, carcinomatous, and sarcomatoid components of each primary tumor. A summary of sequencing metrics is shown in Table S2. Normal, carcinomatous, and sarcomatoid components were sequenced to a mean depth of 135, 177, and 171 independent reads per targeted base in the exome. Median tumor purity was estimated at 62% (range 33–82%) for the sarcomatoid and 46% (range 18–75%) for the carcinomatous components; these values are similar to estimates in other ccRCC cohorts such as TCGA (median purity 54%, range 18–87%; ref. 28). There was no correlation between median read depth per sample and the number of somatic mutations detected in either carcinomatous or sarcomatoid regions (both $r^2 < 0.01$, $P > 0.9$), consistent with high sensitivity for calling of somatic mutations.

Landscape of Mutation Burden. Somatic single nucleotide variants (SSNVs) and chromosome segments showing LOH were called in each tumor as described in *Materials and Methods*. In 19 tumors, the mean total number of SSNVs, including both shared and component-specific, was 108 ± 33 (range 41–163; Fig. 1*A*). The other two tumors were >5 SD outliers in both tumor components, with a total mutation burden of 597 in one tumor and 434 SSNVs in the other. These two tumors also had a mutational signature characteristic of mismatch repair (MMR) deficiency, with an abundance of C:G > T:A transitions and a paucity of A:T > C:G, A:T > T:A, and C:G > G:C transversions (30) (Fig. 1*B*). These tumors were considered to have hypermutation based on prior definitions (31, 32) and evidence of mismatch repair deficiency. Consistent with this classification, one hypermutated tumor had a heterozygous somatic truncating mutation at R389 in mutS homolog 2 (*MSH2*) in both carcinomatous and sarcomatoid elements with sarcomatoid-specific LOH at this locus and a sarcomatoid-specific heterozygous E1085K mutation in polymerase ε (*POLE*). Neither of these samples had a rare germ-line protein-altering variant (minor allele frequency <0.001) in MMR genes (*MSH2-6, MLH1, MLH3, PMS1-2, PSMP3, POLE*). In contrast, there were no examples of hypermutation in the ccRCCs studied by TCGA (no tumor with more than 128 SSNVs) (28, 32) ($P = 0.002$) (Fig. S2). Both hypermutated tumors had homozygous/hemizygous von Hippel–Lindau tumor suppressor (VHL) SSNVs, making misclassification unlikely, suggesting that these hypermutated tumors may be more likely to develop sarcomatoid features. Because these hypermutated tumors may differ from the others biologically, these tumors were separately analyzed. Cancer genes with somatic mutations in these tumors are shown in Fig. S3.

Common Origin of Carcinomatous and Sarcomatoid Elements. Among the nonhypermutated tumors, sarcomatoid and carcinomatous elements shared a mean of 45/108 (41.7%) SSNVs, providing

Fig. 1. Somatic mutations in 21 renal tumors with sarcomatoid features. (*A*) Somatic mutation counts in 21 tumors by tumor component. Sample IDs labeled on bottom axis. (*B*) Somatic mutation pattern by single nucleotide change. (*C*) Presence of somatic mutations and LOH for significantly mutated and genes of interest. (*D*) Frequency of LOH events by chromosome region in the carcinomatous (green) and sarcomatoid (red) tumor components for 14 nonhypermutated tumors with complete genome-wide LOH data. (*E*) Presence of LOH in chromosomal segments with significant sarcomatoid-specific LOH.

unequivocal evidence that these elements arise from a common cell of origin that bears many somatic mutations. The known cancer genes (using the Pan-Cancer gene set; ref. 27) that most frequently shared somatic mutations in both carcinomatous and sarcomatous elements were *VHL* (SSNV+LOH in both elements in 11/19 tumors), polybromo 1 (*PBRM1*) (SSNV+LOH in both elements in 4/19 tumors), and SET domain containing 2 (*SETD2*) (SSNV+LOH in both elements in 4/19 tumors) (Fig. 1*C* and Table S3). Moreover, these three genes are linked to one another on chromosome 3p; this segment shows LOH in every tumor. Somatic SSNVs in these three genes and LOH of 3p are hallmarks of ccRCC (28), and the evidence that these mutations predate the split of carcinomatous and sarcomatoid elements provides strong evidence that these tumors initially arise as ccRCC.

Increased Burden of Cancer Driver Mutations in Sarcomatoid Elements. Among somatic mutations that were specific to either sarcomatoid or carcinomatous elements, sarcomatoid components had a significantly higher burden of unique SSNVs (mean 45 vs. 18 SSNVs per tumor, $P = 6.2 \times 10^{-4}$; Fig. 2*A*). Similarly, sarcomatoid components had nearly twice the length of element-specific somatic LOH (median 913 Mb vs. 460 Mb, $P < 0.05$; Fig. 1*D*).

The minor allele frequencies (MAFs) of component-specific SSNVs were significantly lower than those of shared SSNVs. Among carcinomatous components, the median MAFs were 15.3% vs. 21.4% for component-specific and shared SSNVs, respectively ($P < 2.2 \times 10^{-16}$ by Mann–Whitney *u* test). Similarly, in sarcomatoid elements the median MAFs were 19.4% vs. 27.0% for component-specific and shared SSNVs, respectively ($P < 2.2 \times 10^{-16}$ by Mann–Whitney *u* test). These findings are consistent with many component-specific mutations arising after clonal lineage separation.

Component-specific, nonsynonymous SSNVs in known cancer genes were significantly more frequent in sarcomatoid than carcinomatous elements (respectively 27 total, mean 1.4 SSNVs per tumor, vs. 5 total, mean 0.26; $P = 0.002$ by Wilcoxon signed rank test; Fig. 2*B*). Nonsynonymous somatic mutations in known cancer genes also occurred more often than expected by chance in sarcomatoid elements ($P = 1.7 \times 10^{-6}$) but not carcinomatous elements ($P = 0.08$). Consistent with this finding, the ratio of nonsynonymous/synonymous (NS/S) SSNVs was 5.4 for cancer genes vs. 3.7 for other genes in sarcomatoid elements, whereas the NS/S ratio was not elevated among Pan-Cancer genes compared with other genes in carcinomatous elements (2.5 vs. 2.9) (Fig. 2*C*). These findings lend further support to the evolution of

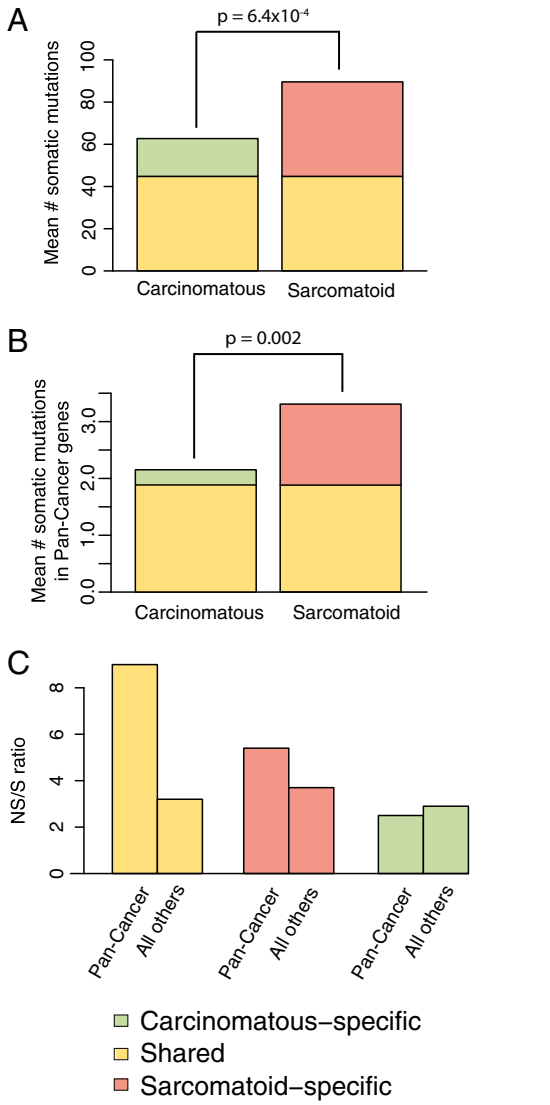

Fig. 2. Comparison of somatic mutations in carcinomatous and sarcomatoid elements. (*A*) Mean number of somatic mutations by tumor component for the 19 nonhypermutated tumors. Among all mutations, 41.7% were shared between tumor components. Sarcomatoid regions had a significantly higher number of component-specific mutations (mean 45 vs. 18, $P = 6.2 \times 10^{-4}$ by Wilcoxon signed-rank text). (*B*) Mean number of nonsynonymous somatic mutations in known Pan-Cancer genes by tumor component. Sarcomatoid regions had a significantly higher number of component-specific mutations (1.42 vs. 0.26, $P = 0.002$ by Wilcoxon signed-rank test). (*C*) Ratio of nonsynonymous to synonymous mutations in known Pan-Cancer genes by tumor component.

sarcomatoid elements from carcinomatous elements by acquisition of somatic mutations in cancer drivers.

Sarcomatoid-Specific Mutations in Tumor Protein p53, AT-Rich Interaction Domain 1A, and BRCA1 Associated Protein 1. Among sarcomatoid-specific SSNVs in known cancer genes, the frequency of mutation in tumor protein p53 (*TP53*) was remarkable (Table S3). There were no SSNVs or segments of LOH involving *TP53* among carcinomatous regions in these tumors. In contrast, six sarcomatoid elements acquired biallelic *TP53* mutations (six NS SSNVs that were all homozygous/hemizygous via LOH), an event highly unlikely to occur by chance ($P = 5.47 \times 10^{-17}$; Fig. 1*C*). Phylogenetic trees of the carcinomatous and sarcomatoid components of these six tumors are shown in Fig. 3. Sarcomatoid-specific mutations also occurred in other cancer driver genes, including two mutations in BRCA1 associated protein 1 (*BAP1*) and three in AT-rich interaction domain 1A (*ARID1A*). With the exception of one *ARID1A* mutation, all were accompanied by LOH ($P = 3.24 \times 10^{-5}$ and $P = 1.54 \times 10^{-5}$ for presence of SSNV and LOH in *ARID1A* and *BAP1*, respectively). Interestingly, all biallelic *TP53*, *ARID1A*, and *BAP1* mutations were mutually exclusive ($P = 0.08$, Monte Carlo simulation), suggesting that these SSNVs may represent alternative pathways toward sarcomatoid transformation (Fig. 1*C*). Consistent with this interpretation, mutual exclusivity of *ARID1A* and *TP53* is commonly observed in ovarian and endometrial malignancies (33, 34).

Among tumors with genome-wide LOH data in both components, several chromosomes showed recurrent segments of sarcomatoid-specific LOH that were unlikely to have occurred by chance. These segments included chromosome 1p (57%, all including *ARID1A*, $q = 0.030$); chromosome 9 (86%, all including *CDKN2A*, $q = 0.007$), chromosome 10 [36%, all including phosphatase and tensin homolog (*PTEN*), $q = 0.108$] chromosome 14 (64%, $q = 0.108$), chromosome 17p (43%, all including *TP53*, $q = 0.030$), chromosome 18 (50%, $q = 0.188$), and chromosome 22 (29% tumors, $q = 0.210$) (Fig. 1 *D* and *E*).

We also sought other genes with SSNVs that occurred more often than expected by chance on either lineage (Table S3). FAT

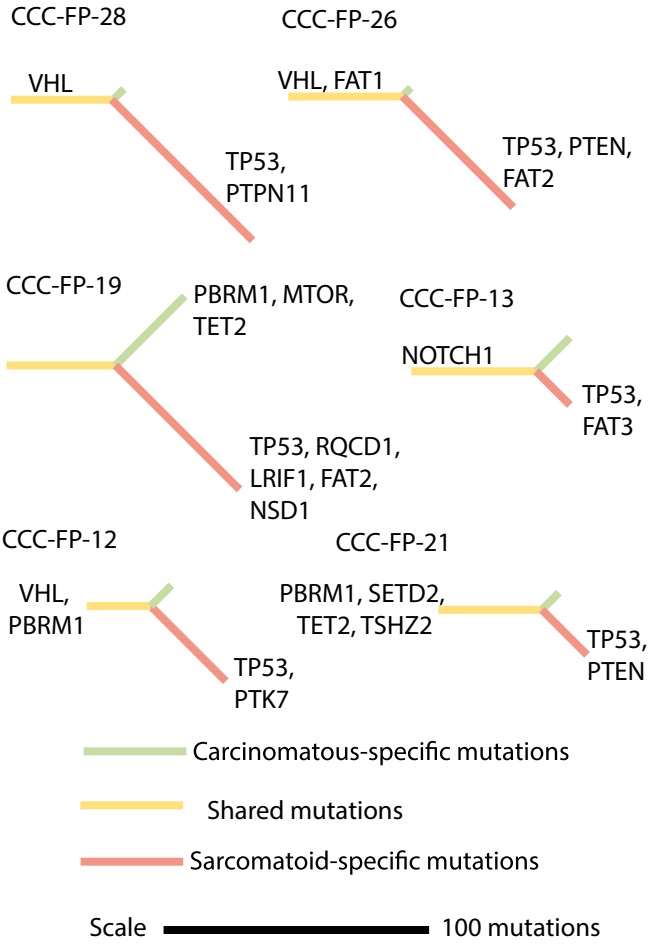

Fig. 3. Phylogenetic trees of six *TP53*-mutant, nonhypermutated ccRCC tumors. Branch and trunk lengths correspond to the number of somatic mutations in each tumor component, including shared, carcinomatous-specific, and sarcomatoid-specific mutations. Mutations in previously described ccRCC genes, Pan-Cancer genes, and other recurrently mutated genes (*TSG101*, *RQCD1*, *LRIF1*, *PTK7*, and *FAT* family) are shown. Sample IDs are labeled at top left of each phylogenetic tree.

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atypical cadherin 2 (*FAT2*) was the second most frequently mutated gene in the sarcomatoid-specific gene burden analysis (four SSNVs) and the top mutated gene not previously implicated in ccRCC ($P = 4.84 \times 10^{-5}$). Six other genes not previously implicated in ccRCC harbored sarcomatoid-specific SSNVs in two tumors each, some in segments of LOH. These genes include two additional *FAT* genes, *FAT1* and *FAT3*, as well as tumor susceptibility 101 (*TSG101*), ligand-dependent nuclear receptor interacting factor 1 (*LRIF1*), required for cell differentiation1 homolog (*RQCD1*), and protein tyrosine kinase 7 (*PTK7*). Details of these and other SSNVs in driver genes are shown in Table S4. Phylogenetic trees of all tumors, by tumor component and including mutated cancer genes, are shown in Fig. S3.

Discussion

The presence of sarcomatoid features has long been recognized as an extremely poor prognostic factor in kidney cancer. However, until now, a genetic basis of sarcomatoid transformation has remained largely unknown. Our finding that 43% of somatic mutations are shared between carcinomatous and sarcomatous elements provides conclusive evidence that these elements arise from a common clonal ancestor. Despite the suggestion of a shared clonal origin, there has previously been little evidence that the sarcomatoid component arose in a process of dedifferentiation from a preexisting carcinomatous component (16). The data herein provides strong evidence of a carcinomatous origin. The most frequently mutated cancer drivers that are shared by carcinomatous and sarcomatous elements are the genes that are characteristically mutated in ccRCC. Second, the burden of component-specific SSNVs in known cancer drivers is more than fivefold higher on the sarcomatoid than carcinomatous regions. Third, there is a highly significant burden of sarcomatoid-specific mutation of *TP53*, implicating a specific gene in development of sarcomatoid elements, along with recurrent mutations and/or segments of LOH affecting other known cancer genes. These findings support a pathogenic sequence in which somatic mutations occurring in a ccRCC drives dedifferention to a sarcomatoid state. Importantly, the finding of highly significant sarcomatoid-specific mutation signals is inconsistent with the observed differences being the result of simple heterogeneity within tumors, in which case differences between carcinomatous and sarcomatoid elements would be expected to be stochastic.

The high frequency of biallelic *TP53* mutations in sarcomatoid elements was striking. These biallelic mutations were found in 6 of 19 (31.5%) nonhypermutated tumors. *TP53* mutations are otherwise rare in ccRCC (28, 35). For example, among 395 ccRCCs reported by TCGA that do not have sarcomatoid elements, only 6 of 395 (1.5%) have *TP53* mutation ($P = 3 \times 10^{-6}$, Fisher exact test, odds ratio 29), and only two of these mutations are in segments of LOH (Fig. S4). These findings are consistent with prior work from Oda et al. who performed a candidate gene study of 14 tumors with sarcomatoid transformation and noted a higher incidence of *TP53* mutations in the sarcomatoid region by using immunohistochemistry and Sanger sequencing (36). *TP53* alterations may link the EMT pathway to sarcomatoid transformation, because p53 loss can reduce expression of miR-200c, which contributes to EMT (37). Additionally, one hypermutated tumor contained an R175H alteration in *TP*53, a known gain-of-function mutation that results in up-regulation of TWIST1, an important EMT transcriptional regulator (38). Interestingly, in Wilms' tumor, another form of renal cancer, loss of *TP53* also leads to histologic dedifferentiation (anaplasia) and a poor prognosis (39, 40).

We identified somatic mutations in genes that are characteristic for ccRCC, including *VHL, PBRM1, SETD2, PTEN, ARID1A*, and *BAP1*. Notably, all *ARID1A* and *BAP1* SSNVs were exclusive to sarcomatoid regions, all but one were in segments of LOH, and were mutually exclusive with each other and with *TP53* mutations. Deficiency of *ARID1A* and *BAP1* has been associated with worse prognosis, higher tumor grade, and a higher incidence of sarcomatoid histology (41, 42). However, many other tumors show only LOH at these loci, suggesting these events may be permissive of, but insufficient for, sarcomatoid transformation. Although mutual exclusivity of mutation in *TP53* and *ARID1A* has been described in ovarian and endometrial cancer, the explanation has been unclear. Recently, their gene products have been shown to form a complex that regulates transcription of *CDKN1A* and *SMAD4* (33), suggesting that mutation of these genes may be equivalent and sufficient to promote tumorigenesis via a common pathway.

The incidence of *VHL* SSNVs was 57.9% (11/19 tumors). Additionally, all tumors had LOH of chromosome 3p. Consistent with its role as an early event in tumorigenesis (43, 44), all *VHL* mutations were shared in carcinomatous and sarcomatoid elements. *VHL* alterations (mutation and hypermethylation) have been considered the hallmark of ccRCC (45, 46). For centrally reviewed ccRCC, the incidence of *VHL* mutation is as high as 81.3% (47), which suggests that our cohort had a lower incidence of *VHL* mutation (35, 47). Similarly, it has been shown that wild-type *VHL* ccRCC has a more aggressive phenotype (48, 49), perhaps related to an increased propensity for sarcomatoid transformation.

Mutations in genes not implicated in ccRCC may be relevant to sarcomatoid transformation. Sarcomatoid-specific mutations in *FAT2* were found in five tumors. Mutations in other members of the FAT family, including *FAT1* and *FAT3*, were in two tumors each. FAT family mutations were rarely found in ccRCC in TCGA (Fig. S4). FAT proteins play multiple roles in cell adhesion, motility, polarity, signaling, and proliferation, and mutations are implicated in a variety of cancers (50–52). Loss of *FAT1* has been shown to promote WNT signaling, a critical mediator of EMT (53). Further exploration of these genes in sarcomatoid elements will be required to assess the significance of the role of *FAT* genes. Mutations in several other genes were of interest, but will also require larger numbers of samples to assess significance. These mutated genes include *TSG101*, a member of the ESCRT-I complex involved in ubiquitinated protein trafficking (54); *PTK7*, a tyrosine kinase regulator of cell motility, adhesion, polarity, and WNT signaling (55); and *RQCD1* and *LRIF1*, both retinoic-acid receptor transcriptional cofactors (56, 57). Several of these genes were enriched in specimens listed as having sarcomatoid transformation in the TCGA dataset (Fig. S4).

Effective systemic therapy for individuals with sarcomatoid renal tumors is an unmet need in oncology. *TP53* and *ARID1A* are among potential sarcomatoid-specific targets for which therapeutics are in development (58, 59). As drugs that mitigate effects of mutation in these genes enter clinical trials, their study in sarcomatoid renal tumors may be warranted. Similarly, hypermutability in sarcomatoid tumors also has implications for treatment of both tumor components. Loss of key mismatch repair genes can sensitize tumors to radiation and some types of chemotherapy (60). These tumors have also shown sensitivity to immunotherapy for several cancer types, perhaps due to the increased burden of somatic mutation-derived epitopes (61, 62). Lastly, PD-1 and PDL-1 expression has also recently been found to be greater in tumors with sarcomatoid features (63), raising the possibility that these tumors may be responsive to immune checkpoint inhibitor immunotherapy.

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