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ORIGINAL ARTICLE

Multiregion sequencing of sarcomatoid renal cell carcinoma arising from autosomal dominant polycystic kidney disease

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Abstract

Background: Autosomal dominant polycystic kidney disease (ADPKD) is an inherited cystic kidney disease associated with a spectrum of various renal and extrarenal manifestations, including increased risk of kidney cancers. Here, we present the initial molecular description of sarcomatoid renal cell carcinoma (sRCC) arising in the setting of ADPKD.

Methods: Multiregion whole-exome sequencing and whole transcriptomic sequencing were used to examine intratumoral molecular heterogeneity among histologically-distinct spindle (sarcomatoid), epithelioid, or biphasic compartments within the tumor and compared with the non-malignant ADPKD component.

Results: Spindle and biphasic components harbored several overlapping driver gene mutations, but do not share any with the epithelioid component. Mutations in *ATM*, *CTNNB1*, and *NF2* were present only in the biphasic and spindle components, while mutations in *BID*, *FLT3*, *ARID1B*, and *SMARCA2* were present only in the epithelioid component. We observed dichotomous evolutionary pathways in the development of epithelioid and spindle compartments, involving early

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mutations in *TP53* and *ATM*/*CTNNB1*/*NF2* respectively. Wnt, PI3K-mTOR, and MAPK signaling pathways, known key mechanisms involved in ADPKD development, featured prominently in the sarcomatoid component.

Conclusion: This highlights that common pro-oncogenic signals are present between ADPKD and sRCC providing insights into their shared pathobiology.

KEYWORDS

genetics, hereditary, phylogeny, tumor evolution

1 | INTRODUCTION

Sarcomatoid renal cell carcinoma (sRCC) is an uncommon but important clinical entity, given its aggressive nature and poor response to contemporary therapeutic options. Approximately 5% of kidney cancers involve sarcomatoid change, and sRCC is now thought to be a terminally dedifferentiated form of RCC which may arise from any histological subtype, rather than a distinct subtype of RCC (Shuch et al., 2012). This current thinking follows the observation that sarcomatoid tumors always contain some proportion of carcinomatous element, and can be found associated with any histological subtype (Peralta-Venturina et al., 2001). Sarcomatoid change had been demonstrated to be an independent poor prognostic factor for RCC, which confers a median survival of 4–9 months. A higher proportion of sarcomatoid cells in a tumor has also been linked to poorer prognosis (Cheville et al., 2004; Shuch et al., 2009).

Despite its clinical significance, the pathogenesis and genomic alterations underlying sRCC remain poorly characterized. A previous study on sarcomatoid change in the setting of clear cell RCC showed that biallelic mutations in *TP53* were associated with sarcomatoid change. Other cancer driver genes *ARID1A* and *BAP1* were significantly mutated in the sarcomatoid components and were mutually exclusive with *TP53* (Bi et al., 2016). Another study showed that cell-cycle pathways were enriched in sarcomatoid versus adjacent clear cell components, suggesting greater cell proliferation. This was accompanied by increased mTOR pathway activation driven by overexpression of *AUKRA* in the sarcomatoid elements as compared to the clear cell components (Pal et al., 2015). Apart from these limited studies, the molecular pathobiology of sarcomatoid change in non-clear cell RCC has been less frequently documented until recently (Malouf et al., 2016; Wang & Zhang, 2020).

Interestingly, there has been a suggestion that sarcomatoid change occurs in a disproportionately large number of patients with RCC on a background of autosomal dominant polycystic kidney disease (ADPKD; Keith et al., 1994; Yu et al., 2016). ADPKD is the commonest monogenic renal disorder that is correlated with mutations in genes including *PKD1* and *PKD2*. It causes the gradual development of innumerable cysts in the kidneys, as well as cysts in the liver, pancreas, and spleen in adulthood, and often results in end-stage renal failure in late adulthood. There are many known complications of ADPKD, such as renal failure, cyst hemorrhage, infection, and malignancy—a rare (1%) but arguably the most serious complication (Cordido et al., 2017). We came across a patient who developed sRCC on a background of ADPKD, providing the opportunity to study its molecular pathogenesis in this rare setting.

In this study, we investigated a case of sRCC in a patient with known ADPKD and end-stage renal failure. Specifically, we examined the evolutionary pathway of distinct components present in the tumor sample. The possible mechanisms by which ADPKD predisposes to sRCC, in this case, are discussed.

2 | METHODS

2.1 | Patient data and biospecimen collection

All clinical information was retrieved from electronic medical records. Demographic data including sex, age, and ethnicity of the affected patient and his family members were verified against their National Registry Identification Cards. All histological parameters were reviewed by an expert pathologist.

2.2 | Genomic DNA extraction

Genomic material from formalin-fixed paraffin-embedded tumor tissue was available from the index patient only. No material was available from the other family members. Following the manufacturer's instructions, genomic DNA was extracted from tumor specimens and matched normal

tissue using the FFPE RNA/DNA Purification Plus Kit (Norgen Biotek). The yield and quality were determined using the 2100 Bioanalyzer (Agilent Technologies).

2.3 | Library construction, whole exome sequencing, and bioinformatic analysis

Whole exome sequencing was performed as described previously (Chan et al., 2020; Chang et al., 2021). Briefly, hybrid selection was done using the Human All Exon kit SureSelect Target Enrichment System (Agilent Technologies) version 6 and sequenced on the Illumina HiSeq X platform (Illumina) as paired-end 150-base pair reads. Read pairs were aligned to the human reference genome NCBI GRC Build 37 (hg19) using Burrows-Wheeler Aligner (BWA MEM; Wellcome Genome Campus, Hinxton; Li & Durbin, 2009). Optical duplicates were marked with Picard followed by base score recalibration using GATK version 4.1.4 (Broad Institute) for post alignment data processing (McKenna et al., 2010). Potential germline variants were screened for by filtering for the following conditions: missense or splice site variants with mapping quality >Q20, sequencing depth >50, alternate allele depth >15, min alt fraction of 0.1. Somatic variants from the resulting normal and tumor BAM files were identified using Mutect2, and subsequently annotated and prioritized using VEP (Wellcome Genome Campus; McLaren et al., 2016). Mutational signature identification was performed using SigProfiler Bioinformatics Tools (Wellcome Genome Campus; Alexandrov et al.,). Copy-number segmentations were processed with TitanCNA v1.17.1 (University of British Columbia; Ha et al., 2014).

2.4 | RNA isolation and gene expression analysis

Total RNA was extracted using the FFPE RNA/DNA Purification Plus Kit (Norgen Biotek). Transcriptomic analysis was performed using the Illumina Ampliseq Transcriptome Human Gene Expression Panel (Illumina). Principal component analysis and Poisson distribution (Witten, 2011) were used to describe the data. For pathway analysis, gene expression was first normalized using an in-house pipeline and filtered against those in the KEGG cancer pathway.

2.5 | Phylogenetic analysis

We constructed a phylogenetic tree based on shared mutations across the various samples examined. In addition, clonal status was estimated from allele frequencies of mutated genes using the SciClone package. SciClone is an R package developed to infer subclonal populations of cells in a tumor sample. The software implements a variational Bayesian mixture model to classify variants into different populations based on their copy number states and allele frequencies, with clusters in the diploid copy number state being potential subclones. A probability is calculated for the presence of each variant in each of the inferred subclones (Miller et al., 2014).

3 | RESULTS

3.1 | Clinical case report

The patient was a 56-year-old Chinese man from Singapore who presented with a 2-month history of worsening painless gross hematuria and low-grade fever. His medical history was significant for ADPKD, complicated by end-stage renal failure requiring renal replacement therapy for the past 6 years, recurrent cyst hemorrhage, urinary tract infections, and hypertension. The patient's mother and a younger brother were also diagnosed with ADPKD (Figure 1a). Computed tomography revealed a solid exophytic mass at the posterior aspect of the right kidney without metastasis (Figure 1b). The patient underwent bilateral radical nephrectomy with clear histological margins. Gross pathological examination of the specimen revealed a solid whitish tumor 5 cm in largest dimension, surrounded by ill-defined, variegated, solid-cystic-hemorrhagic areas, in total measuring 10.7 by 8 by 5 cm. These lesions were on a background of innumerable thin-walled cysts (Figure 1c). Microscopic examination of the lesion demonstrated sarcomatoid carcinoma with tubulopapillary epithelial elements. Perinephric fat invasion was seen but renal vein invasion was absent. Sections from the whitish tumor showed distinct regions of heterogeneity (Figure 1d), consisting of sarcomatoid components with spindle cells (30%), epithelial components with tubulopapillary architecture and a variable/mixed component consisting of cells with elongated, high-grade pleomorphic nuclei. Non-tumor areas showed cysts and atrophic parenchyma in keeping with ADPKD. Immunohistochemical staining for epithelial markers MNF116 and AE 1/3 were positive in both spindle and epithelial areas. In epithelial areas, AMACR, vimentin, CAIX, EMA, and CD10 stains were at least patchily weak positive, while CK7, CK20, and P63 stains were negative. Following the diagnosis of stage 2 RCC, the patient did not receive any adjuvant treatment and remained disease-free until 8 months later, when he relapsed with retrocaval nodal, adrenal, and lung metastases. The patient received sunitinib (37.5 mg daily

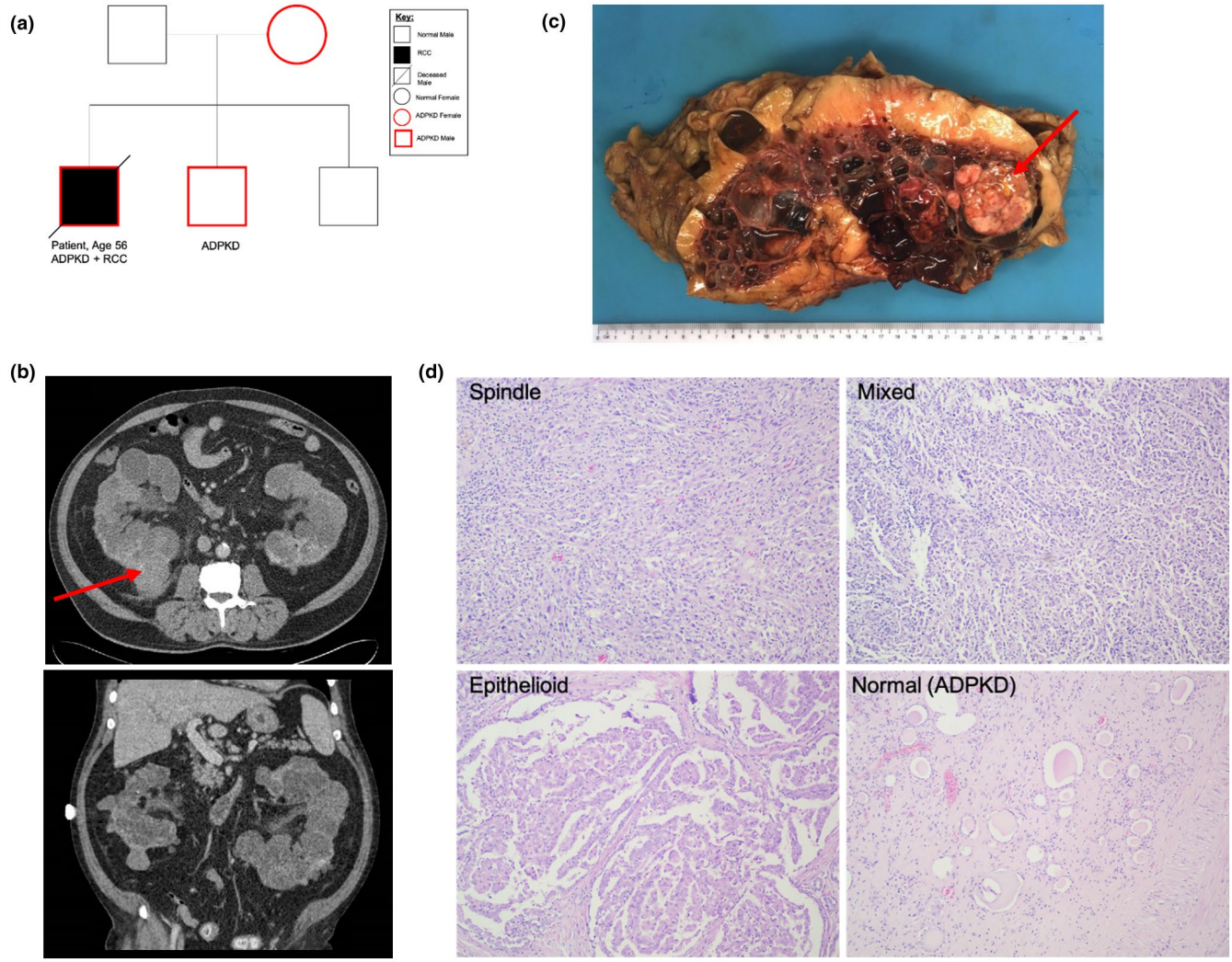

FIGURE 1 Clinical characterization of the patient with sRCC arising from ADPKD. (a) Pedigree chart describing the patient's family history with ADPKD. (b) Images of the RCC tumor on the CT scans showing a mass on the right kidney. (c) Photograph of the affected kidney after radical nephrectomy. (d) Immunohistochemistry (IHC) profiles of the three tumor sections and normal (ADPKD) tissue from the patient samples

2 weeks on, 1 week off) for 17 months, followed by three cycles of nivolumab (3 mg/kg given monthly), as well as 1 month of everolimus (5 mg daily), before succumbing to disease 15 months from the time of relapse.

3.2 | Genomic analysis by whole-exome sequencing

The top 10 cancer driver gene mutations in the three tumor components: spindle, epithelioid, and mixed are represented in an oncoplot (Figure 2a). We observed that the mixed and spindle components contain several overlapping driver gene mutations, but do not share any with the epithelioid component. The top three recurrently-mutated genes were *ATM* (67%), *CTNNB1* (67%), and *NF2* (67%) which were only present in the mixed and spindle components. Missense mutations in *BID* (33%), *FLT3* (33%), *ARID1B* (33%), and *SMARCA2* (33%) were only present in the epithelioid component but not in the mixed and spindle elements. Mutation count in the epithelioid component was the highest (894), followed by mixed (535) then spindle (385), with intronic mutations being the most common across all elements. The most common single nucleotide variation that occurred was C > T (1184), while the dominant COSMIC mutational signatures were SBS 1 and SBS 5 (both clock-like signatures). We identified a putative pathogenic germline splice site mutation in the *PKD1* gene (c.7490-2A>G; ClinVar accession number VCV000562314.1; Figure S1a). The copy number landscapes of the three tumor components are shown in Figure S1b.

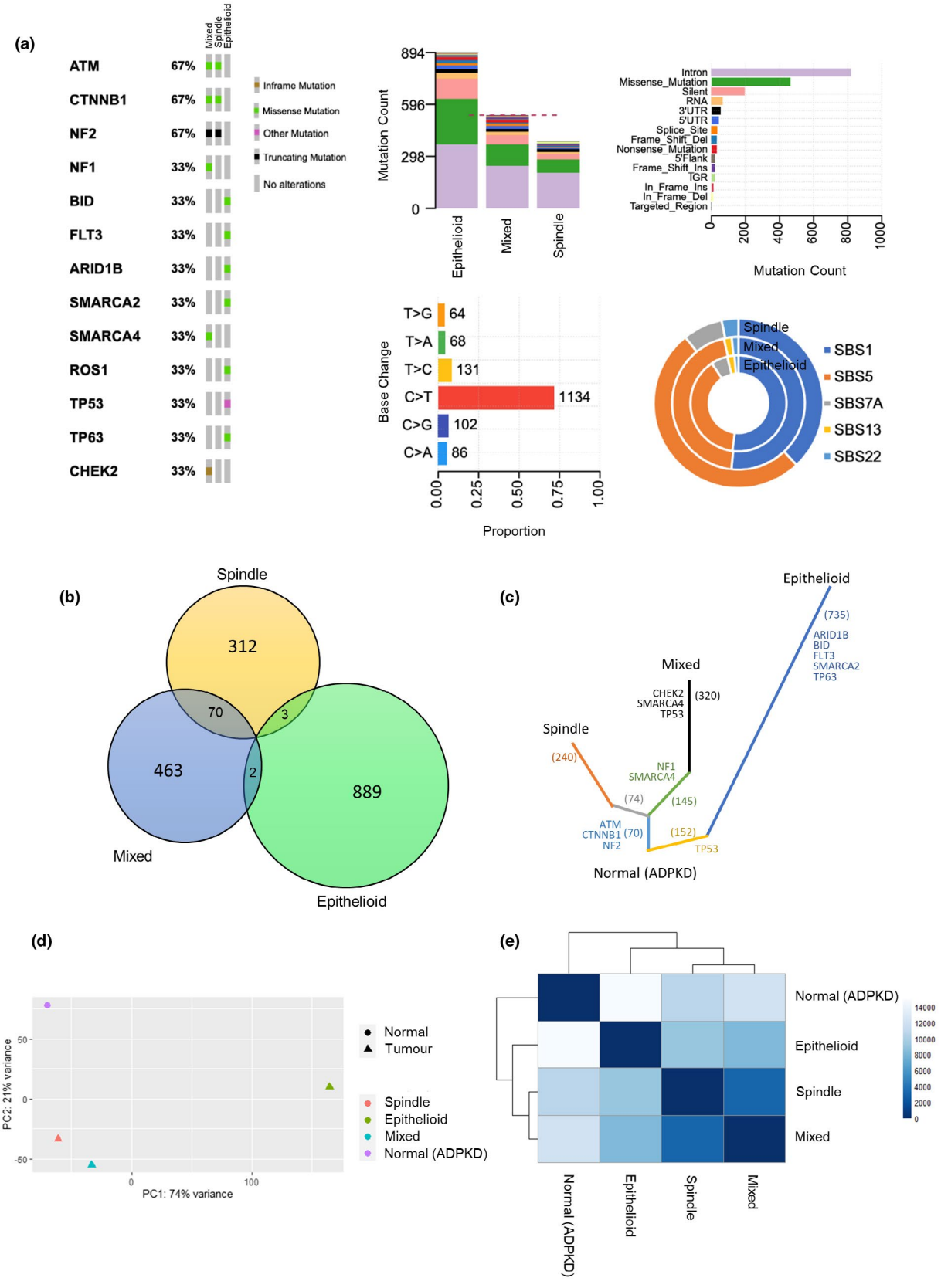

FIGURE 2 Mutational and phylogenetic analysis. (a) Oncoplot showing the mutations found in the samples. Graphs showing the mutation count; mutations sorted by mutation type; base changes; and mutational signatures. (b) Venn diagram describing the overlap of all mutations between the three components. (c) Phylogenetic tree describing the evolution of the mutations in the samples from the normal. (d) Principal component analysis of gene expression profiles in tumor and normal components. (e) Poisson distribution of gene expression profiles in the individual components

3.3 | Phylogenetic analysis

As shown in a Venn diagram, we observed that the largest number of overlapping mutations (70) occurred between the spindle and mixed components, as compared to only 2 between mixed and epithelioid and 3 between spindle and epithelioid. There were no shared mutations among all three components. A similar pattern was observed by examining only the non-synonymous mutations (Figure S1c), supporting distinct evolutionary pathways driving the epithelioid component as compared to the spindle and mixed elements. A phylogenetic tree of the epithelioid, spindle, and mixed components is shown in Figure 2c. The spindle and mixed components share common truncal mutations which included *ATM* (c.9156G>C), *CTNNB1* (c.1187A>T), and *NF2* (c.1021C>T). Mutations in *NF1* (c.5C>T), *CHEK2* (c.180_181ins), *SMARCA4* (c.2851G>A, c.3539C>T), *TP53* (c.375G>A) were unique to the mixed component. Mutations unique to the epithelioid component included *TP53* (c.277del), and further branch mutations that include *ARID1B* (c.2249G>A), *BID* (c.301G>A), *FLT3* (c.2899C>T), *SMARCA2* (c.1201C>T), and *TP63* (c.1528A>T).

3.4 | Gene expression profiling

Gene expression profiles of each tumor component and the matched normal tissue were obtained. Via Principal Component Analysis (PCA) on the data generated from gene expression, we observed that the epithelioid cluster was most different from the rest (Figure 2d). The spindle component was also closest to the normal component. The same pattern was also observed by Poisson clustering (Figure 2e). This relationship was further emphasized using SciClone, which plots the variant allele frequency against density and read depth for copy numbers, identifying six unique clusters of mutations. From this, we observed an overlap of clusters (one and three) found in spindle versus mixed, while there was none found for spindle versus epithelioid and the epithelioid versus mixed (Figure S2).

We explored the differential expression of cancer pathway-associated genes (KEGG cancer pathway) in the different tumor components compared against the normal tissue. Ten clusters were identified following unsupervised hierarchical clustering, including Clusters 1 and 8 which were upregulated and downregulated, respectively, in all tumor components relative to normal. Interestingly, one of the upregulated genes within Cluster 1 was interleukin-6 (IL-6), a pleiotropic proinflammatory cytokine known to promote multidrug resistance by activating oncogenic pathways, contributing to poor prognosis in patients with RCC (Wang & Zhang, 2020). On the other hand, downregulation of *CDKN2A* was observed within Cluster 8, consistent with previous reports in both sRCC and non-sRCC (Malouf et al., 2016, 2020). Among genes upregulated specifically in the spindle component (Clusters 3, 4, 10) include several members of the Wnt, PI3K-mTOR, and MAPK signaling pathways (Figure S3).

4 | DISCUSSION

ADPKD is a well-characterized inherited cystic kidney disease and a leading cause of end-stage renal disease worldwide. In addition, patients with ADPKD experience various renal and extrarenal manifestations, including increased risk of cancers arising from the kidney, colon, and liver (Yu et al., 2016). A previous study reported that the occurrence of sRCC was disproportionally higher in the setting of ADPKD compared to the general population (33% vs. 1%–5%), although most kidney cancers that developed were still non-sarcomatoid RCCs (Keith et al., 1994). At the molecular level, the upregulation of *MYC* oncogene expression and abrogation of *TP53* expression within renal cells of ADPKD patients have been previously observed (Harris & Torres, 2014), while deregulation of the mTOR signaling pathway has been implicated in cyst formation (Shillingford et al., 2006). In our patient with a rare occurrence of sRCC arising from ADPKD, we examined the molecular landscape of histologically-distinct elements present in the tumor sample, revealing the possible mechanisms by which ADPKD predisposes to sRCC.

The mutational landscape of sRCC, particularly in the setting of non-clear cell RCC, has not been well examined until recently. Interestingly, a high frequency of *TP53* mutation has been previously described in sRCC, suggesting that the p53 signaling pathway may be important in the process of sarcomatoid change (Bi et al.,; Oda et al., 1995). More recently, Wang et al. conducted a multiplatform and genome-wide analysis using sRCC (n = 55) and non-sRCC of various subtypes (n = 598) and showed that sRCC exhibits subtype-specific differences in their molecular profiles. Specifically, they described that *TP53* and *NF2* mutations were associated with sRCC within papillary RCCs, which is in keeping with the mutational profile of our case presented. However, in contrast to our case, they had observed a higher overall mutational burden in the spindle as compared to the epithelioid components within the same tumor (Wang et al., 2017). In an earlier study, Malouf et al., (2016) had found a high frequency of *TP53* (42.3%) and *NF2* (20%) mutations in sRCC as compared to non-sRCC. *NF2* mutations were mutually exclusive with *TP53* mutations. In our case, *NF2* mutation was

exclusively found in the spindle component while *TP53* mutations were only found in the epithelioid component. Taken together, this suggests that *TP53* and *NF2* mutations might characterize unique pathways related to sRCC development.

Another mechanism that might encourage sarcomatoid change in ADPKD relates to mTOR signaling. A study of tissues from metastatic sRCC patients found elevated levels of phosphorylated mTOR expression in sarcomatoid areas compared to areas with clear cell histology, suggesting that alterations in mTOR signaling might be involved in the process of sarcomatoid change (Pal et al., 2015). This is significant because polycystin-1 is involved in the regulation of the mTOR pathway, and its inactivation in ADPKD in both mouse models and human patient samples has been shown to result in pathological activation of the mTOR pathway in cyst linings. Inhibition of mTOR using rapamycin also abrogated cyst formation, further establishing the pathway as a key mechanism in ADPKD (Shillingford et al., 2006). In the case presented, we similarly observed the significant expression of genes involved in the mTOR pathway, suggesting that shared pro-oncogenic signals are present in ADPKD and sRCC. Nonetheless, as sRCC remains a rare complication of ADPKD, additional pro-tumorigenic processes, such as the mutations described earlier, must be acquired in order for sRCC to be formed.

To our best knowledge, there has been no reported molecular description of sRCC arising in the setting of ADPKD in the literature. With the caveat that this report involves only a single patient, we provided initial insights into the molecular pathobiology of ADPKD and sRCC. Further characterization of a link between the two entities might allow for a better understanding of the mechanisms underlying both disease processes, and eventually permit the development of more effective treatment options.

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CONFLICT OF INTERESTS

The authors have declared no conflict of interest.

AUTHOR CONTRIBUTIONS

EL, PG, and JYC generated the experimental data and drafted the manuscript; PG, AHL, and JWL performed the bioinformatics analyses; TL provided pathological assessment of tissues; DYXN, JYL, SG, WL, and CCN were involved in sample preparation and provided technical expertise for various experiments; GFT and JYC provided patient samples and clinical data; JYC and BTT conceived the study, interpreted the results, and revised the manuscript; JYC directed the study and supervised the research; and all authors read and approved the final version of the manuscript.

ETHICS COMPLIANCE

Written informed consent from the patient for the use of biospecimens and clinical data were obtained in accordance with the Declaration of Helsinki. Tissue collection and consent protocols were under ethics approval from the SingHealth Centralized Institution Review Board.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

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