

An explorative study using next generation sequencing of papillary renal cell carcinoma, type 2

Ji-Yeon Kim*, Jeong-eun Lee**, Soojin Cha***, Se-Hoon Lee****, Jong-Il Kim*****

*Department of Internal Medicine, Cancer Research Institute, Seoul National University Hospital, Seoul, Korea

**Cancer Research Institute, Seoul National University Hospital, Seoul, Korea

***Cancer Research Institute, Seoul National University Hospital, Seoul, Korea

**** Department of Internal Medicine, Cancer Research Institute, Seoul National University Hospital, Seoul, Korea

*****Department of Biochemistry of Seoul National University Hospital, Seoul, Korea ; Genomic Medicine Institute(GMI), Medical Research Center, Seoul National University, Seoul, Korea; Life Science Institute, Macrogen Inc ,Seoul, Korea

Abstract

Purpose

Papillary renal cell carcinoma (PRCC) is the most common type of non-clear cell renal cell carcinoma (ccRCC). Among the PRCCs, type 2 PRCC (PRCC2) often presents with highly aggressive tumor behavior and relative resistance to chemotherapy. In contrast to clear cell renal cell carcinoma (ccRCC), the genomic alteration landscape of PRCC2 has been poorly understood. Therefore, we aimed to investigate genetic alterations involved in the pathogenesis of PRCC2.

Methods

We performed whole exome sequencing (WES) of DNA samples from 9 PRCC2 patients, matched with their normal tissue as control. Somatic mutations, somatic copy number alterations(SCNAs) from the WES data were analyzed. We also investigated the relationship between relevant genetic alterations and clinical prognosis.

Results

Average non-silent mutation rate was 0.74 mutations per Mb(range, 0.31 to 1.30 mutations per Mb). Fourteen somatic mutations including SRRD (four), and PKD1L2 (three) were detected in multiple samples. SCNAs were predominant in high stage PRCC2, which had relatively small number of somatic mutations.

Conclusion

Application of WES for patients with PRCC2 showed possibility of genomics-guided cancer treatment. This study shows that PRCC2 is characterized by a heterogeneous genetic background. Further large scaled comprehensive genetic analysis of PRCC2 is warranted to develop personalized treatment options for PRCC2.

Key words- Next generation sequencing; Papillary renal cell carcinoma, type2 (PRCC2); Somatic mutation; Whole exome sequencing (WES)

I. INTRODUCTION

Kidney cancer is the eighth most common cancer in the world. In contrast to the favorable prognosis of patients who received curative resection, 5 year survival for patients with metastatic kidney cancer is only about ten percent(1).

A quarter of kidney cancer is non-clear cell renal cell carcinoma (ccRCC) consisting of papillary renal cell carcinoma type1 and type2 (12%), chromophobe renal cell carcinoma (8%; including oncocytoma), collecting duct renal cell carcinoma (<1%) and renal cell carcinoma, unclassified (3-5%)(2).

Loss of *Von Hippel Lindau (VHL)*, a well-known tumor suppressor gene, is a traditional genetic hallmark of clear cell renal cell carcinoma (ccRCC). Loss of *VHL* function leads to activate *Hypoxia Inducible Factor 1* and *2 (HIF1* and *HIF2)* facilitating cell proliferation and angiogenesis. Recent comprehensive genetic analysis of next generation sequencing(NGS) data in ccRCC identified that *PBRM1* is the second most common genetic alteration of ccRCC(3) and the alteration of ubiquitin-mediated proteolysis pathway (*KDM6A*, *KDM5C*, *SETD2* and *PBRM1*) leads to ccRCC without *VHL* inactivation(4). Recently, The Cancer Genome Atlas (TCGA) presented comprehensive genetic and epigenetic portrait of ccRCC(5).

Although ccRCC has unique genetic alteration and metabolic pathway disturbance, pathogenesis of nccRCC is largely unknown. Whether hereditary type or not, sporadic papillary renal cell carcinoma, type1 (PRCC1) exclusively harbors alterations in c-MET oncogene(2, 6, 7). Hereditary type of PRCC2 is also associated with germ line mutation of Fumarate hydratase (FH) gene(8-11).

In contrast of ccRCC, efforts to characterize the genetic alterations of nccRCC are still ongoing. Therefore, we

investigated this genomic analysis of PRCC2 to reveal the landscape of genomics of PRCC2.

II. PATIENTS AND METHODS

Patients and sample preparation

From the prospectively collected, surgically removed fresh frozen samples of cancer and paired normal tissues in the Seoul National University Hospital tissue bank, seven PRCC2 samples were used in this study. Two other formalin-fixed, paraffin-embedded samples from patients with metastatic PRCC2 were also included. These nine samples were reviewed by qualified pathologists and histologically classified as PRCC2. We obtained the informed consent from all subjects. This study was reviewed and approved by the Institutional Review Board of Seoul National University Hospital (IRB No: 1204-026-403).

Genomic DNA was extracted from FFPE samples using QIAGEN FFPE Tissue DNA kit. From the fresh frozen samples, DNA and RNA were extracted using GeneAll exgene cell SV kit and Ambio PureLink RNA mini kit. Concentrations of DNA and RNA were measured using Qubit.

Exome capture, library construction and sequencing

Up to 3 µg of genomic DNA was sheared with a Covaris SS Ultrasonicator and adaptors were then ligated to both ends of the fragments. Adaptor-ligated templates were purified using Agencourt AMPure SPRI beads, and fragments with an insert size of ~200 bp were isolated. Exons were captured from adaptor-ligated DNAs using SureSelect Human All Exon v4+UTRs kit (71Mb) according to the manufacturer's instructions (Agilent Technologies). PCR amplification of the libraries was carried out for four cycles in the pre-capture step and for ten cycles after capture. Paired-end sequencing, resulting in sequences of 101 base pairs from each end of the fragments, was performed on the HiSeq 2000 platform (Illumina) following the manufacturer's instructions. Image analysis and base calling were performed using the Illumina pipeline with default settings.

Sequencing data analysis

Burrows-Wheeler Aligner (BWA)(12) was used to align sequencing reads to the human reference genome GRCh37/hg19. We used SAMtools(13), bamtools and Picard to make clean up BAM file. Local realignment and base quality recalibration using Genome Analysis Tool Kit (GATK)(14) v.2.6-5. We used MuTect 1.1.6 to call somatic single nucleotide variants (SNVs) from whole exome sequencing data for tumor and matched normal tissue. To obtain reliable candidates, we listed candidate somatic mutations by excluding synonymous SNVs. To detect somatic insertion and deletion, we applied Somatic indel detector GATK v.2.2-8. We removed data that is composed of less than two altered reads and ten percent of total reads to get faithful result.

Somatic copy number alterations (SCNAs) were analyzed from sequencing data using VarScan2(15). In order to correct unequal sequencing depth of tumor and normal samples, we revised copy number value from both sequencing data ratio. Significant SCNAs are defined as tumor versus normal tissue copy number ratio is over absolute number 1 on log 2 scale.

III. RESULTS

Patient characteristics and treatment

Six out of seven patients who surgically resected PRCC2 did not recur. Four patients were diagnosed as stage I disease, one patient and another one were diagnosed as stage II and III, respectively. Six patients were male and four male patients had smoking history. Median age of diagnosis of PRCC2 was 55 year old (Table 1).

Table 1: Clinical and pathological baseline characteristics of study population

	Age (YO) ¹	Sex	Smoke (PY)	Stage	Grade	Tumor	Normal
1	55	M	10	1	3	Kidney*	Kidney
2	74	M	60	1	3	Kidney*	Kidney
3	63	M	Never	1	3	Kidney*	Kidney
4	51	M	20	1	3	Kidney*	PB ³
5	72	F	Never	2	3	Kidney*	PB
6	82	M	Never	4	3	Kidney*	Kidney
7	30	M	13	3	4	Kidney*	Kidney
8	26	F	Never	4	3	Kidney [†]	PB
9	36	F	Never	4	2	Kidney [†]	PB

¹Year-old; ²Pack per years; ³Peripheral blood; *Surgical specimen; [†]Percutaneous needle biopsy specimen

Somatic alterations of PRCC2

From the whole exome sequencing (WES) data of nine paired samples, we identified 332 somatic non-synonymous point mutations and 142 somatic insertion or deletion events. Average non-silent mutation rate is 0.74 mutations per Mb (range, 0.31 to 1.30 mutations per Mb).

Fourteen somatic mutations were detected in multiple samples (Figure 1). *SRRD* (four mutations), *PKDIL2* (three) were the most frequently mutated genes. *GNAQ*, a well-known driver oncogene of uveal melanoma(16), was detected in two samples. One mutation is an identical mutation that discovered in lung adenocarcinoma and prostate cancer and other one is a novel stopgain mutation. Two somatic mutations of Mastermind-like3(*MAML3*)(17), a transcriptional co-activator modulating notch signaling pathway, was also discovered. Somatic mutations of *FAT4*, *NOTCH2*, *NRAS*, and *PTEN* were also identified.

SCNAs were significantly enriched in stage IV samples (average number of chromosome where SCNA events happen; 8.3 vs.1.3, P-value=0.095 by Mann-Whitney test). One specimen from a 36 year-old female patient showed multiple

CN loss and another specimen from a young female was associated with CN gain in multiple chromosomes (Figure 2). Interestingly, these two samples had a low number of somatic

mutations, although this comparison did not reach the level of statistical significance (Average number of mutation/Mb; 0.38 vs.0.89, P-value=0.071 by Mann-Whitney test).

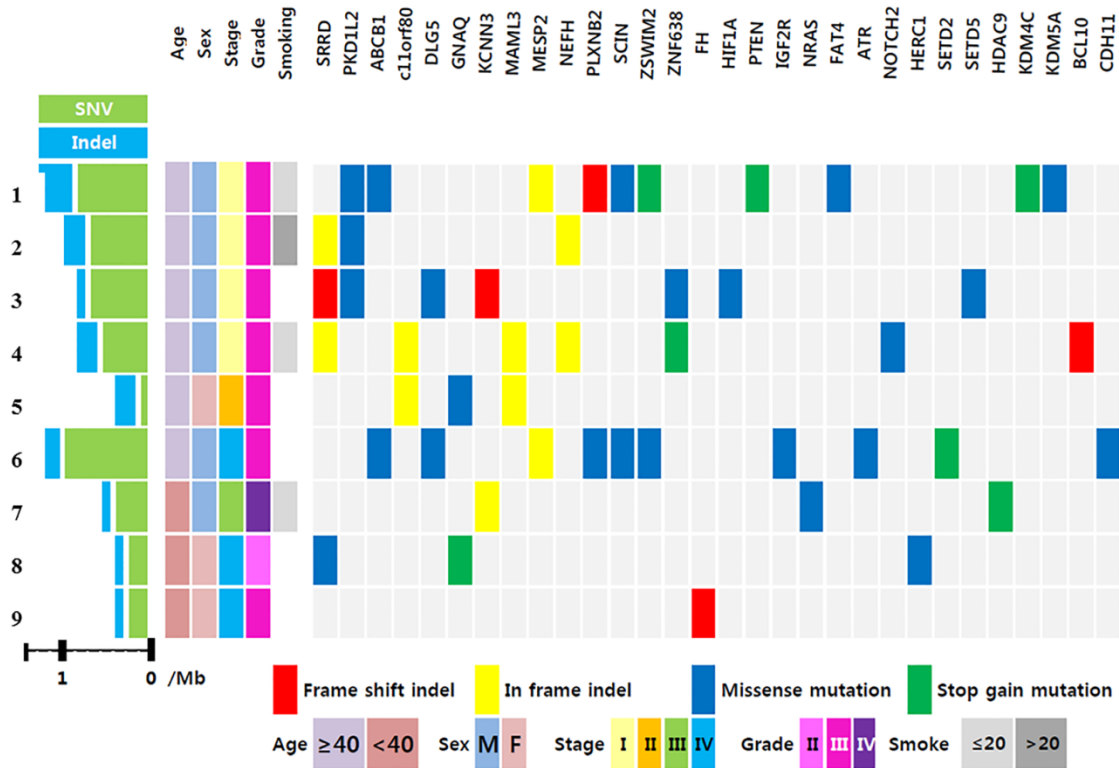


Figure1: Clinicopathological characteristics and genetic aberrations across nine type 2 papillary renal cell carcinomas (PRCC2).

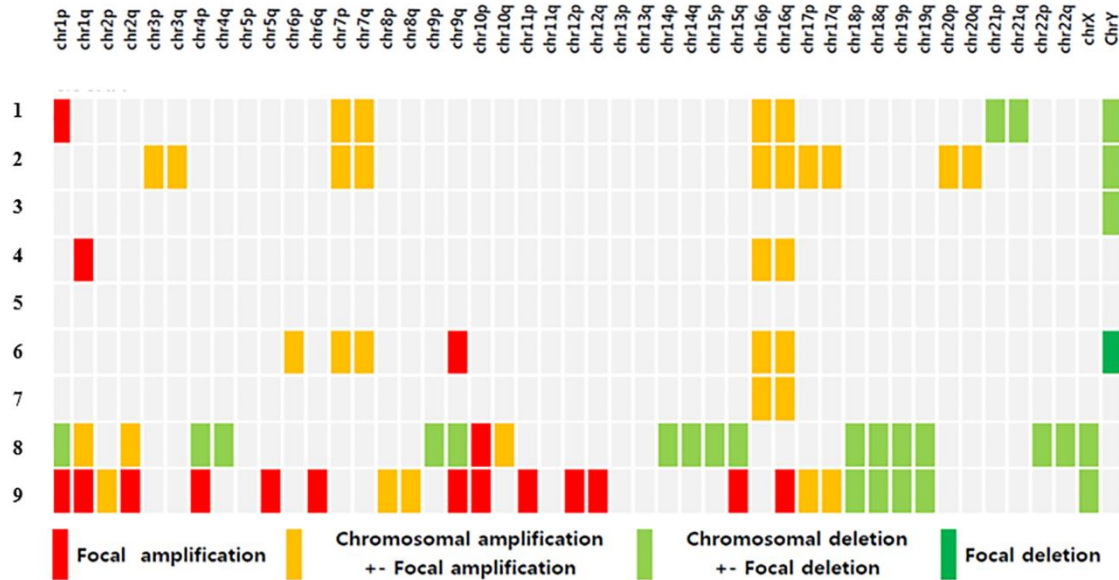


Figure2: Somatic copy number alterations.

IV. DISCUSSION

In this study, we identified multiple genetic alterations of PRCC2 that could contribute to pathogenesis of this largely uncharacterized disease. Therefore, whole exome sequencing, rather than target sequencing, is required to discover the druggable target of PRCC2.

SRRD, the most commonly mutated gene in our study, was previously reported in multiple cancer genome analysis(5, 18, 19). However, the overall incidence of *SRRD* mutation is low (about 0.5-1% of tested specimens in TCGA cohort), and further studies to validate the functional consequence of this mutation have not been performed yet. *PKDIL2*, a G-protein binding protein that was known as associated with primary cilia of kidney epithelial cell(20), was also detected in about 2 to 3 percent of renal cell carcinoma(5). Until now, there is no supporting evidence that these somatic mutations drive the PRCC2 biology and no therapeutic agents targeting these mutations exist.

We found somatic *GNAQ* mutations in two samples. Because the mitogen-activated protein kinase (MAPK) pathway activated by *GNAQ* is critical for the development of uveal melanoma and pre-clinical studies showed that MEK inhibition resulted in uveal melanoma cell death in cell lines harboring mutant *GNAQ*(21-23), *GNAQ* mutation may be a potential target in PRCC2.

In spite of the low incidence of mutation in PRCC2, stopgain mutation of *PTEN* could be a potential target of PI3K inhibitors and mutant *MAML3* and *NOTCH2* also could be inhibited by newly invented drugs(24, 25).

Non-clear cell renal cell carcinoma, which consists of PRCC1, PRCC2, chromophobe and collecting duct type of renal cell carcinoma, is a heterogeneous subtype of renal cell carcinoma(26-30). Because comprehensive study to understanding nccRCC has not been performed yet, the differences among the subtypes of nccRCC were not clearly understood. Moreover, due to non-clear cell subtype is an exclusion criterion in large scaled randomized controlled trials, we could not identify optimal therapeutic options for nccRCC. The ARCC trial(31), phase III trial including nccRCC, showed temsirolimus is more effective to other type of RCC than clear cell type and Sunitinib EAT(32) study told that nccRCC patient has poor prognosis. For this reason, only temsirolimus is the acknowledged the first line treatment for nccRCC(33).

Recently, clinical trials suggest that traditional VEGFR TKIs have effectiveness to nccRCC as well as ccRCC. Phase II study of sunitinib treatment in nccRCC(34) and the USA EAP of sorafenib(35) told that VEGFR inhibition is a good strategy to conquer nccRCC. Especially, patients with PRCC and chromophobe histologies could be obtain a durable advantage using VEGFR TKIs.

This study could suggest that the PRCC2 have heterogenous genetic background and this genetic exploration may be helpful to choose the optimal treatment of PRCC2 patients.

Our study was the first exploratory study to apply comprehensive genomic analysis of PRCC2 for patient treatment in real clinic. Even though this is a small-sized study,

our effort provides a clinical perspective for treating patients with cancer using the insights from the analysis of genetic information.

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AUTHORS

First Author – Ji-Yeon Kim, M.D, Department of Internal Medicine, Seoul National University Hospital, medijiyeon@gmail.com

Second Author – Jeong-eun Lee, Ph.D course candidate, Cancer Research Institute, Seoul National University Hospital, jeleedict@gmail.com.

Third Author – Soojin Cha, Ph.D course candidate, Cancer Research Institute, Seoul National University Hospital, soojincha36@gmail.com

Correspondence Author

Se-Hoon Lee, M.D, Ph.D, Division of Hematology and Medical Oncology, Department of Internal Medicine, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 110-744, Korea. Tel: +82-2-2072-0832; Fax: +82-2-764-2199; E-mail: sehoon.lee119@gmail.com

Jong-Il Kim, M.D, Ph.D, Department of Biochemistry, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 110-744, Korea. Tel:+82-2-740-8251; Fax:+82-2-744-4534; E-mail: jongil@snu.ac.kr