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# Practical Laboratory Medicine



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Registered Report Stage II

# Genetic study of the *CDKN2A* and *CDKN2B* genes in renal cell carcinoma patients

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ARTICLE INFO

Keywords: Renal cell carcinoma Clear-cell RCC CDKN2A and CDKN2B mutations MLPA

# ABSTRACT

*Objectives*: While recent studies have demonstrated several genetic alterations are associated with pathogenesis of RCC, the significance of cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and cyclin-dependent kinase inhibitor 2B (*CDKN2B*) in tumorigenesis of RCC is less clear. We investigate the distribution of *CDKN2A* and *CDKN2B* mutations in patients with RCC and analyze the impact of *CDKN2A* and *CDKN2B* mutations on RCC. *Methods*: A pathological examination was conducted using thirty fresh renal tissue samples with

renal masses that had undergone partial or radical nephrectomy. Multiplex ligation-dependent probe amplification (MLPA) was used to detect genetic aberrations of *CDKN2A* and *CDKN2B* in genomic DNA isolated from samples. Subsequently, *CDKN2A* and *CDKN2B* mutations were confirmed using chromosomal microarray technique.

*Results:* Twenty-one patients were diagnosed with RCC, eight with benign diseases, including angiomyolipoma (AML) and oncocytoma, and one with mucinous adenocarcinoma of renal pelvis. Two of twenty-one patients (9.5 %) with clear-cell RCC were positive for *CDKN2A* and *CDKN2B* gene deletions. Interestingly, patients with *CDKN2A* and *CDKN2B* mutations were associated with sarcomatoid patterns of RCC (2 out of 4, 50 %). In contrast, no *CDKN2A* or *CDKN2B* deletions were detected in samples from benign renal tumors, papillary RCC, or other kidney cancers.

*Conclusions*: This study demonstrated the potential use of *CDKN2A* and *CDKN2B* as biomarkers for the prognostic and molecular classification of renal cancer. *CDKN2A* and *CDKN2B* mutations may be associated with RCC development and sarcomatoid changes. Further research is needed to understand the underlying molecular mechanisms of *CDKN2A* and *CDKN2B* in the pathogenesis of RCC.

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https://doi.org/10.1016/j.plabm.2024.e00410

Received 28 March 2024; Received in revised form 21 May 2024; Accepted 25 May 2024

Available online 25 May 2024

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#### 1. Introduction

Renal cell carcinoma (RCC) originates in the renal cortex and is the most common type of kidney cancer [1]. Moreover, the disease is the deadliest cancer of the common urinary tract and accounts for 5 % of all cancers worldwide [2]. RCC is predominant in males (male-to-female ratio is 1.5:1.0), with the average age of onset being 60–70 years [3]. Despite continuous improvements in therapeutic interventions for RCC, the disease incidence rate is estimated to increase and lead to more than 300,000 deaths by 2040 [2,4].

RCC comprises different types of tumors with broad ranges of histological findings, clinical representations, treatment responses, and genetic alterations [5,6]. There are three common subtypes of RCC, namely clear cell RCC (ccRCC), papillary RCC (pRCC), and chromophobe RCC (crRCC), each with a distinct prognosis and treatment response [7,8]. Several genetic alterations of both germline and acquired mutations have been reported to be associated with the pathogenesis of RCC. Germline mutations in several genes, including *VHL*, *FH*, *SDHB*, *SDHC*, *SDHD*, *MET*, *FLCN*, *PTEN*, *TSC1*, *TSC2*, *MITF*, *BAP1*, *PBRM1*, and *CDKN2B*, are associated with an increased risk of developing RCC [5,9–13]. Studies that used sequencing technology on tissues derived from RCC have revealed that many driving mutations are involved in sporadic RCC, including those of *BAP1*, *PBRM1*, *TCEB1*, *SETD2*, *KDM6A*, *TP53*, and *PTEN* [14–19]. Notably, *VHL* mutations are frequently observed in RCC of the clear-cell histological subtype (>80 %) and in both sporadic and germline forms of RCC. These further underline the complexity of genetic alterations in the disease.

Recent studies have reported the occurrence of cyclin-dependent kinase inhibitor 2A (CDKN2A) gene deletion in several tumors, which is associated with poor health outcomes. CDKN2A is located in chromosome 9p21.3 and encodes two proteins, p14ARF and p16INK4a. CDKN2A is predominantly expressed in several organs and tissues, including the adrenal, bladder, testis, stomach, spleen, and fat tissue. The encoded proteins play an essential role in cell division by regulating TP53 and RB1 [20]. Thus, CDKN2A deletions facilitate cell proliferation and promote tumor formation in several cancers [21]. The prevalence of CDKN2A mutations varies in different types of tumors; they are observed in approximately 3 % of patients with RCC [20]. CDKN2A has been extensively studied in patients treated with immune checkpoint inhibitors (ICI) [22]. For instance, it has been demonstrated that melanoma cells with CDKN2A gene deletions are not sensitive to immune checkpoint inhibitors [23]. However, there are conflicting data on the impact of CDKN2A gene deletion on ICI treatments for several cancers, including RCC [24,25]. The cyclin-dependent kinase inhibitor 2B (CDKN2B) gene lies adjacent to CDKN2A and usually undergoes concomitant deletion with CDKN2A. CDKN2B functions as a tumor suppressor gene that encodes the cyclin-dependent kinase inhibitors involved in cell cycle regulation through CDK4 or CDK6. CDKN2B is highly expressed in the colon, small intestine, esophagus, duodenum, lung, fat tissue, and skin. CDKN2B alterations have been reported in the germline form of RCC, suggesting that detecting mutations in this gene can improve familial RCC screening [26]. Additionally, a homozygous loss of CDKN2A/CDKN2B is associated with the progression of mucinous tubular and spindle cell carcinoma (MTSCC), a rare subtype of RCC [27]. However, there is limited evidence about the distribution of CDKN2A and CDKN2B and the clinical manifestations of RCC subtypes regarding CDKN2A and CDKN2B deletion statuses in our study population. Therefore, this study investigated the genetic deletion of CDKN2A and CDKN2B genes (ch9p21.3 loss) in patients with renal cell carcinoma using the multiplex ligation-dependent probe amplification (MLPA) technique and confirmed the deletions of those genes using chromosome microarrays. Additionally, the impact of CDKN2A and CDKN2B mutations on renal cell carcinoma was analyzed.

# 2. Materials and methods

### 2.1. Patients and samples

Fresh renal mass samples (n = 30) were collected from patients who had undergone partial nephrectomy or radical nephrectomy between February 2019 and September 2020 at Ramathibodi Hospital in Bangkok, Thailand. Based on a pathological examination of the samples, RCC was histologically confirmed in twenty-one patients. One patient was diagnosed with mucinous adenocarcinoma of the renal pelvis. Eight patients were diagnosed with benign renal masses, five had angiomyolipoma, two had oncocytoma, and one had benign spindle cell neoplasm. Demographic and clinical data were obtained from the patient's medical records. This study was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University, based on the Declaration of Helsinki (MURA2019/401).

# 2.2. Multiplex ligation-dependent probe amplification (MLPA) analysis

Genomic DNA was isolated from fresh tissue samples using the QIAamp® DNA Micro Kit according to the manufacturer's instructions. The amount and purity of the extracted DNA were measured using NanoDrop<sup>TM</sup> ND-2000 (Thermo Fisher Scientific, Waltham, MA, USA). Fifty nanograms of gDNA were subjected to MLPA analysis with SALSA® MLPA® Probemix P419 *CDKN2A/2B*-*CDK4* (MRC-Holland, Amsterdam, Netherlands), which was developed to detect the duplication or deletion of *CDKN2A*, *CDKN2B*, and *CDK4* genes. In detail, the probe mix contained 57 MLPA probes for the detection of the following regions: 14 probes for *CDKN2A*, nine probes for *CDKN2A*, nine probes for *CDK4*, ten flanking probes in total for *CDKN2A* and *CDKN2B*, one probe for the *MITF* p.E318K (c.952G > A) point mutation, one wildtype probe for *CDK4* codon 24, and 13 reference probes. Additionally, nine quality control probes, including four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, one chromosome X, and one chromosome Y-specific fragment, which those generally included in MLPA reaction for internal quality control were included into this assay. Normal DNA samples extracted from the peripheral blood of the healthy individual and positive DNA samples with known deletion of *CDKN2A* and *CDKN2B* from patients with acute lymphoblastic leukemia tested by MLPA P355-ALL-IKZF1 were also included in this study. Five reference DNA samples (two blood samples and three normal adjacent tissue samples) were used during the assay validation steps. MLPA reactions, including those for the internal quality controls and negative controls, were performed according to the manufacturer's instructions. The PCR products were subsequently analyzed using an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and Coffalyser.net software (MRC Holland, Amsterdam, Netherlands), according to the respective manufacturer's instructions. For the MLPA data interpretation, the final ratio (FR) indicating *CDKN2A* and *CDKN2B* statuses followed normal; 0.80 < FR < 1.20, homozygous deletion; FR = 0, heterozygous deletion; 0.40 < FR < 0.65, Heterozygous duplication; 1.30 < FR < 1.65, Heterozygous triplication/homozygous duplication; 1.75 < FR < 2.15, and ambiguous copy number; all other values. Notably, the level of tumor burden, tumor subclonal, and mutation patterns (somatic/germline) could affect the samples' FR. Samples with positive for *CDKN2A* and *CDKN2B* were subsequently confirmed by chromosomal microarray technique.

# 2.3. Chromosome microarray analysis (CMA)

CMA was performed using Affymetrix CytoScan 750 K arrays (Thermo Fisher Scientific, Santa Clara, CA), in accordance with the manufacturer's instructions, to further confirm the copy number variations in the *CDKN2A* and *CDKN2B* genes. The intensities of probe hybridization were analyzed using Affymetrix GeneChip® Operating Software (GCOS), and copy number analysis was performed using Affymetrix Chromosome Analysis Suite (ChAS).

# 2.4. Statistical analysis

Patient characteristics with categorical variables were compared using Pearson's chi-square test or Fisher's exact test, whereas continuous variables were compared using the Kruskal–Wallis test. A *p*-value <0.05 was considered statistically significant. All statistical analyses were performed using Stata version 14 (StataCorp LP, College Station, Texas, USA).

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Variable	Total
Gender, n (%)	
Male	20 (66.7)
Female	10 (33.3)
Age (year), mean $\pm$ SD	$57.6 \pm 16.4$
Body mass index, mean $\pm$ SD	$27.3\pm5.0$
Smoking status, n (%)	
No	20 (66.7)
Yes	10 (33.3)
Hypertension, n (%)	
No	16 (53.3)
yes	14 (46.7)
Chronic kidney disease, n (%)	
No	13 (43.3)
Yes	17 (56.7)
Patient history of other cancer, n (%)	
No	28 (93.3)
Yes	2 (6.7)
Family history of cancer, n (%)	
No	26 (86.7)
Yes	4 (13.3)
Pathology of renal mass, n (%)	
Benign renal tumour	8 (26.7)
Mucinous adenocarcinoma of renal pelvis	1 (3.3)
RCC	21 (70.0)
Clear-cell RCC	19 (63.3)
Papillary RCC	2 (6.7)
Tumour size (cm), median (Interquartile range (IQR))	4 (3, 5.9)
$\leq$ 4 cm	16 (53.3)
4.1–7 cm	9 (30.0)
7.1–10 cm	2 (6.7)
> 10 cm	3 (10.0)
Histological grade, n (%)	
1	6 (28.6)
2	10 (47.6)
3	2 (9.5)
4	3 (14.3)

 Table 1

 Clinical characteristics and clinicopathological data of cohort study participants.

#### 3. Results

# 3.1. Cohort characteristics and clinicopathological data

Thirty patients (20 men and 10 women) with a mean  $\pm$  SD age of onset of 57.6  $\pm$  16.4 years were enrolled in this study. About half of the patients had hypertension and chronic kidney disease (CKD) (53.3 % and 56.7 %, respectively). 30 % of the patients were smokers. Moreover, four patients had a history of familial cancer. The pathological findings revealed that RCC (70 %) was the most common cancer cell type among the participants in this study. Additionally, clear-cell RCC (ccRCC) was the most common subtype in the patient group (63.3 %). Half of the patients had tumors less than 4 cm (53.3 %) in size and a nuclear grade of two (47.6 %). The patient characteristics and clinicopathological data are given in Table 1.

# 3.2. The incidence and spectrum of CDKN2A and CDKN2B alterations

An MLPA assay was performed to detect the genetic gain or loss of *CDKN2A* and *CDKN2B* gene sequences. Two of the thirty renal mass tissue samples were positive for both *CDKN2A* and *CDKN2B* deletions. As an example, the MLPA results for samples RCC03 and RCC28 are given in Fig. 1. Interestingly, the two samples mentioned earlier were initially diagnosed with RCC, drawing a frequency of 9.5 % (2 out of 21) among the RCC subgroup. Moreover, the subsequent chromosomal microarray analysis further confirmed the loss of *CDKN2A* and *CDKN2B* in the two samples (Fig. 2). No *CDKN2A* or *CDKN2B* deletions were detected in samples derived from other benign renal tumors, papillary RCC, or different types of kidney cell cancer. This finding suggests that the loss of *CDKN2A* and *CDKN2B* functions may contribute to the tumorigenesis of RCC.

# 3.3. CDKN2A and CDKN2B were associated with poor clinical outcomes in RCC patients

The impact of *CDKN2A* and *CDKN2B* deletions on the clinical features of patients who tested positive for these mutations was further analyzed. A comparison of the clinical characteristics and possible outcomes of patients associated with the loss of *CDKN2A* and *CDKN2B* is given in Table 2. Genetic deletions of both *CDKN2A* and *CDKN2B* were predominantly observed in elderly patients. While



**Fig. 1.** The chromatogram results of an MLPA conducted on genomic DNA isolated from a tissue sample of RCC03 indicate the loss of *CDKN2A* and *CDKN2B* genes. The SALSA MLPA Probemix P419–B1 CDKN2A/2B-CDK4 contains 57 MLPA probes with amplification products between 121 and 504 nucleotides. The area between the blue and red lines indicates the normal range of final ratio, 0.80 < FR < 1.20 (FR (former, dosage quotient (DQ)). The red circle dots under the red line indicate targeted-specific probes with genetic deletion (homozygous deletion FR = 0, heterozygous deletion = 0.40 < FR < 0.65). The blue dots upper the blue line indicate targeted-specific probes with duplication patterns (heterozygous duplication = 1.30 < FR < 1.65, heterozygous triplication/homozygous duplication = 1.75 < FR < 2.15). The ambiguous copy number results are other FR values from the above ranges. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

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Fig. 2. A microarray analysis of genomic DNA isolated from tumor tissue samples of RCC03 and RCC28 revealed *CDKN2A* and *CDKN2B* gene deletions (9p21.3) and control DNA isolated from adjacent normal tissue samples (NAT28). This result is concordant with that of the MLPA.

no statistical differences were found in several characteristics, *CDKN2A* and *CDKN2B* gene deletions (100 % vs. 100 %; p = 0.023 vs. p = 0.018) were strictly observed in RCC with sarcomatoid differentiation. There were 2 of 4 (50 %) sarcomatoid changes, samples RCC03 and RCC28, positive for *CDKN2A* and *CDKN2B*. Meanwhile, other sarcomatoid samples, RCC31 and RCC35, were negative for the deletion of *CDKN2A* and *CDKN2B* (Fig. 3). These findings further highlight the impact of *CDKN2A* and *CDKN2B* on tumorigenesis and provide potential explanations for the pathogenesis of RCC driven by *CDKN2A* and *CDKN2B* mutations.

#### Table 2

A comparison of patients' clinical characteristics and outcomes associated with the loss of CDKN2A and CDKN2B.

No variation (n = 4)Deletion (n 2)Deletion (n valuePeriod valueDeletion (n valueDeletion (n value <th>Variable</th> <th colspan="3">CDKN2A</th> <th></th> <th></th> <th colspan="2">CDKN2B</th> <th colspan="2"></th>	Variable	CDKN2A					CDKN2B			
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		No variation	Deletion (n	Duplication (n	p-	No variation	Deletion (n	Duplication (n	p-	
Pachage<		(n = 4)	= 2)	= 24)	value	(n = 5)	= 2)	= 23)	value	
lening008 (33.3)0.554008 (3.4)0.4.2Muchicons denoration01 (4.2)001 (4.3)1 (4.3)RCC4 (100)2 (100)1 5 (6.2.5)5 (100)2 (100)1 4 (6.0.9)1 (6.0.7)PRCC4 (100)2 (100)1 3 (8.5.7)0.9994 (80.0)2 (100)1 3 (5.5.7)0.130OppleC001 (2.1.3)0.2585 (100)2 (100)1 3 (5.5.7)0.474Seq (year) mean ± 505 3.2 ± 1.6.86 3.6 ± 4.25 6.5 ± 17.00.4745 7.8 ± 1.6.86 6.8 ± 4.35 6.5 ± 17.00.4745 7.8 ± 1.6.86 6.8 ± 1.20.400.300.330.330.330.430.430.5550.0700.330.430.350.4747.8 ± 0.477	Pathology of renal mass, n (%)									
Inductions adenocariant of the set of	Benign	0	0	8 (33.3)	0.554	0	0	8 (34.8)	0.426	
RCCA (100)C (100)15 (6.2.5)S (70)C (100)14 (6.9.7)L (6.9.7)RCCA (100)2 (100)15 (6.2.7)0.9994 (80.0)2 (100)13 (6.2.7)0.57PRCCA (100)2 (100)14 (5.8.3)0.2581 (20)2 (100)13 (5.5.7)0.100Parala0010 (1.7)0010 (3.5.7)1.45Parala3 (2.9.7)8 (3.11)4 (3.6.7)0.445.8.6 (1.7.8)6.6.9 ± 36.6.	Mucinous adenocarcinoma	0	0	1 (4.2)		0	0	1 (4.3)		
RC         4 (100         2 (100)         15 (6.5)         5 (100)         2 (100)         14 (6.9)           rcRC         0         0         2 (13.3)         120.00         2 (100)         13 (6.7)         0.73           Gender, r(s)         1         120.00         12 (100)         14 (58.3)         0.28         5 (100)         2 (100)         13 (6.5)         .030           Female         0         0         0         10 (1.7)         0         0         10 (7.5)         .0467           Age (ray, men ± 5D         53 ± 16.8         68 0 ± 4.2         55 ± 17.0         0.474         57.8 ± 16.3         86 ± 4.3         56 6 ± 17.2         0.467           Tumour size (rum, median         0         0         9 (37.5)         1 (26.0)         1 (20.0)         1 (3.6,0) <td< td=""><td>of the renal pelvis</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>	of the renal pelvis									
cRCC4 (100)2 (100)13 (8.6.7)0.994 (8.0.0)2 (100)13 (02.9)0.567Gender, n (%)11 <td>RCC</td> <td>4 (100)</td> <td>2 (100)</td> <td>15 (62.5)</td> <td></td> <td>5 (100)</td> <td>2 (100)</td> <td>14 (60.9)</td> <td></td>	RCC	4 (100)	2 (100)	15 (62.5)		5 (100)	2 (100)	14 (60.9)		
pRC Gender, 1%022110.000111 <th< td=""><td>ccRCC</td><td>4 (100)</td><td>2 (100)</td><td>13 (86.7)</td><td>0.999</td><td>4 (80.0)</td><td>2 (100)</td><td>13 (92.9)</td><td>0.567</td></th<>	ccRCC	4 (100)	2 (100)	13 (86.7)	0.999	4 (80.0)	2 (100)	13 (92.9)	0.567	
Generation of targets and targets an	pRCC	0	0	2 (13.3)		1 (20.0)	0	1 (7.1)		
Male4 (100)2 (100)14 (3.3)0.285 (100)2 (100)13 (56.5)0.13Fenale0010 (41.7)00010 (47.7)Age (var), mean ± SD53.4 ± 16.868 (4 ± 1)4 (3, 6)37.8 ± 16.368.0 ± 4.356.6 ± 17.20.473Tumour size (cm), median3 (2, 9)8 (4, 1)4 (3, 6)0.5310 (50.0)1 (50.0)1 (50.2)0.473(007)3 (75.0)1 (50.0)1 (50.0)1 (50.0)1 (50.2)0.47310 (50.0)1 (50.2)0.473(107)1 (25.0)1 (50.0)1 (42.0)1 (20.0)1 (50.0)	Gender, n (%)									
Fende0010 (17.)0010 (73.5)Age (vca7), median59.3 ± 16.868.0 ± 4.256.5 ± 17.00.47457.8 ± 16.366.014.6.00.637Tumour size (cm), median3 (2, 9)8 (4, 11)4 (3, 6)0.4493 (3, 5)8 (4, 11)4 (3, 6)0.637URN1 (50.0)1 (50.0)1 (20.0)08 (34.8)2 4 cm002 (8.3)002 (8.7)-1 (20.0)1 (4.3)-7.1-10 cm002 (8.3)1 (20.0)1 (20.0)1 (20.0)1 (4.3)10 cm1 (25.0)001 (20.0)1 (20.0)1 (20.0)2 (100)2.3 (100)0.2 (3.7)Yes1 (25.0)1 (50.0)1 (4.1)11 (25.0)1 (50.0)7 (46.7)2 (40.0)04 (28.6)0.733002 (13.3)002 (14.3)41 (25.0)1 (50.0)1 (6.7)1 (20.0)1 (50.0)1 (7.1)3001 (6.7)1 (20.0)1 (50.0)1 (7.1)<	Male	4 (100)	2 (100)	14 (58.3)	0.258	5 (100)	2 (100)	13 (56.5)	0.130	
Age (yar), mean ± 50         53 ± 16.8         80 ± 4.2         56.5 ± 17.0         0.474         57.8 ± 16.3         68.0 ± 4.3         56.4 ± 17.2         0.437           Tumour size (cm), median         3 (2, 9)         8 (4, 11)         4 (3, 6)         0.439         3 (50.0)         8 (4, 11)         4 (3, 6)         0.535           ≤ 4 cm         3 (75.0)         1 (50.0)         1 (20.0)         0.8         3 (60.0)         1 (50.0)         1 (50.0)         2 (57.2)           > 10 cm         0         0         2 (37.5)         0         0         2 (8.7)           > 10 cm         1 (25.0)         1 (50.0)         1 (4.0)         2 (100)         2 (100)         2 (100)         2 (100)         2 (100)         2 (100)         2 (100)         2 (100)         0.203           Yes         1 (25.0)         1 (50.0)         1 (20.0)         0         1 (20.0)         1 (20.0)         1 (20.0)         2 (100.1)	Female	0	0	10 (41.7)		0	0	10 (73.5)		
$\begin{array}{c c c c c c } \hline \begin{tabular}{c c c c c c } \hline \begin{tabular}{c c c c c c } \hline \begin{tabular}{c c c c c c c } \hline \begin{tabular}{c c c c c c c c } \hline \begin{tabular}{c c c c c c c c c c c c c c c c c c c $	Age (year), mean $\pm$ SD	$59.3 \pm 16.8$	$68.0\pm4.2$	$56.5 \pm 17.0$	0.474	$57.8 \pm 16.3$	$68.0\pm4.3$	$56.6 \pm 17.2$	0.467	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Tumour size (cm), median	3 (2, 9)	8 (4, 11)	4 (3, 6)	0.449	3 (3, 5)	8 (4, 11)	4 (3, 6)	0.535	
< 4 m         3 (75.0)         1 (50.0)         9 (37.5)         1 (20.0)         0         8 (34.8)           4.1-7 cm         0         0         2 (8.7)         1 (20.0)         0         8 (34.8)           7.1-10 cm         0         0         2 (8.7)         1 (20.0)         1 (0.0)         1 (4.3)           VC thrombus, (9%)          1 (25.0)         2 (100)         24 (100)         0.200         4 (0.0)         2 (100)         2.33 (100)         0           VS         1 (25.0)         0         0         0         0         0         0           Histological grade, n (%)         1         2 (50.0)         1 (50.0)         7 (66.7)         2 (40.0)         1 (50.0)         7 (50.7)           3         0         0         2 (13.3)         0         0         2 (14.3)         0           4         1 (25.0)         1 (50.0)         1 (6.7)         1 (20.0)         1 (7.1)         1 (7.1)           Sarcenatioi, n (%) n = 21          -         1 (20.0)         1 (50.0)         1 (7.1)         1 (7.1)           Absent         1 (25.0)         2 (100)         1 (6.7)         0         1 (50.0)         1 (7.1)         1 (7.1)	(IQR)									
1-7 cm         0         0         9 (37.5)         1 (20.0)         0         8 (3.4).           7.1-10 cm         1 (25.0)         1 (50.0)         1 (42.0)         1 (50.0)         1 (43.0)         1 (43.0)           NC         1 (25.0)         2 (100)         2 (4 (100)         0.200         4 (80.0)         2 (100)         2 (3.0)         0.233           Yes         1 (25.0)         0         5 (33.3)         0.671         2 (40.0)         1 (50.0)         7 (55.0)	$\leq 4 \text{ cm}$	3 (75.0)	1 (50.0)	12 (50.0)	0.180	3 (60.0)	1 (50.0)	12 (52.2)	0.455	
7.1-0 cm002 (8.3)0002 (8.7)> 10 cm1 (25.0)1 (50.0)1 (4.2)1 (20.0)1 (50.0)1 (4.3)NC thrombus, n(%)11 (25.0)001 (20.0)2 (100)2.3 (100)2.33Ne1 (25.0)001 (20.0)0001 (20.0)00Histological grade, n (%)11 (25.0)0002 (40.0)1 (50.0)7 (50.0)7 (50.0)22 (50.0)1 (50.0)7 (46.7)2 (40.0)1 (50.0)7 (50.0)1 (7.1)1Sarcomatoli, n (%) n = 2111 (25.0)1 (50.0)1 (6.7)1 (20.0)1 (50.0)1 (7.1)1Absent3 (75.0)2 (100)1 (6.7)1 (20.0)1 (50.0)1 (7.1)12.1001 (7.1)1Recrosis, n (%) n = 214 (100)1 (50.0)1 (50.0)1 (50.0)1 (7.1)12.1001 (7.1)12.1001 (7.1)12.1001 (7.1)12.1001 (7.1)12.1001 (7.1)12.1001 (7.1)12.1001 (7.1)1112.1001 (7.1)12.1001 (7.1)1111111111111111111111111111111111111 <td>4.1–7 cm</td> <td>0</td> <td>0</td> <td>9 (37.5)</td> <td></td> <td>1 (20.0)</td> <td>0</td> <td>8 (34.8)</td> <td></td>	4.1–7 cm	0	0	9 (37.5)		1 (20.0)	0	8 (34.8)		
> 10 cm1 (25.0)1 (30.0)1 (4.2)1 (20.0)1 (50.0)1 (4.3)IVC thrombus, n (%)3 (75.0)2 (100)24 (100)0.2004 (80.0)2 (100)0.213No1 (25.0)05 (33.3)0.6712 (40.0)04 (28.6)0.73511 (25.0)1 (50.0)7 (46.7)2 (40.0)04 (28.6)0.73522 (50.0)1 (50.0)7 (46.7)002 (14.3)1 (50.0)3002 (13.3)002 (14.3)041 (25.0)1 (6.7)1 (50.0)1 (50.0)1 (7.1)1 (50.0)Sarcomatch, n (%) n = 2111 (50.0)1 (6.7)1 (50.0)1 (50.0)1 (7.1)Absent3 (75.0)2 (100)1 (6.7)01 (50.0)1 (7.1)0.18Present01 (50.0)1 (6.7)01 (50.0)1 (7.1)0.23Rhabdid, n (%) n = 2111 (50.0)1 (50.0)1 (7.1)0.23Present01 (50.0)1 (6.7)01 (50.0)1 (7.1)Necrosis, n (%) n = 2112 (40.0)01 (50.0)1 (7.1)Nether1 (25.0)2 (100)6 (40.0)3 (60.0)2 (100)6 (42.9)Nether1 (25.0)2 (100)2 (100.0)2 (100.0)2 (100.0)2 (100.0)Nether1 (25.0)2 (100.0)2 (100.0)2 (100.0)2 (100.0)2 (100.0)2 (100.0)Nether1 (25.0)	7.1–10 cm	0	0	2 (8.3)		0	0	2 (8.7)		
IVC thrombus, n (%)       No       3 (75.0)       2 (100)       24 (100)       0.200       4 (80.0)       2 (100)       0       0.233         Yes       1 (25.0)       0       0       1 (20.0)       0       0       0       0       0       0         1       1 (25.0)       0       0       5 (33.3)       0.671       2 (40.0)       1 (50.0)       7 (50.0)       0       2 (14.3)         3       0       0       2 (25.0)       1 (50.0)       1 (6.7)       1 (20.0)       1 (25.0)       1 (7.1)       3 (75.0)       0       1 (4 (93.3)       0.023       4 (80.0)       0       1 (39.9)       0.018         Present       1 (25.0)       2 (100)       1 (6.7)       1 (20.0)       1 (50.0)       1 (7.1)       2 (100)       1 (7.1)       1 (20.0)       1 (7.1)       1 (20.0)       1 (7.1)       1 (20.0)       1 (7.1)       1 (20.0)       1 (7.1)       1 (20.0)       1 (7.1)       1 (20.0)       1 (7.1)       1 (20.0)       1 (7.1)       1 (20.0)       1 (7.1)       1 (20.0)       1 (7.1)       1 (20.0)       1 (7.1)       1 (20.0)       1 (7.1)       1 (20.0)       1 (7.1)       1 (20.0)       1 (7.1)       1 (20.0)       1 (7.1)       1 (20.0)       1 (7.1)	> 10 cm	1 (25.0)	1 (50.0)	1 (4.2)		1 (20.0)	1 (50.0)	1 (4.3)		
No3 (75.0) (25.0)2 (100) 024 (100) 00.200 (20.0)4 (80.0) (20.0)2 (100) 023 (100) 00.233 0Yes1 (25.0)05 (33.3) (15.0)0.671 (24.0)2 (40.0)04 (28.6) (20.0)0.735 (50.0)3002 (13.3)002 (14.3)141 (25.0)1 (50.0)1 (6.7)1 (20.0)1 (50.0)1 (50.0)5arcomatoid, n (%) n = 21111 (25.0)1 (16.7)1 (20.0)1 (10.0)1 (7.1)Absent3 (75.0)01 4 (93.3)0.234 (80.0)01 (3.2,9)0.018Present1 (25.0)2 (100)1 (6.7)01 (50.0)1 (7.1)1Rhabeid, n (%) n = 2111111111Absent01 (50.0)1 (6.7)01 (50.0)1 (7.1)11Necrosis, n (%) n = 211111111111Absent3 (75.0)2 (100)1 (6.7)01 (50.0)1 (50.0)111	IVC thrombus, n (%)						. ,			
Yes1 (25.0)001 (20.0)00Histolizatal grade, n (%)11 (25.0)05 (33.3)0.6712 (40.0)04 (28.6)0.73522 (50.0)1 (50.0)7 (46.7)2 (40.0)1 (50.0)7 (50.0)7 (50.0)3002 (13.3)01 (50.0)1 (50.0)1 (7.1)1Sarcomatoid, n (%) n = 21Absent1 (25.0)2 (100)1 (6.7)1 (20.0)2 (100)1 (7.1)Rhabdoid, n (%) n = 211 (20.0)2 (100)1 (7.1)Absent4 (100)1 (50.0)1 (6.7)01 (50.0)1 (7.1)	No	3 (75.0)	2 (100)	24 (100)	0.200	4 (80.0)	2 (100)	23 (100)	0.233	
Histological grade, n (%)11 (25.0)05 (33.3)0.6712 (40.0)04 (28.6)0.73522 (50.0)1 (50.0)7 (46.7)2 (40.0)1 (50.0)7 (50.0)7 (50.0)3002 (13.3)002 (14.3)141 (25.0)1 (50.0)1 (6.7)1 (20.0)1 (50.0)1 (7.1)Sarcomatoid, n (%) n = 2111 (25.0)2 (100)1 (6.7)1 (20.0)2 (100)1 (7.1)Rhabdid, n (%) n = 2111 (50.0)1 (6.7)01 (50.0)1 (50.0)1 (92.9)0.233Present01 (50.0)1 (6.7)01 (50.0)1 (50.0)1 (7.1)1Necrosis, n (%) n = 21Absent1 (25.0)09 (60.0)0.182 (40.0)08 (57.1)0.538Present3 (75.0)2 (100)6 (40.0)3 (60.0)2 (100)6 (42.9)-Heamaturia, n (%)No3 (75.0)2 (100)2 (18.3)0.9993 (60.0)2 (100)8 (34.8)Present3 (75.0)2 (100)1 (56.2)0.9993 (60.0)1 (50.0)1 (60.9)No3 (75.0)1 (50.0)1 5 (62.5)0.3454 (80.0)1 (50.0)1 (60.9)No1 (25.0)1 (50.0)1 4 (58.3)0.5341 (20.0)1 (50.0)9 (31.3)No1 (	Yes	1 (25.0)	0	0		1 (20.0)	0	0		
111105533.30.671220428.60.735222(50.0)11(50.0)77(50.0)7(50.0)7(50.0)7(50.0)7(50.0)7(50.0)7(50.0)7(50.0)7(50.0)7(50.0)1(7.1)7(50.0)1(7.1)7(50.0)1(7.1)7(50.0)1(7.1)0013(92.9)0.01801(50.0)1(16.7)1(20.0)2(10.0)11(50.0)1(9.0)01(9.0)0110011011(50.0)1(9.0)11(50.0)11(50.0)11(50.0)11(50.0)11(50.0)11(50.0)11(50.0) <t< td=""><td>Histological grade, n (%)</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>	Histological grade, n (%)									
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	1	1 (25.0)	0	5 (33.3)	0.671	2 (40.0)	0	4 (28.6)	0.735	
3002 (13.3)0002 (14.3)41 (25.0)1 (50.0)1 (6.7)1 (20.0)1 (50.0)1 (7.1)Sarcomatoid, n (%) n = 2111 (20.0)2 (100)1 (6.7)1 (20.0)2 (100)1 (7.1)Rhabdoid, n (%) n = 2111 (20.0)2 (100)1 (50.0)1	2	2 (50.0)	1 (50.0)	7 (46.7)		2 (40.0)	1 (50.0)	7 (50.0)		
41 (25.0)1 (50.0)1 (6.7)1 (20.0)1 (50.0)1 (7.1)Sarcomatoid, n (%) n = 21	3	0	0	2 (13.3)		0	0	2 (14.3)		
Sarcomatoid, n (%) n = 21Absent3 (75.0)014 (93.3)0.0234 (80.0)013 (92.9)0.018Present1 (25.0)2 (100)1 (6.7)1 (20.0)2 (100)1 (7.1)0Rhabdoid, n (%) n = 21Absent4 (100)1 (50.0)14 (93.3)0.2145 (100)1 (50.0)13 (92.9)0.233Present01 (50.0)1 (50.0)13 (92.9)0.233Necrosis, n (%) n = 21 </td <td>4</td> <td>1 (25.0)</td> <td>1 (50.0)</td> <td>1 (6.7)</td> <td></td> <td>1 (20.0)</td> <td>1 (50.0)</td> <td>1 (7.1)</td> <td></td>	4	1 (25.0)	1 (50.0)	1 (6.7)		1 (20.0)	1 (50.0)	1 (7.1)		
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Necrosis, n (%) n = 21Absent1 (25.0)09 (60.0)0.1812 (40.0)08 (57.1)0.538Present3 (75.0)2 (100)6 (40.0)3 (60.0)2 (100)6 (42.9)Haematuria, n (%)VNo3 (75.0)2 (100)20 (83.3)0.9993 (60.0)2 (100)20 (87.0)0.453Yes1 (25.0)04 (16.7)2 (40.0)03 (13.0)3 (13.0)Smoking status, n (%)VVVVVNo4 (100)1 (50.0)15 (62.5)0.3454 (80.0)1 (50.0)15 (65.2)0.999Yes01 (25.0)1 (50.0)14 (58.3)0.5341 (20.0)1 (50.0)14 (60.9)0.299Yes3 (75.0)1 (50.0)14 (58.3)0.5341 (20.0)1 (50.0)14 (60.9)0.299Yes3 (75.0)2 (100)12 (50.0)0.5123 (60.0)010 (43.5)0.447Yes3 (75.0)2 (100)12 (50.0)0.5123 (60.0)010 (43.5)0.447Yes3 (75.0)2 (100)12 (50.0)2 (40.0)2 (100)13 (56.5)20.447Yes3 (75.0)2 (100)12 (50.0)2 (40.0)2 (100)13 (56.5)20.447Yes002 (100)2 (291.7)0.9995 (100)2 (100)21 (91.3)0.999Yes002 (100)2 (8.3)002 (100)2 (8.7)	Present	0	1 (50.0)	1 (6.7)		0	1 (50.0)	1 (7.1)		
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Haematuria, n (%)No3 (75.0)2 (100)20 (83.3)0.9993 (60.0)2 (100)20 (87.0)0.453Yes1 (25.0)04 (16.7)2 (40.0)03 (13.0)Smoking status, n (%) $V$ $V$ $V$ $V$ $V$ $V$ $V$ No4 (100)1 (50.0)15 (62.5) $0.345$ 4 (80.0)1 (50.0)15 (65.2) $0.999$ Yes01 (50.0)15 (62.5) $0.345$ 4 (80.0)1 (50.0)15 (65.2) $0.999$ Yes3 (75.0)1 (50.0)14 (58.3) $0.534$ 1 (20.0)1 (50.0)14 (60.9) $0.299$ Yes3 (75.0)1 (50.0)14 (58.3) $0.534$ 1 (20.0)1 (50.0)9 (39.1)CKD, n (%) $V$ $V$ $V$ $V$ $V$ $V$ $V$ No1 (25.0)012 (50.0) $0.512$ 3 (60.0)010 (43.5) $0.447$ Yes3 (75.0)2 (100)12 (50.0) $2 (40.0)$ 2 (100)13 (56.5) $0.447$ No1 (25.0)012 (50.0)2 (40.0)2 (100)13 (56.5) $0.811$ Patient cancer history, n (%) $V$ No4 (100)2 (100)22 (91.7) $0.999$ 5 (100)2 (100)21 (91.3) $0.999$ $Y$ $Y$ $V$	Present	3 (75.0)	2 (100)	6 (40.0)		3 (60.0)	2 (100)	6 (42.9)		
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Hypertension, n (%)No1 (25.0)1 (50.0)14 (58.3)0.5341 (20.0)1 (50.0)14 (60.9)0.299Yes3 (75.0)1 (50.0)10 (41.7)4 (80.0)1 (50.0)9 (39.1)CKD, n (%)VNo1 (25.0)012 (50.0)0.5123 (60.0)010 (43.5)0.447Yes3 (75.0)2 (100)12 (50.0)0.5123 (60.0)010 (43.5)0.447Patient cancer history, n (%)28.7 $\pm$ 5.727.1 $\pm$ 2.727.1 $\pm$ 5.20.77625.3 $\pm$ 3.827.1 $\pm$ 2.727.8 $\pm$ 5.40.581No4 (100)2 (100)22 (91.7)0.9995 (100)2 (100)21 (91.3)0.999Yes002 (8.3)002 (8.7)0.3174 (80.0)1 (50.0)21 (91.3)0.225No4 (100)1 (50.0)21 (87.5)0.3174 (80.0)1 (50.0)21 (91.3)0.225Yes01 (50.0)21 (87.5)0.3174 (80.0)1 (50.0)21 (91.3)0.225	Yes	0	1 (50.0)	9 (37.5)		1 (20.0)	1 (50.0)	8 (34.8)		
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$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Yes	3 (75.0)	1 (50.0)	10 (41.7)		4 (80.0)	1 (50.0)	9 (39.1)		
	CKD, n (%)						. ,			
	No	1 (25.0)	0	12 (50.0)	0.512	3 (60.0)	0	10 (43.5)	0.447	
BMI, mean $\pm$ SD Patient cancer history, n (%)28.7 $\pm$ 5.727.1 $\pm$ 2.727.1 $\pm$ 5.20.77625.3 $\pm$ 3.827.1 $\pm$ 2.727.8 $\pm$ 5.40.581No4 (100)2 (100)22 (91.7)0.9995 (100)2 (100)21 (91.3)0.999Yes002 (8.3)002 (8.7)Family cancer history, n (%)No4 (100)1 (50.0)21 (87.5)0.3174 (80.0)1 (50.0)21 (91.3)0.225Yes01 (50.0)3 (12.5)1 (20.0)1 (50.0)2 (8.7)	Yes	3 (75.0)	2 (100)	12 (50.0)		2 (40.0)	2 (100)	13 (56.5)		
Patient cancer history, n (%)       No       4 (100)       2 (100)       22 (91.7)       0.999       5 (100)       2 (100)       21 (91.3)       0.999         Yes       0       0       2 (8.3)       0       0       2 (8.7)         Family cancer history, n (%)         No       4 (100)       1 (50.0)       21 (87.5)       0.317       4 (80.0)       1 (50.0)       21 (91.3)       0.225         Yes       0       1 (50.0)       3 (12.5)       1 (20.0)       1 (50.0)       2 (8.7)	BMI, mean $\pm$ SD	$28.7 \pm 5.7$	$27.1 \pm 2.7$	$27.1 \pm 5.2$	0.776	$25.3 \pm 3.8$	$27.1 \pm 2.7$	$27.8 \pm 5.4$	0.581	
No         4 (100)         2 (100)         22 (91.7)         0.999         5 (100)         2 (100)         21 (91.3)         0.999           Yes         0         0         2 (8.3)         0         0         2 (8.7)           Family cancer history, n (%)         Ves         0         1 (50.0)         21 (87.5)         0.317         4 (80.0)         1 (50.0)         21 (91.3)         0.225           Yes         0         1 (50.0)         3 (12.5)         1 (20.0)         1 (50.0)         21 (91.3)         0.225	Patient cancer history, n (%)									
Yes         0         2 (8.3)         0         0         2 (8.7)           Family cancer history, n (%)           No         4 (100)         1 (50.0)         21 (87.5)         0.317         4 (80.0)         1 (50.0)         21 (91.3)         0.225           Yes         0         1 (50.0)         3 (12.5)         1 (20.0)         1 (50.0)         2 (8.7)	No	4 (100)	2 (100)	22 (91.7)	0.999	5 (100)	2 (100)	21 (91.3)	0.999	
Family cancer history, n (%)         Image: Constraint of the second	Yes	0	0	2 (8.3)		0	0	2 (8.7)		
No         4 (100)         1 (50.0)         21 (87.5)         0.317         4 (80.0)         1 (50.0)         21 (91.3)         0.225           Yes         0         1 (50.0)         3 (12.5)         1 (20.0)         1 (50.0)         2 (8.7)	Family cancer history, n (%)									
Yes 0 1 (50.0) 3 (12.5) 1 (20.0) 1 (50.0) 2 (8.7)	No	4 (100)	1 (50.0)	21 (87.5)	0.317	4 (80.0)	1 (50.0)	21 (91.3)	0.225	
	Yes	0	1 (50.0)	3 (12.5)		1 (20.0)	1 (50.0)	2 (8.7)		

Abbreviation: n, number of samples; ccRCC, clear cell renal cell carcinoma; pRCC, papillary renal cell carcinoma; SD, standard deviation; IQR, interquartile range; IVC thrombus, Inferior vena cava thrombus; *CDKN2A*, cyclin-dependent kinase inhibitor 2A, *CDKN2B*, cyclin-dependent kinase inhibitor 2B.

# 4. Discussion

Renal cell carcinoma is a heterogeneous disease in terms of both clinical representation and genetic background. The present study mainly focused on establishing a routine, robust MLPA technique for the detection of deleted/amplified *CDKN2A* and *CDKN2B* genes in tissue samples derived from patients with RCC. Reliable data from several recent studies revealed that molecular genetic alterations indicate a predisposition to the development of familial RCC [28–32]. However, the underlying mechanisms of the genes involved in sporadic kidney cancer remain unclear. Similar to the *VHL* gene, which has predominantly been identified in familial RCC, *CDKN2B* 





Fig. 3. The chromatogram results of an MLPA conducted on genomic DNA isolated from a tissue sample of RCC31 (A) and RCC35 (B) indicate the sarcomatoid samples without CDKN2A and CDKN2B deletion.

point mutations have been reported to be involved in cell cycle dysregulation in patients with RCC [26]. Furthermore, evidence indicates that extensive genetic alterations can be observed in patients with RCC, such as a gain/loss of *CDKN2B* and *CDKN2A* DNA fragments. An MLPA assay was performed, and the gain/loss of *CDKN2A* and *CDKN2B* was subsequently confirmed using the chromosomal microarray technique. Concomitant losses of *CDKN2A* and *CDKN2B* were found in two out of 21 patients (9.5 %) with RCC. This finding is similar to that of Schraml et al. [33], who used a Fluorescence in situ hybridization (FISH) technique specifically for the detection of chromosome 9p deletion and found allelic losses in the *CDKN2A* region in 10 % of patients with clear-cell RCC, and those patients exhibited poor outcomes. The incidences of chromosome 9p deletion (presumably with concomitant losses of *CDKN2A* and *CDKN2B*) were found to diverge in different cohorts, depending on the discerning subjects and investigating tools. Furthermore, El-Mokadem et al. used tissue microarrays and interphase FISH to investigate chromosome 9p deletion in patients with clear-cell RCC. 44 % of the patients were positive for chromosome 9p deletion and were associated with poor prognosis phenotypes, including higher stages of cancer, larger tumors, necrosis, microvascular and renal vein invasion, higher stage, size, grade, and necrosis (SSIGN) scores, detained higher risk of recurrence, and RCC-specific mortality [34]. Interestingly, Beroukhim et al. [35] reported that 29 % of patients with sporadic clear-cell RCC were positive for *CDKN2A* and *CDKN2B* deletion with homozygous loss patterns. Notably, the *CDKN2A* and *CDKN2B* regions encode several tumor suppressor proteins, including p14, p15, and p16, which are essential for regulating cell cycle arrest during the G1 phase. Therefore, alterations of those genes may be involved in the pathogenesis of several cancers, including RCC. Recently, Bakouny et al. demonstrated that the deletion of *CDKN2A* and *CDKN2B* are significantly enriched in sarcomatoid or rhabdoid RCC compared with patients who do not exhibit sarcomatoid or rhabdoid phenotypes [36]. While previous sophisticated studies used a mouse model to study the loss of *CDKN2A* and *VHL* in the establishment of RCC, it is hard to make a conclusion about the exact phenotypes of the tumors derived from those mice because of a lack of histological data [37]. Thus, further study in both animal and human-derived samples needs to investigate the oncogenic potential of *CDKN2A* and *CDKN2B* and *CDKN2B* in the establishment of sarcomatoid and other aggressive forms of RCC. Taken together, these data further indicate that germline mutations of *CDKN2A* and *CDKN2B* and the perturbance loss of those genes could contribute to the tumorigenesis of both familial and sporadic *RCC*.

The genetic deletions of CDKN2A and CDKN2B were further analyzed in relation to the clinical outcomes of patients with RCC. The characteristics of patients with deletions versus those with no deletions or with duplications showed no significant differences (p > 10.05), except in the sarcomatoid presentation (p < 0.05). Furthermore, it was found that these gene deletions occurred in clear-cell RCC samples that exhibited sarcomatoid patterns. No gene deletion was detected in the other types of kidney cell tumors. However, it is important to note that the small sample size may have affected the lack of statistically significant differences. While the overall trend of multiple comparisons showed coherence with no statistical significance, genetic alterations of CDKN2A/CDKN2B were associated with the aggressive, sarcomatoid patterns of RCC with a stringent significance at 0.05 for CDKN2A and lower down to 0.01 for CDKN2B. Moreover, our findings are consistent with recent reports that showed genetic alterations of CDKN2A/CDKN2B are associated with aggressive forms of RCC and are common in sarcomatoid RCC [38-41]. We believe the number of samples is sufficient for our statistical analyses, but we also acknowledge that future study is needed to add to the body of knowledge about the role of CDKN2A/CDKN2B in the establishment of RCC and the applications of CDKN2A/CDKN2B mutation analysis in clinical practice. Interestingly, CDKN2A deletions were found to be associated with CDKN2B deletions (concomitant loss). Finally, patients with deletions of these two genes were found to have an increased risk of RCC development. At present, in our setting, we have not pursued the association between the deletion of CDKN2A/CDKN2B and the treatment outcomes of the patients. However, in literature reviews, it has been suggested that RCC patients who proceed with CDKN2A/CDKN2B deletion are associated with aggressive phenotypes and could benefit from treatment with immunotherapeutic agents [36,40]. The present study further highlights the application of CDKN2A and CDKN2B deletion detection for the prognostication of aggressive forms of RCC.

At present, treatment of RCC includes surgery removal (radical or partial nephrectomy) and targeted therapy and immunotherapy in patients who experience metastatic recurrence post-surgery [42]. RCC with sarcomatoid change (approximately 4–5% of all RCCs) is frequently associated with advanced or metastasis disease (20% of all patients with sarcomatoid pattern) [43] and has shorter survival (less than 1 year) [44,45]. Treatment of RCC with advanced sarcomatoid RCC has slowly progressed during the past decades. A recent 2022 European Association of Urology (EAU) update on guidelines for managing RCC has summarised the recommendation for treating some rare RCCs in which treatment of high malignant potential sarcomatoid RCC comprise of surgery, nivolumab and ipilimumab, immune checkpoint inhibitors (ICI) and tyrosine kinase inhibitor (TKI) combinations, and sunitinib, gemcitabine plus doxorubicin is an option [46]. Although current genetic analysis could not precisely identify key diving mutations in the tumorigenesis of the disease, sarcomatoid RCC predominantly overexpressed PD1 and PDL1 compared to another subtype of RCC. This further highlights the potential use of immunotherapies to treat those patients [47]. Recent reports demonstrated that metastasis RCC and metastasis sarcomatoid RCC have beneficial responses to the treatment with PD-1/PDL-1 targeted immunotherapies [48,49]. Currently, Pembrolizumab (PD-1) has been approved for treating high-risk RCC with or without sarcomatoid differentiation after nephrectomy, significantly improving progression-free survival [50]. These data emphasize the beneficial use of morphological and histological studies of these tumors for diagnostic recognition and predictive value to select appropriate therapeutic options, particularly immunotherapy for high-risk RCC. Additionally, it is critical to identify and validate the biomarker that could identify those patients who may benefit from single or combined therapy with available effective tyrosine kinase inhibitors and immunotherapies.

# 5. Conclusion

A robust MLPA assay was created specifically for the detection of *CDKN2A* and *CDKN2B* duplication/deletion. Genetic aberrations were subsequently confirmed using the chromosomal microarray technique. *CDKN2A* and *CDKN2B* genes were found to undergo concomitant losses in 9.5 % of the RCC samples (2 of 21) and displayed sarcomatoid patterns (2 of 4, 50 %), which were recognized as one of the aggressive RCC forms and may benefit for immunotherapy. The present study's findings further highlight the impact of *CDKN2A* and *CDKN2B* on the tumorigenesis of RCC and the potential use of *CDKN2A* and *CDKN2B* as biomarkers for the prognostication and molecular classification of RCC.

# CRediT authorship contribution statement

Nattaradee Kiatprungvech: Writing – original draft, Investigation, Data curation. Premsant Sangkum: Supervision, Methodology, Conceptualization. Rozita Malinee: Validation, Methodology. Suchada Sommaluan: Validation, Methodology. Veerawat Korkiatsakul: Software, Methodology, Formal analysis. Suchin Worawichawong: Conceptualization. Budsaba

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**Rerkamnuaychoke:** Resources, Conceptualization. **Adcharee Kongruang:** Formal analysis. **Suraida Aeesoa:** Software, Formal analysis. **Panuwat Lertsithichai:** Formal analysis, Data curation. **Kittinut Kijvikai:** Formal analysis, Data curation. **Wisoot Kong-chareonsombat:** Formal analysis, Data curation. **Teerapong Siriboonpiputtana:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

#### Declaration of competing interest

The authors declare no competing interests.

### Data availability

Data will be made available on request.

#### Acknowledgments

We thank the urology staff at the Division of Urology, Ramathibodi Hospital, for granting permission to collect surgical cases for this research. We also thank the staff of the Human Genetic Laboratory, Department of Pathology, Ramathibodi Hospital, for their valuable advice on the MLPA and microarray techniques. Teerapong was supported by Mahidol University (New Discovery and Frontier Research). Busaba Rerkamnuaychoke was supported by the Faculty of Medicine Ramathibodi Hospital, Mahidol University. Nattaradee Kiatprungvech was supported by the Faculty of Medicine Ramathibodi Hospital, Mahidol University (RF 62097).

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