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Original Article

Chromophobe renal cell carcinoma with and without sarcomatoid change: a clinicopathological, comparative genomic hybridization, and whole-exome sequencing study

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Abstract: Chromophobe renal cell carcinomas (CRCC) with and without sarcomatoid change have different outcomes; however, fewstudies have compared their genetic profiles. Therefore, we identified the genomic alterationsin CRCC common type (CRCC C) (n=8) and CRCC with sarcomatoid change (CRCC S) (n=4) using comparative genomic hybridization (CGH) and whole-exome sequencing. The CGH profiles showed that the CRCC C group had more chromosomal losses (72 vs. 18) but fewer chromosomal gains (23 vs. 57) than the CRCC S group. Losses of chromosomes 1p, 8p21-23, 10p16-20, 10p12-ter, 13p20-30, and 17p13 and gains of chromosomes 1q11, 1q21-23, 1p13-15, 2p23-24, and 3p21-ter differed between the groups. Whole-exome sequencing showed that the mutational status of 270 genes differed between CRCC (n=12) and normal renal tissues (n=18). In the functional enrichment analysis, the missense-mutated genes were classified into 6 biological processes (38 functions) and 5 pathways. The biological processes included cell adhesion, cell motility, ATP metabolism, sensory perception, carbohydrate and lipid metabolism and transport. The pathways included ATP-binding cassette transporter, extracellular matrix-receptor interaction, olfactory transduction, chondroitin sulfate biosynthesis, and hypertrophic cardiomyopathy. Whole-exome sequencing analysis revealed that the missense mutation statuses of 49 genes differed between the CRCC C and CRCC S groups. Furthermore, genetic alterations in metastasis suppressor 1, serine peptidase inhibitor Kazal type 8, transient receptor potential cation channel super family M member 6, Rh family B glycoprotein, and mannose receptor C type 1 were located in different chromosomal regions. These alterations may provide clues regarding CRCC tumorigenesis and provide a basis for future targeted therapies.

Keywords: Chromophobe renal cell carcinoma, chromophobe renal cell carcinoma with sarcomatoid change, comparative genomic hybridization, whole-exome sequencing, genetic alteration

Introduction

Renal cell carcinoma (RCC) accounts for 2-3% of all adult malignancies and is the seventh and twelfth most common malignancy in men and women, respectively [1]. Chromophobe renal cell carcinoma (CRCC) is the third most common histological subtype of RCC and carries a better prognosis than conventional RCC [2]. However, CRCC with sarcomatoid change (CRCC S) is associated with poor clinical outcomes, with most cases of sarcomatoid RCCs being diagnosed at TNM stages III and IV, and with a median survival time of only 19 months [3-5]. Sarcomatoid RCCs account for about 1-8% of all renal neoplasms; however, it is not a distinct histological entity, as it arises from all subtypes of RCC [6, 7]. Molecular genetic testing is of great importance for the CRCC and CRCC S diagnosis and prognosis and may help guide treatment. However, little is currently known about genetic alterations in CRCC S, and few molecular genetics studies have compared CRCC and CRCC S. Furthermore, until recently,

whole-genome sequencing of CRCC has been little investigated. Therefore, we here identified and compared the genomic alterations in CRCC common type (CRCC C; 8 cases) and CRCC S (4 cases) using comparative genomic hybridization (CGH) and whole-exome sequencing.

Material and methods

Ethics statement

Ethical approval was obtained from Institutional Ethics Review Board (IERB), The First Affiliated Hospital, Shihezi University School of Medicine and all participants provided written informed consent for themselves.

Specimens

Paraffin-embedded CRCC (n=12) and normal renal tissues (n=18) were obtained from the archives of the Department of Pathology, School of Medicine, Shihezi University. The clinicopathological data of these cases were collected from the patients' medical records after obtaining patient consent and approval by the Institutional Research Ethics Committee. CRCCs were classified according to the 2004 World Health Organization Classification of Renal Tumors, 2010 American Joint Committee on Cancer guidelines, and International Union against Cancer tumor/lymph node metastasis/distal metastasis (TNM) classification system.

Immunohistochemistry

Paraffin-embedded CRCC tissue sections were stained using the 2-step Envision method (Dako, Glostrup, Denmark). The following primary antibodies were used: cluster of differentiation (CD) 10 (GT200410, 1:100), cytokeratin (CK) AE1/3 (AE1/AE3, 1:100), vimentin (Vim3B4, 1:100), CD117 (104D2, 1:300), P504S (13H4, 1:100), and CK7 (OV-TL12/30, 1:50) (all from Dako). Briefly, thetissue sections were incubated with primary antibodies according to the manufacturer's instructions. Immunodetection was performed using 3,3'-diaminobenzidine as the chromogenic horseradish peroxidase substrate, followed by hematoxylin counterstaining.

DNA extraction

Total DNA was extracted from CRCC (n=12) and normal renal (n=18) tissue samples using a standard phenol/chloroform extraction method. DNA quality was checked on a 1% agarose gel, and the amount of extracted DNA was measured spectrophotometrically at 260 nm (impurity and the DNA to non-DNA ratio were also crosschecked at 280 nm). Extractions were stored at -80°C until nick translation labeling.

Comparative genomic hybridization (*CGH*)

CGH was performed using a commercially available kit according to the manufacturer's protocol (Vysis Inc., Downers Grove, IL, USA). Briefly, tumor DNA was labeled with fluorescein isothiocyanate (FITC), while the normal reference DNA was labeled with cyanine 3 (Cy3). The hybridization mixture consisted of approximately 200 ng Spectrum Green-labeled test DNA and 200 ng Spectrum Red-labeled total genomic reference DNA coprecipitated with 10 μg human Cot-1 DNA (Invitrogen, Carlsbad, CA, USA) and was dissolved in hybridization buffer before hybridization to metaphase chromosomes. The probe mixtures were denatured at 73°C for 5 min and then competitively hybridized to the denatured normal metaphase chromosomes in a 37°C humidified chamber for 3 days. After washing, the chromosomes were counterstained with 4'6-diamidino-2-phenylindoledihydrochloride (DAPI) II (Vysis Inc.) and embedded in an anti-fading agent to reduce photobleaching. To provide a control in case of artifacts, normal female DNA (labeled green) was used as a negative control. DNA extracted from the MPE600 breast cancer cell line was used as a positive control (labeled green). Threshold levels of 1.25 and 0.8 were used to score gains and losses, respectively. High-level amplification was indicated by a ratio greater than 1.5. All centromeres, chromosome p35-36, and heterochromatic regions of chromosomes Y, 16, 19, and 22 were excluded from further analysis because these regions can yield unreliable hybridization owing to incompletely suppressed repetitive DNA sequences.

Whole-exome sequencing

DNA (1 μg) extracted from CRCC tissues (n=12) and normal renal tissues (n=18) were labeled with Illumina reagents and hybridized to Human Exome BeadChips (Illumina, San Diego, CA, USA). Illumina Expression Console software was used to perform the quality assessment. The significance analysis of microarrays algo-

Table 1. Clinical characteristic of the 12 CRCC cases

Case	Age (y)/Sex	Diagnosis	Stage (tumor diameter, comment)	Follow-up
1	52/M	CRCC C	PT2M0N0 stage 2 (7.5 cm, primary)	Alive
2	36/M	CRCC C	PT1M0N0 stage 1 (6 cm, primary)	Alive
3	56/F	CRCC C	pT1M0N0 stage 1 (5 cm, primary)	Alive
4	53/M	CRCC C	pT3M0N0 stage 3 (20 cm, primary)	Died 5 years after the operation
5	55/F	CRCC C	pT1M0N0 stage 1 (6.5 cm, primary)	Alive
6	25/M	CRCC C	pT2M0N0 stage 2 (8 cm, primary)	Alive
7	64/M	CRCC C	pT1M0N0 stage 1 (5.5 cm primary)	Alive
8	60/M	CRCC C	pT2M0N0 stage 2 (8.5 cm, primary)	Alive
9	39/M	CRCC S	pT3M0N1 stage 3 (15 cm, primary, 3/4 lymph nodes positive)	Developed brain metastasis and died 1 month after the operation
10	52/F	CRCC S	pT3M0N1 stage 3 (15 cm, primary, 1/4 lymph nodes positive)	Died 3 years after the operation
11	36/F	CRCC S	pT2M1N0 stage 4 (9.5 cm, primary, lung metastasis)	Died 8 months after the operation
12	72/F	CRCC S	pT2M1N1 stage 4 (8.2 cm, primary, bone metastasis, 1/5 lymph nodes positive)	Died 1 year after the operation

Abbreviations: CRCC, chromophobe renal cell carcinoma; CRCC C, CRCC common type; CRCC S, CRCC with sarcomatoid change; F, female; M, male.

Table 3. Chromosome aberrations in the 12 cases of CRCC

Case	Gains	Losses
1	1q31, 15q12-18, Xp21-ter	1p30-ter, 2q12-21, 6q13, 6q31, 11p12-13, 10p13-20, 13q12-13, 13p20, 13p31, 17p13, 21q22
2	3q13, 9q21, 16p24, 20q21	1p14-20, 1p21, 1p24, 2q31-32, 13p20, 13p26-32, 17p26
3	4q31-32, 15p13	1p14, 13p26-ter, 13p12, 13q14, 10p15-30, 17p13
4	1q21-23, 3q24	1p16, 1p34-ter, 2q14, 2q24, 2q34-ter, 6q13, 10p12-26, 11p, 13p11, 13q12-13, 13p13-ter, 17p13, 21q21-22
5	3q13-21, 4q28	1p14, 1p16-18, 1p22-25, 10p12-ter, 13p15, 13p16-25, 17p13
6	16p31, Xq21-22	2q12, 6q32, 10p12, 10p13, 10p16-ter, 11p11.2, 11p12-15, 13q15, 13p17, 13p20-ter, 17p13,
7	1q31, 5q16, 5q25, 19q13,	1p16, 1p21-22, 1p25-34, 8p11, 10p12-ter, 13p17, 13p26-28, 17p13
8	4p12-13, 8p22-25, 12p12, 19p12,	1p, 6q14, 6q22, 10p16-33, 13q12-13, 13q21, 13q22-24, 17p13, 21q
9	1p13-15, 1q20-28, 2p23-24, 3p14, 3q12-13, 3q22, 3p23-ter, 4q21, 4q24, 6p12-20, 6p23, 16p21, 16p23-25, 20q12, Xp16-ter	1p20-25, 2q16, 2q31, 8p21-12, 8p23-ter
10	1p13-32, 1q13, 1q15-26, 1q21-23, 1q30-32, 2p23-24, 3p11-12, 3p23, 3p36, 3q24, 3q13, 3q25, 9q21, 20q21, 20q32	1q13-16, 2q13-14, 8p22, 11p11.2, 9p21
11	1p12, 1p13-20, 1p22-30, 1q11-15, 1q18, 1q19-24, 1q21-23, 2p13-ter, 3q13-21, 3q24, 3p24-ter, 4q16, 6p14-ter, 9q12	8p23, 21q12-13
12	1p12-15, 1p22, 1p24-30, 1q11-20, 1q21-23, 1q23-30, 2p14, 2p28-34, 3p16, 3p21-ter, 9q31, 16p20-24, Xp26-30	8p11, 9p21, 11p11.2, 11p12-15, 21q21

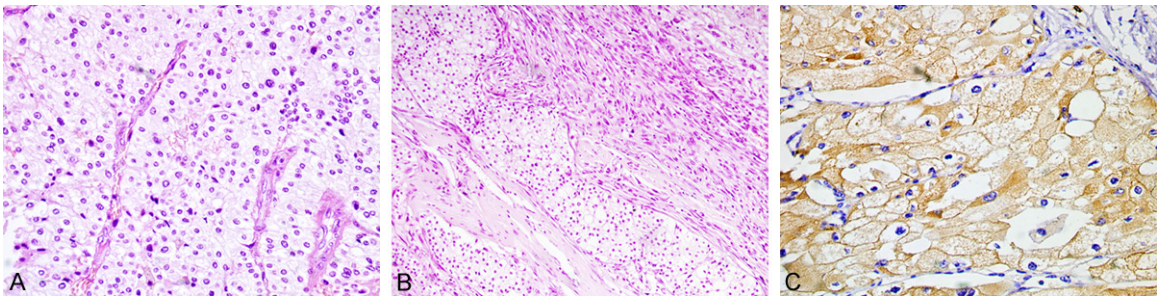

Figure 1. Microscopic and immunohistochemical findings in chromophobe renal cell carcinoma common type (CRCC C) and CRCC with sarcomatoid change (CRCC S). A. CRCC C tumors showed sheet-like and solid structures with variable proportions of translucent and granular eosinophilic cells (×200). B. CRCC S showed diffuse malignant spindle cells similar to fibrosarcoma (×100). C. Immunohistochemically, CRCC showed diffuse, intense plasma membrane staining for CD117 (×200).

Table 2. Immunohistochemical analyses of CK, CD117, CD10, vimentin, CK7, and AMACR in CRCC

Tumor type	n	CK	CD117	Vimentin	CD10	CK7	AMACR
CRCC	12	12 (100)	9 (75)	2 (16.7)	2 (16.7)	3 (25)	3 (25)
CRCC C	8	8 (100)	7 (87.5)	0	0	2 (25)	2 (25)
CRCC S	4	4 (100)	2 (50)	2 (50)	2 (50)	1 (25)	1 (25)

Values are presented as n (%). Abbreviations: CRCC, chromophobe renal cell carcinoma; CRCC C, CRCC common type; CRCC S, CRCC with sarcomatoid change; CK, cytokeratin; CD117, c-kit-encoded proto-oncogene; CD10, cluster of differentiation 10; AMACR, alpha-methylacyl-CoA racemase.

rithm was used to identify differences in gene mutationstatus between CRCC and normal renal tissues. Gene Ontology enrichment based on the biological process was used to identify mutated genes associated with cell cycle regulation and other biological functions. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used to identify pathways associated with CRCC.

Statistical analysis

Fisher's exact test was used to compare differences between the groups (CRCC C vs. CRCC S, CRCC tissue vs. normal renal tissue). The Gene Ontology (biological processes andmolecular function) functional enrichment toolwas used, and KEGG pathway analysis was performed using the Database for Annotation, Visualization, and Integrated Discovery. *P* values < 0.05 were considered statistically significant.

Results

Clinical features

The clinical characteristics of the 12 CRCC cases are summarized in **Table 1**. The overall male-to-female ratio was 7:5 (1:3 and 6:2 for the CRCC S and CRCC C groups, respectively). The mean age at diagnosis for all CRCC cases was 50 years (range, 25-72 years). The mean age at diagnosis was lower for CRCC S patients than for CRCC C patients (49 vs. 54 years). In the CRCC C group, 5 patients presented with painless hematuria, and 3 patients presented with asymptomatic kidney stones detected on B-mode ultrasonography. In the CRCC S group, 2 patients presented with kidney pain and 1 patient presented with pulmonary metastases and fever. Most CRCC C cases (7/8) were TNM stage I-II. The remaining CRCC C case was TNM stage III. Conversely, all CRCC S cases (4/4) were TNM stage III-IV. The 5-year survival rates were 87.5% (7/8) and 0% (0/4) in the CRCC C and CRCC S groups, respectively.

Histopathology

All tumors demonstrated typical CRCC morphology. The mean tumor size was 6.8 cm (range, 3-15 cm). All tumor masses were located in the renal cortex or the junction between the renal cortex and medulla and were solid and well circumscribed with light brown-tan (8/12) or colorful (4/12) cut surface. Necrotic areas were found in all 4 CRCC S cases. In one of these cases, the necrotic area protruded through the fatty renal capsule and invaded into the rectum. Microscopically, the CRCC tumors showed sheet-like and solid structures with variable proportions of translucent and granular eosinophilic cells (**Figure 1A**). Eosinophilic cytoplasmic granular bodies were ob-

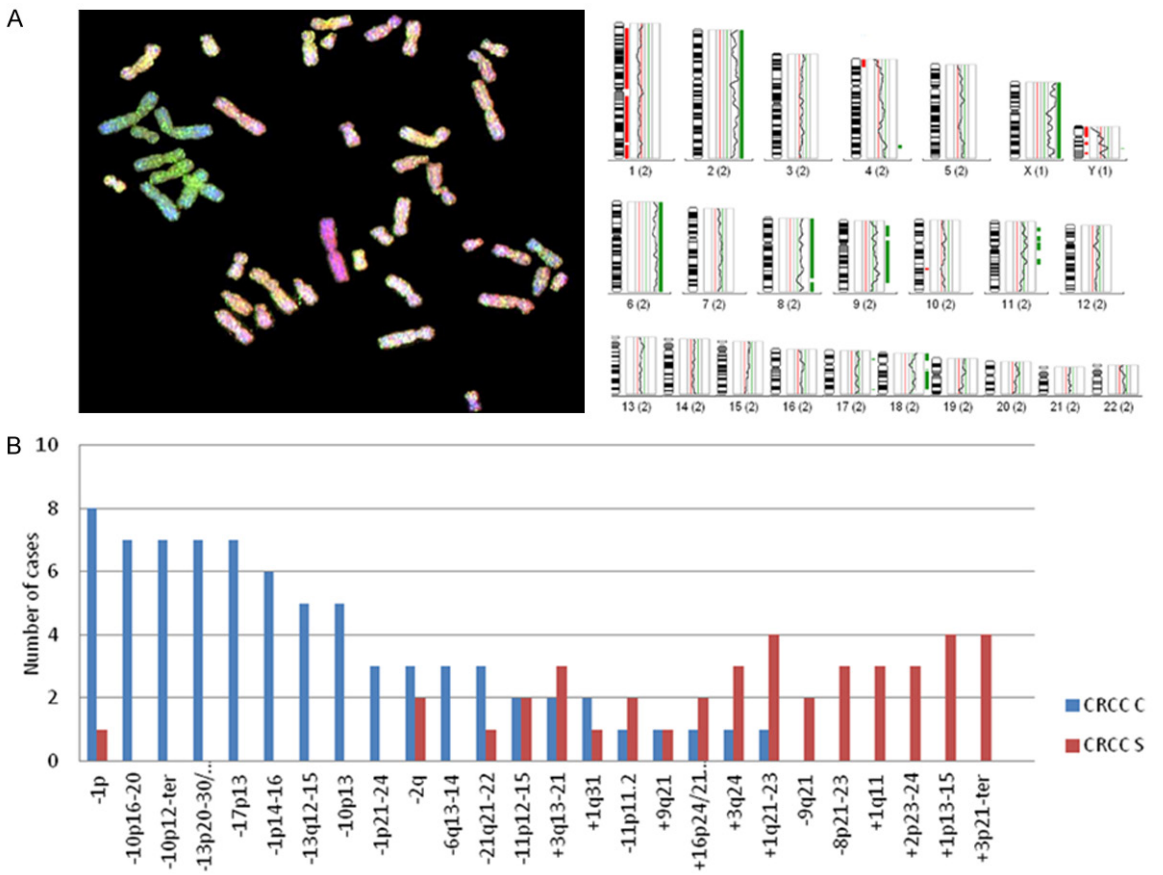

Figure 2. Comparative genomic hybridization (CGH) analysis of chromosomal abnormalities in chromophobe renal cell carcinoma common type (CRCC C) and CRCC with sarcomatoid change (CRCC S). A. CGH detected metaphase spreads in 2 CRCC cases. Green areas, gains; red areas, losses; yellow/yellowish areas, normal; and blue areas, heterochromatin. Hybridization to repetitive sequences/heterochromatin was blocked by unlabeled human Cot-1 DNA and stained with 4,6-diamidino-2-phenylindole dihydrochloride. B. Chromosomal alterations in CRCC C and CRCC S. +, chromosomal gain; -, chromosomal loss.

served in 41.6% (5/12) of CRCC cases. All 4 CRCC S cases showed focal or diffuse malignant spindle cells (**Figure 1B**) and vascular and/or lymphovascular invasion.

Immunohistochemical analysis

The immunohistochemical findings of the 12 CRCC cases are summarized in **Table 2**. Positive CKAE1/3 (100%, 12/12) and CD117 (75%, 9/12) expressions were observed in a high proportion of CRCC cases (**Figure 1C**), whereas a lower proportion of cases showed positivevimentin (16.7%, 2/12), CD10 (16.7%, 2/12), P504s (25%, 3/12), and CK7 (16.7%, 2/12) expressions.

CGH findings

The CGH profiles of all 12 CRCC cases showed chromosomal imbalances, with 80 gains and 90 losses (**Table 3**; **Figure 2**). The mean number of gains and losses per tumor sample was 6.67 and 7.5, respectively. Gains of chromosomes 1q21-23 and 3q13-21 were observed in 5 of the 12 CRCC cases. The most frequent losses occurred on chromosome 1p (9/12). Losses of chromosomes 10p16-20, 17p13, and 13p20-30 were observed in 7 of 12 cases, and losses of chromosomes 13q12-15 and 10p13 were observed in 5 of 12 cases.

Several significant differences in chromosomal gains and losses were observedbetween the CRCC C and CRCC S groups (**Table 4**; **Figure 2B**). The CRCC C group had 72 chromosomal losses and 23 chromosomal gains. Compared with the CRCC C group, the CRCC S group had more chromosomal gains (n=57) but fewer chromosomal losses (n=18). Fourteen significantly different chromosomal alterations were

Table 4. The most common chromosome aberrations in CRCC C vs. CRCC S cases

Chr.	CRCC C (n)	CRCC S (n)	*p**	Stage 1-2	Stage 3-4	*p**
-1p	8	1	0.00202	7	2	0.020979
-10p16-20	7	0	0.010101	6	1	0.07197
-10p12-ter	7	0	0.010101	7	0	0.010101
-13p20-30	7	0	0.010101	6	1	
-17p13	7	0	0.010101	6	1	
-1p14-16	6	0	0.060606	5	1	
-13q12-15	5	0		4	1	
-10p13	5	0		4	1	0.001263
-1p21-24	3	0		3	0	0.151515
-2q	3	2		3	2	0.045455
-6q13-14	3	0		3	0	0.045455
-21q21-22	3	1		3	1	0.045455
-11p12-15	2	2		2	2	0.010101
+3q13-21	2	3		2	3	0.010101
+1q31	2	1		1	2	
-11p11.2	1	2		0	3	
+9q21	1	1		1	1	
+16p24/21-25	1	2		1	2	
+3q24	1	3	0.066667	0	4	0.00303
+1q21-23	1	4	0.010101	0	5	
-9q21	0	2	0.090909	0	2	
-8p21-23	0	3	0.018182	0	3	
+1q11	0	3	0.018182	0	3	
+2p23-24	0	3	0.018182	0	3	
+1p13-15	0	4	0.00202	0	4	
+3p21-ter	0	4	0.00202	0	4	

Abbreviations: Chr., chromosome; CRCC, chromophobe renal cell carcinoma; CRCC C, CRCC common type; CRCC S, CRCC with sarcomatoid change; +, chromosomal gain; -, chromosomal loss. *CRCC C vs. CRCC S, Fisher's exact test.

observed between the CRCC C and CRCC S groups, including losses of chromosomes 1p, 8p21-23, 10p16-20, 10p12-ter, 13p20-30, and 17p13 (p=0.0020, p=0.0182, p=0.0101, p=0.0101, p=0.0101, and p=0.0101, respectively) and gains of chromosomes 1q11, 1q21-23, 1p13-15, 2p23-24, and 3p21-ter (p=0.018182, p=0.0101, p=0.00202, p=0.018182, p=0.0101, p=0.018182, and p=0.00202, respectively). The losses of chromosomes 1p, 8p21-23, and 10p12-ter (p=0.009, p=0.045, p=0.0101, respectively) and gains of chromosomes 1q11, 1q21-23, 1p13-15, 2p23-24, 3p21-ter, and 3q24 (p=0.045, p=0.001263, p=0.0101, p=0.045, p=0.0101, p=0.00303, respectively) were significantly different between TNM stage I-II and III-IV tumors.

Exome sequencing findings

In the whole-exome sequencing data analysis, the mutational statuses of 270 genes were found to significantly differ ($p < 0.05$) between CRCC and normal renal tissues. In the CRCC tissues, 189 missense, 73 silent, 4 nonsense, and 4 synonymous mutations were detected (**Table 5**). In the functional enrichment analysis, the missense-mutated genes were classified into 6 biological process categories (comprised of 38 functions) and 5 pathways. The mutated genes in the CRCC tissues were mainly involved in cell adhesion, cell motility, ATP metabolic process, sensory perception, and carbohydrate and lipid metabolism and transport. The associated pathways includedthe ATP-binding cassette (ABC) transporter, extracellular matrix (ECM)-receptor interaction, olfactory transduction, chondroitin sulfate biosynthesis, and hypertrophic cardiomyopathy pathways (**Table 6**; **Figure 3**).

The missense mutation statuses of 49 genes significantly differed ($p < 0.05$) between the CRCC C and CRCC S groups (**Table 7**). The functional enrichment analysis revealed that these genes were involved in embryonic digestive tract morphogenesis andsignal transduction regulation. Alterations in the metastasis suppressor 1 (MTSS1), serine peptidase inhibitor Kazal type 8 (SPINK8), transient receptor potential cation channel superfamily M member 6 (TRPM6), Rh family B glycoprotein (RHGB), and mannose receptor C type 1 (MRC1) genes were located in different chromosomal regionsin the CRCC C and CRCC S groups (**Table 8**).

Discussion

CRCC was first described in 1985 and is characterized by recognizable pathognomonic features [8]. CRCC has been reported to account for 1.6-6.5% of all RCCs [9]. CRCC can occur at any age, with the average age at diagnosis being 54 years, andthe incidence rate of CRCC

Table 5. The 211 genes containing missense mutations detected in the CRCC tissues ($p < 0.05$)*

Name	Chr	Alleles	Mutation(s)	Gene
exm142861	1	[T/C]	Missense_A198T	PM20D1
exm124419	1	[C/G]	Missense_E10D	ANKRD45
exm104144	1	[A/G]	Missense_E301K, Missense_E334K	ATP8B2
exm114173	1	[T/C]	Missense_E319G	OR10J3
exm139708	1	[T/C]	Missense_G102S, Silent, Silent, Silent	CHIT1
exm100981	1	[T/C]	Missense_G480S	CRNN
exm112525	1	[T/C]	Missense_I137M, Missense_K384R	OR10T2
exm104672	1	[T/C]	Missense_K89R, Missense_K384R, Missense_K384R, Missense_K89R	ADAR
exm105993	1	[C/G]	Missense_L780V, Missense_L900V, Missense_L891V	THBS3
exm140633	1	[T/G]	Missense_M1008L	PLEKHA6
exm128173	1	[A/G]	Missense_N328S, Silent	LHX4
exm135301	1	[C/G]	Missense_P1633A	KIF14
exm125929	1	[G/C]	Missense_P719A, Missense_P719A	ASTN1
exm137376	1	[A/G]	Missense_R144W	LAD1
exm132690	1	[A/G]	Missense_R254C	FAM5C
exm103827	1	[T/C]	Missense_R27Q	C1orf189
exm106273	1	[T/C]	Missense_R523Q, Missense_R619Q	FAM189B
exm142673	1	[A/G]	Missense_R86W	SLC45A3
exm114755	1	[T/G]	Missense_T94K	CCDC19
exm118460	1	[C/G]	Missense_X427S	FCRLB
exm137881	1	[A/G]	Silent, Missense_I159M	SHISA4
exm1009217	12	[A/G]	Missense_A227T	HOXC12
exm1037476	12	[A/G]	Missense_A536T	SH2B3
exm1006802	12	[A/G]	Missense_A72V	KRT4
exm1011126	12	[A/G]	Missense_A974V, Missense_A970V, Missense_A877V	ITGA7
exm1010557	12	[A/G]	Missense_C130Y	OR6C1
exm1026600	12	[G/C]	Missense_G260A	PLXNC1
exm1007512	12	[A/G]	Missense_G959E, Missense_G1093E	TENC1
exm1008942	12	[C/G]	Missense_G97A, Missense_G76A, Missense_G76A	TARBP2
exm1010567	12	[G/C]	Missense_H165D	OR6C1
exm1040411	12	[T/A]	Missense_K351N, Missense_K351N, Missense_K351N	RBM19
exm1038106	12	[T/C]	Missense_K876R	NAA25
exm1019806	12	[T/C]	Missense_L191P	HELB
exm1013918	12	[C/G]	Missense_P1254A	BAZ2A
exm1031057	12	[A/G]	Missense_R243Q	STAB2
exm1019751	12	[A/G]	Missense_R412Q, Missense_R351Q	IRAK3
exm1042551	12	[T/C]	Missense_R48Q, Missense_R48Q	CIT
exm1040015	12	[T/C]	Missense_R69Q	SLC24A6
exm1013851	12	[G/C]	Missense_S1854T	BAZ2A
exm1016108	12	[A/C]	Missense_T166P	INHBC
exm1010716	12	[A/G]	Missense_T222A	OR6C65
exm1010581	12	[A/G]	Missense_V246I	OR6C1
exm1039119	12	[A/G]	Missense_V248I, Missense_V248I	OAS2
exm1000913	12	[A/G]	Missense_P822S	FAM186B
exm1055958	13	[T/C]	Missense_A140T	N6AMT2
exm1075777	13	[C/G]	Missense_E646D	FARP1
exm1077991	13	[T/C]	Missense_M103V	TEX30
exm1069663	13	[T/C]	Missense_R168W	WDFY2
exm1067772	13	[T/C]	Missense_R244K	LCP1
exm1058122	13	[A/G]	Missense_R523H, Missense_R517H	ATP12A
exm1061399	13	[A/G]	Missense_R536Q, Missense_R512Q	RXFP2
exm1078264	13	[A/G]	Missense_V1394M, Missense_V940M	BIVM-ERCC5
exm1055253	13	[A/G]	Missense_V74I	MPHOSPH8
exm1069713	13	[A/G]	Missense_P244L	DHRS12

exm1129474	14	[A/G]	Missense_A983V	CDC42BPB
exm1114137	14	[T/A]	Missense_D314V, Missense_D339V	COQ6
exm1096254	14	[A/T]	Missense_F2171Y	AKAP6
exm1087032	14	[G/C]	Missense_G746R	SALL2
exm1113028	14	[T/G]	Missense_M666R	PAPLN
exm1096237	14	[A/G]	Missense_N2035D	AKAP6
exm1114533	14	[A/G]	Missense_R136W, Silent	ABCD4
exm1094536	14	[T/C]	Missense_R151K	CMA1
exm1090676	14	[T/C]	Missense_R204H	MYH6
exm1128206	14	[T/C]	Missense_R2398C	DYNC1H1
exm1083928	14	[A/G]	Missense_R302Q	OR4L1
exm1092102	14	[A/G]	Missense_R521H	PCK2
exm1129309	14	[T/C]	Missense_S92L	AMN
exm1169486	15	[T/C]	Missense_A141V	ANKDD1A
exm1176048	15	[T/G]	Missense_A384S	ISLR2
exm1148492	15	[T/C]	Missense_E1110K	PLCB2
exm1188945	15	[G/C]	Missense_E130D	C15orf38-AP3S2
exm1152778	15	[T/C]	Missense_K879E	SPTBN5
exm1193803	15	[A/G]	Missense_M386V	ALDH1A3
exm1183603	15	[A/G]	Missense_N740S	WHAMM
exm1170292	15	[A/G]	Missense_P358L	IGDCC3
exm1185068	15	[A/C]	Missense_Q237K	SLC28A1
exm1191113	15	[T/C]	Missense_R107Q	VPS33B
exm1184386	15	[A/G]	Missense_R514H	ZSCAN2
exm1193622	15	[A/C]	Missense_R680S	LINS
exm1188286	15	[A/G]	Missense_R746W	KIF7
exm1162087	15	[A/G]	Missense_S125N	SCG3
exm1153786	15	[A/G]	Missense_S427N	GANC
exm1190321	15	[T/C]	Missense_T274M, Missense_T332M	FES
exm1173024	15	[A/G]	Missense_T453A	GLCE
exm1185057	15	[A/G]	Missense_V189I	SLC28A1
exm1170496	15	[T/C]	Missense_V196I	IGDCC4
exm1186080	15	[A/G]	Missense_V849M	AGBL1
exm1153366	15	[T/G]	Silent, Missense_H602Q	PLA2G4F
exm1192644	15	[A/G]	Silent, Missense_R1386G	SYNM
exm1161949	15	[T/C]	Silent, Missense_V936I, Missense_V936I	DMXL2
exm1241823	16	[T/G]	Missense_A157E, Missense_A128E	CES5A
exm1199140	16	[T/C]	Missense_A465T	MSLNL
exm1242656	16	[A/G]	Missense_G338D, Missense_G337D	SLC12A3
exm1261804	16	[A/C]	Missense_G813V	PKD1L2
exm1216469	16	[T/C]	Missense_G85S	CARHSP1
exm1223321	16	[A/G]	Missense_H238Y	ACSM1
exm1228348	16	[A/G]	Missense_I75V	IL4R
exm1198490	16	[T/C]	Missense_P151S	FAM173A
exm1263429	16	[A/G]	Missense_P163S	SLC38A8
exm1196373	16	[A/G]	Missense_P201S	TMEM8A
exm1260509	16	[G/C]	Missense_P218R	ADAMTS18
exm1262402	16	[T/C]	Missense_P236L	PLCG2
exm1261070	16	[A/G]	Missense_P3S	CMC2
exm1202171	16	[A/C]	Missense_R135S	PTX4
exm1221023	16	[T/C]	Missense_R147Q	XYLT1
exm1228660	16	[T/C]	Missense_R1630H	GTF3C1
exm1222362	16	[T/C]	Missense_R28Q	C16orf88
exm1246104	16	[A/G]	Missense_R393W	GOT2
exm1268976	16	[T/C]	Missense_R407G	PIEZO1
exm1230665	16	[A/G]	Missense_R515H, Missense_R514H	CD19

exm1206599	16	[T/C]	Missense_R699Q	CASKIN1
exm1244502	16	[A/G]	Missense_R836H	CCDC135
exm1236667	16	[T/C]	Missense_R842C	ITGAD
exm1197516	16	[C/G]	Missense_S165T	WDR90
exm1208984	16	[A/G]	Missense_T145M, Silent	TCEB2
exm1263247	16	[C/G]	Missense_T235S	NECAB2
exm1228589	16	[T/C]	Missense_V2034M	GTF3C1
exm1265676	16	[T/C]	Silent, Missense_L16F	KIAA0182
exm1210664	16	[T/C]	Synonymous_L590L, Missense_D438N	MEFV
exm1341024	17	[T/G]	Missense_A308S	RAD51C
exm1358102	17	[A/G]	Missense_A30T	SPHK1
exm1350530	17	[A/G]	Missense_A522T	C17orf80
exm1275652	17	[A/G]	Missense_A88V	SMYD4
exm1320079	17	[T/C]	Missense_C35Y	KRTAP1-5
exm1367854	17	[T/C]	Missense_D292N	P4HB
exm1360711	17	[T/C]	Missense_E3253K	DNAH17
exm1295318	17	[A/G]	Missense_E337G	C17orf48
exm1350497	17	[T/C]	Missense_F356L	C17orf80
exm1337714	17	[C/G]	Missense_H243D, Missense_H100D	SPAG9
exm1339307	17	[A/C]	Missense_L29I	OR4D2
exm1341176	17	[T/A]	Missense_L453M	TRIM37
exm1361017	17	[T/C]	Missense_M1986V	DNAH17
exm1275649	17	[T/C]	Missense_N101D	SMYD4
exm1350304	17	[A/G]	Missense_N392S	COG1
exm1301264	17	[A/G]	Missense_N636S	SMCR8
exm1332014	17	[T/C]	Missense_P186L	ITGB3
exm1318328	17	[C/G]	Missense_P228R	WIPF2
exm1336082	17	[A/C]	Missense_P334H	ACSF2
exm1316186	17	[T/C]	Missense_Q116R	ARL5C
exm1339993	17	[A/G]	Missense_R1149W, Missense_R1209W	BZRAP1
exm1306150	17	[T/C]	Missense_R1663H	KIAA0100
exm1281583	17	[T/C]	Missense_R172C	PLD2
exm1319172	17	[T/C]	Missense_R267H	KRT25
exm1321638	17	[A/G]	Missense_R282W	KRT35
exm1310223	17	[A/C]	Missense_S201R	LRRC37B
exm1321696	17	[A/G]	Missense_S36P	KRT35
exm1308572	17	[T/C]	Missense_S443L, Missense_S499L	EFCAB5
exm1304879	17	[A/G]	Missense_S608L	NOS2
exm1312166	17	[T/C]	Missense_S713N	SLFN11
exm1297587	17	[A/G]	Missense_T1507I, Missense_T1491I	NCOR1
exm1312358	17	[A/G]	Missense_T675I	SLFN13
exm1326022	17	[A/G]	Missense_V411M	AOC3
exm1369568	17	[T/C]	Missense_V480M	CCDC57
exm1312493	17	[T/C]	Missense_Y383C	SLFN12L
exm1298766	17	[T/C]	Silent, Missense_G303R	FLCN
exm1274634	17	[A/G]	Missense_A599V, Silent, Missense_A513V	SCARF1
exm1374533	18	[A/C]	Missense_A1133D	CCDC165
exm1392810	18	[A/G]	Missense_E117K	CNDP1
exm1371494	18	[A/G]	Missense_E173K	CLUL1
exm1387358	18	[A/G]	Missense_E374K	SMAD4
exm1372922	18	[T/G]	Missense_H54Q	C18orf42
exm1386887	18	[C/G]	Missense_P351A, Missense_P401A, Missense_P352A Missense_P345A, Missense_P378A, Missense_P370A, Missense_P426A, Missense_P425A	MBD1
exm1382469	18	[T/C]	Missense_P529L, Missense_P526L, Missense_P533L, Missense_P586L, Missense_P276L, Missense_P238L, Missense_P234L	DTNA
exm1372633	18	[A/G]	Missense_S181P, Missense_S181P	MYOM1
exm1373657	18	[T/C]	Missense_V1768M	LAMA1

exm1381205	18	[A/G]	Missense_V515I	DSG2
exm1393249	18	[T/C]	Missense_Y115C	ZADH2
exm1388566	18	[G/C]	Silent, Missense_T1242S	ATP8B1
exm1405682	19	[A/G]	Missense_A174T	S1PR4
exm1404924	19	[A/G]	Missense_A248T	ZNF556
exm1412203	19	[T/C]	Missense_D248N	LONP1
exm1395580	19	[T/C]	Missense_D329N, Missense_D353N	THEG
exm1430165	19	[C/G]	Missense_F292L, Missense_F289L	ZNF20
exm1410034	19	[A/G]	Missense_L279F	LRG1
exm1424151	19	[T/C]	Missense_M202T	RDH8
exm1422650	19	[T/A]	Missense_M277L	OR7D2
exm1418085	19	[T/G]	Missense_P1958H	FBN3
exm1408551	19	[T/C]	Missense_P240S	CCDC94
exm1424879	19	[A/G]	Missense_R136C, Missense_R136C	DNMT1
exm1424463	19	[A/G]	Missense_R410Q	PPAN-P2RY11
exm1417876	19	[T/C]	Missense_R50W	CCL25
exm1427986	19	[A/G]	Missense_R924C	DOCK6
exm1420707	19	[T/C]	Missense_S11104N	MUC16
exm1401998	19	[A/G]	Missense_S759P	REXO1
exm1420836	19	[A/G]	Missense_T10155I	MUC16
exm1422205	19	[A/G]	Missense_T617I	MUC16
exm1420710	19	[T/C]	Missense_V11097M	MUC16
exm1422472	19	[T/C]	Missense_V247I	OR7G3
exm1428070	19	[A/G]	Missense_G26S	C19orf80

Abbreviations: CRCC, chromophobe renal cell carcinoma; Chr., chromosome; SNP, single nucleotide polymorphism. *CRCC vs. normal renal tissue group, Fisher's exact test.

is higher in males than in females (approximately 1.2:1) [10]. CRCC has a better prognosis than conventional RCC; the reported 5-year overall survival rates for CRCC and conventional RCC are 87.5% and 100%, respectively [11]. Only 1.3% of CRCC patients present with distant metastases at diagnosis, and the reported 5- and 10-year cancer-specific survival rates are 93% and 88.9%, respectively [12]. Sarcomatoid RCCs account for about 1-8% of all renal neoplasms; this cancer type is not a distinct histological entity, as it arises from all RCC subtypes. Sarcomatoid RCCs are most frequently diagnosed at TNM stages III and IV, and are associated with a median survival of only 19 months. Similarly, our findingsindicate that CRCC S maybehave more aggressively than CRCC C, highlighting the importance of further comparative studies.

Herein, although thehistological and macroscopic characteristics were consistent, the presence of necrosis and its invasion into adjacent tissues were more common in CRCC S than CRCC C cases. Microscopically, CRCC S cases showed mixed regions containing both CRCC C-type cells and diffuse malignant spindle cells. Additionally, vascular and/or lymphovascular invasion was present in all CRCC Scases.

Immunohistochemically, we found that, similar to in previous studies, CRCC stained positive for CD117 (c-kit-encoded proto-oncogene [KIT]) [13-16], a type III receptor tyrosine kinase involved in signal transduction in several cell types. KIT activation (phosphorylation) upon ligand binding initiates asignal transduction cascade that ultimately leads to the activationof various transcription factors involved in the regulation of apoptosis, cell differentiation, proliferation, chemotaxis, and cell adhesion [17]. In the present study, the tumors tended to show amembranous/cytoplasmic KIT expression pattern, suggesting that abnormal transmembrane signal transduction mayexist in CRCC. Tyrosine kinase inhibitors have been shown to be effective in the treatment of CRCC in clinical trials. For example, Choueiri et al. reported that tyrosine kinase inhibitor therapy with sunitinib and sorafenibmay prolong progression-free survival in CRCC patients [18, 19].

In terms of chromosomal events, CRCC is characterized by multiple chromosomal changes, especially losses. The most common losses

Table 6. Gene Ontology (GO) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of the 211 missense mutated genes of chromophobe renal cell carcinoma ($p < 0.05$) detected by Human Exome BeadChip technology

Cluster	Term	Gene Count	%	*P* Value	Genes
Cell adehesion	GO:0022610~biological adhesion	46	6.7449	6.70789E-05	PLXNC1, AEBP1, COL21A1, ADAMTS13, CLSTN1, ASTN1, DSCAML1, ITGB3, CRNN, RADIL, PKD1L1, SCARF1, VCL, NRCAM, ALCAM, PCDHB16, TRO, COL6A3, FAT2, COL12A1, THBS3, TECTA, HAPLN1, PPFIBP1, HSPG2, NID1, MSLNL, STAB2, ECM2, GPR98, MUC4, ITGA9, VWF, LAMA1, DSG2, TSC1, TMEM8A, CDON, CPXM1, ITGA7, ITGAD, PDZD2, DST, CHL1, MUC16, AOC3
	GO:0007155~cell adhesion	46	6.7449	6.72522E-05	PLXNC1, AEBP1, COL21A1, ADAMTS13, CLSTN1, ASTN1, DSCAML1, ITGB3, CRNN, RADIL, PKD1L1, SCARF1, VCL, NRCAM, ALCAM, PCDHB16, TRO, COL6A3, FAT2, COL12A1, THBS3, TECTA, HAPLN1, PPFIBP1, HSPG2, NID1, MSLNL, STAB2, ECM2, GPR98, MUC4, ITGA9, VWF, LAMA1, DSG2, TSC1, TMEM8A, CDON, CPXM1, ITGA7, ITGAD, PDZD2, DST, CHL1, MUC16, AOC3
	GO:0007160~cell-matrix adhesion	10	1.4663	0.0040253	TECTA, TSC1, ADAMTS13, ITGA7, ITGAD, NID1, ITGB3, ECM2, THBS3, MUC4
	GO:0007229~integrin-mediated signaling pathway	8	1.1730	0.0113399	ADAMTS18, ITGA9, ADAMTS13, ITGA7, ITGAD, ITGB3, DST, ADAMDEC1
	GO:0031589~cell-substrate adhesion	11	1.6129	0.0023249	TECTA, VWF, TSC1, ADAMTS13, ITGA7, ITGAD, NID1, ITGB3, ECM2, THBS3, MUC4
	GO:0035023~regulation of Rho protein signal transduction	11	1.6129	0.0025070	MCF2L2, OBSCN, SYDE2, TSC1, GMIP, MCF2, AKAP13, TTN, VAV2, FARP1, KALRN
Cell motility	GO:0007010~cytoskeleton organization	28	4.1056	0.0032135	TRIOBP, ANLN, TTN, OFD1, KRT25, HOOK2, CTTNBP2, NISCH, DMD, TNKS, KRT4, DYNC1H1, PLD2, SPTBN5, CEP135, MAP1B, SPTBN4, MYH6, PLK1S1, PCM1, MAP2, SYNM, DST, NCOR1, LCP1, ARHGAP10, SGCB, CDC42BPB
	GO:0007018~microtubule-based movement	9	1.3197	0.0458527	KIF14, KIF7, DNAH17, KIF20B, DYNC1H1, DNAH7, DNAH8, DST, DNAH5
	GO:0007017~microtubule-based process	19	2.7859	0.003692	KIF14, CEP135, DNAH17, MAP1B, PLK1S1, DNAH7, PCM1, DNAH8, DNAH5, OFD1, CTTNBP2, HOOK2, KIF7, MAP2, KIF20B, TNKS, DYNC1H1, NCOR1, DST
	GO:0000226~microtubule cytoskeleton organization	12	1.7595	0.0147775	OFD1, CTTNBP2, HOOK2, CEP135, MAP2, MAP1B, TNKS, PLK1S1, DYNC1H1, PCM1, DST, NCOR1
	GO:0048870~cell motility	18	2.6393	0.0438898	SGPL1, PLXNB1, HMGCR, DNAH17, PODXL, ASTN1, VAV2, DNAH7, DNAH8, TGFB1, DNAH5, NRCAM, VEGFC, CCL25, CTTNBP2, LAMA1, APOB, PLAU
	GO:0001539~ciliary or flagellar motility	4	0.5865	0.0117961	DNAH17, DNAH7, DNAH8, DNAH5
ATP metabolic process	GO:0046034~ATP metabolic process	10	1.4663	0.0116586	ATP6V1C2, LONP1, ATP8B1, ATP8B2, ATP10D, MYH6, ATP12A, ATP6V0A4, ATP13A5, TGFB1
	GO:0006754~ATP biosynthetic process	8	1.1730	0.0371490	ATP6V1C2, ATP8B1, ATP8B2, ATP10D, ATP12A, ATP6V0A4, ATP13A5, TGFB1
	GO:0009205~purine ribonucleoside triphosphate metabolic process	10	1.4663	0.0222429	ATP6V1C2, LONP1, ATP8B1, ATP8B2, ATP10D, MYH6, ATP12A, ATP6V0A4, ATP13A5, TGFB1
	GO:0009199~ribonucleoside triphosphate metabolic process	10	1.4663	0.0233604	ATP6V1C2, LONP1, ATP8B1, ATP8B2, ATP10D, MYH6, ATP12A, ATP6V0A4, ATP13A5, TGFB1
	GO:0009144~purine nucleoside triphosphate metabolic process	10	1.4663	0.0282323	ATP6V1C2, LONP1, ATP8B1, ATP8B2, ATP10D, MYH6, ATP12A, ATP6V0A4, ATP13A5, TGFB1
	GO:0009141~nucleoside triphosphate metabolic process	10	1.4663	0.0417108	ATP6V1C2, LONP1, ATP8B1, ATP8B2, ATP10D, MYH6, ATP12A, ATP6V0A4, ATP13A5, TGFB1
Carbohy-drate and lipid metabolism and transport	GO:0016051~carbohydrate biosynthetic process	9	1.3196	0.0349184	GPD2, B3GNT8, RBP4, XYLT1, PLCG2, CHST15, PCK2, DSE, GLCE
	GO:0016042~lipid catabolic process	12	1.7595	0.0422305	SGPL1, PLD2, APOB, CHKB, PLCG2, SPHK1, SMPD1, PLA2G4F, HSD17B4, PLCB2, PHLDB2, SCARF1
	GO:0006869~lipid transport	11	1.6129	0.0321866	GOT2, RBP4, APOB, ABCG5, STAR, PSAP, CHKB, ATP8B1, ATP8B2, OSBPL10, ATP10D
	GO:0044242~cellular lipid catabolic process	8	1.1730	0.0173021	SGPL1, APOB, CHKB, PLCG2, SPHK1, SMPD1, PLA2G4F, HSD17B4
Sensory perception	GO:0007600~sensory perception	51	7.4780	7.37E-05	OR10T2, RP1L1, OR4L1, OR6C65, OR2V2, RDH8, OR4D2, HMCN1, OR7G3, OR4C15, OR52L1, OR6C1, OR14J1, TAS2R7, TAS2R20, PLCB2, CRYBB3, SCN10A, CRYBB2, MYO3A, OR10J3, CNGA4, OR51G1, GPR98, SAG, EYA4, TAS2R19, OR6V1, OR7D2, OR5AR1, RBP4, OR8U8, OR2T1, OR2L13, OR2L2, OR10G9, SCNN1A, OR10W1, TECTA, DFNA5, OR2A12, OR8G2, SPTBN4, OR10H1, ALMS1, KCNV2, TULP1, EYS, ATP6V0A4, OR8D4, OPN4

	GO:0050890~cognition	52	7.6246	0.0006084	HMGCR, OR10T2, RP1L1, OR4L1, OR6C65, OR2V2, RDH8, OR4D2, HMCN1, OR7G3, OR4C15, OR52L1, OR6C1, OR14J1, TAS2R7, TAS2R20, PLCB2, CRYBB3, SCN10A, CRYBB2, MYO3A, OR10J3, CNGA4, OR51G1, GPR98, SAG, EYA4, TAS2R19, OR6V1, OR7D2, OR5AR1, RBP4, OR8U8, OR2T1, OR2L13, OR2L2, OR10G9, SCNN1A, OR10W1, TECTA, DFNA5, OR2A12, OR8G2, SPTBN4, OR10H1, ALMS1, KCNV2, TULP1, EYS, ATP6V0A4, OR8D4, OPN4
	GO:0050877~neurological system process	64	9.3842	0.0010017	IGDCC3, HMGCR, OR10T2, RP1L1, OR4L1, OR6C65, TGFB1, OR2V2, RDH8, CTTNBP2, OR4D2, HMCN1, OR7G3, OR4C15, OR52L1, OR6C1, OR14J1, TAS2R7, TAS2R20, PLCB2, CRYBB3, CRYBB2, SCN10A, MYO3A, ALDH5A1, OR10J3, OR51G1, CNGA4, GPR98, SAG, EYA4, TAS2R19, SBF2, OR6V1, OR7D2, OR5AR1, RBP4, OR8U8, OR2T1, OR2L13, APLP2, OR2L2, OR10G9, DMD, PCDHB16, SCNN1A, DTNA, OR10W1, TECTA, DFNA5, OR2A12, NTF3, DLGAP2, OR8G2, SPTBN4, OR10H1, ALMS1, KCNV2, TULP1, EYS, TSC1, ATP6V0A4, OR8D4, OPN4
	GO:0007606~sensory perception of chemical stimulus	31	4.5455	0.0016093	OR8U8, OR2T1, OR10T2, OR4L1, OR6C65, OR2L13, OR2V2, OR2L2, OR4D2, OR7G3, OR4C15, OR52L1, OR10G9, OR6C1, OR14J1, TAS2R7, TAS2R20, SCNN1A, PLCB2, OR10W1, OR2A12, OR8G2, OR10H1, OR10J3, CNGA4, OR51G1, TAS2R19, OR6V1, OR7D2, OR8D4, OR5AR1
	GO:0050953~sensory perception of light stimulus	15	2.1994	0.0206174	RBP4, MYO3A, RP1L1, ALMS1, KCNV2, GPR98, SAG, EYA4, TULP1, RDH8, EYS, HMCN1, CRYBB3, CRYBB2, OPN4
	GO:0007608~sensory perception of smell	26	3.8123	0.0099784	OR8U8, OR2T1, OR10T2, OR4L1, OR6C65, OR2L13, OR2V2, OR2L2, OR4D2, OR7G3, OR4C15, OR10G9, OR52L1, OR6C1, OR14J1, OR10W1, OR2A12, OR8G2, OR10H1, OR10J3, OR51G1, CNGA4, OR6V1, OR7D2, OR8D4, OR5AR1
	GO:0007601~visual perception	15	2.1994	0.0206174	RBP4, MYO3A, RP1L1, ALMS1, KCNV2, GPR98, SAG, EYA4, TULP1, RDH8, EYS, HMCN1, CRYBB3, CRYBB2, OPN4
Other process	GO:0051674~localization of cell	18	2.6393	0.0438898	SGPL1, PLXNB1, HMGCR, DNAH17, PODXL, ASTN1, VAV2, DNAH7, DNAH8, TGFB1, DNAH5, NRCAM, VEGFC, CCL25, CTTNBP2, LAMA1, APOB, PLAU
	GO:0010720~positive regulation of cell development	7	1.0264	0.0341482	VEGFC, STAR, NTF3, PLXNB1, MAP1B, SMAD4, TGFB1
	GO:0030030~cell projection organization	21	3.0792	0.0361906	NTF3, MCF2, SPTBN4, MAP1B, ALMS1, DSCAML1, PCM1, VAV2, SCARF1, GPR98, VCL, NRCAM, ALCAM, OFD1, DNAI1, TSC1, DMD, MAP2, LHX4, DST, KALRN
	GO:0006023~aminoglycan biosynthetic process	4	0.5865	0.0454816	B3GNT8, XYLT1, DSE, GLCE
	GO:0043244~regulation of protein complex disassembly	6	0.8798	0.0329035	TRIOBP, IRAK3, SPTBN5, MAP2, SPTBN4, MAP1B
	GO:0043242~negative regulation of protein complex disassembly	6	0.8798	0.0139389	TRIOBP, IRAK3, SPTBN5, MAP2, SPTBN4, MAP1B
	GO:0033043~regulation of organelle organization	15	2.1994	0.0214477	TRIOBP, SPTBN5, MAP1B, EDN1, SPTBN4, CENPF, CTTNBP2, CUL7, TSC1, MAP2, KIF20B, DNMT1, TNKS, DST, IL1A
	GO:0007588~excretion	7	1.0264	0.0158675	SLC26A3, ABCG5, EDN1, AQP1, AMN, ATP6V0A4, SCNN1A
	GO:0043062~extracellular structure organization	12	1.7595	0.0292123	
KEGG Pathways	hsa02010:ABC transporters	7	1.0264	0.0048980	ABCA8, ABCG5, ABCB11, ABCD4, ABCC2, ABCA6, ABCB4
	hsa04740:Olfactory transduction	25	3.6657	0.0053120	OR2T1, OR8U8, OR10T2, OR4L1, OR6C65, OR2L13, OR2V2, OR2L2, OR4D2, OR7G3, OR4C15, OR10G9, OR52L1, OR6C1, OR14J1, OR2A12, OR8G2, OR10H1, OR10J3, OR51G1, CNGA4, OR6V1, OR7D2, OR8D4, OR5AR1
	hsa04512:ECM-receptor interaction	8	1.1730	0.0331190	ITGA9, LAMA1, VWF, COL6A3, ITGA7, HSPG2, ITGB3, THBS3
	hsa05410:Hypertrophic cardiomyopathy (HCM)	8	1.1730	0.0350186	ITGA9, DMD, ITGA7, ITGB3, MYH6, TTN, TGFB1, SGCB
	hsa00532:Chondroitin sulfate biosynthesis	4	0.5865	0.0444765	CHSY3, XYLT1, CHST15, DSE

Abbreviations: ECM, extracellular matrix.

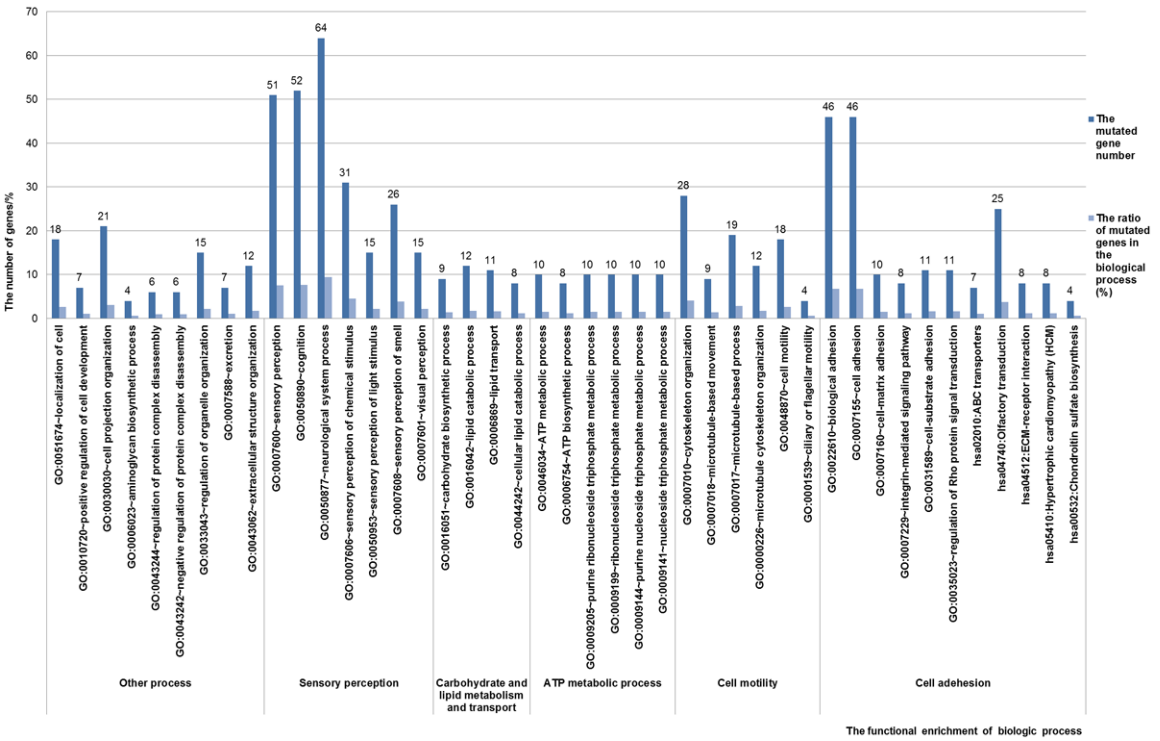

Figure 3. Functional enrichment analysis related biological process categories of the 211 missense-mutated genes detected by exome sequencing in chromophobe renal cell carcinoma (CRCC).

include chromosomes 1, 2, 6, 10, 13, 17, and 21, and the most frequently detected gains involve chromosomes 4, 7, 15, 19, and 20 [20-22]. Brunelli et al. found that over 60% of classic and metastatic CRCCs (without sarcomatoid change) showed chromosome 1, 2, 10, 13, 17, and 21 deletions, whereas chromosome 1, 2, 6, 10, and 17 gains were detected in more than 60% of CRCC S cases [23]. Our findings regarding chromosomal alterations in CRCC C are consistent withthese previous studies. However, we also identified differences in the chromosomal alterations between CRCC C and CRCC S. CRCC S had more chromosomal losses but fewer chromosomal gains compared with CRCC C. Chromosome 1p13-15, 3p21-ter, 2q23-24, and 1q11 gains and chromosome 8q21-23 loss were unique to CRCC S cases, where as chromosome 1p14-16, 10p12-ter, 10p16-20, 13p20-30, 17p13, 13q12-15, and 1p21-24 losses were only observed in CRCC C cases. Hence, genes on these chromosomes may be related to the development of CRCC C and CRCC S.

To further investigate the genetic changes in CRCC, we used Human Exome BeadChip technology. The mutated genes in CRCC were mainly involved in cell adhesion, cell motility, ATP metabolic processes, sensory perception, carbohydrate and lipid metabolism and transport, integrin-mediated signaling pathway regulation, and Rho protein signal transduction regulation. Additionally, some mutated genes were also associated with the ECM-receptor interaction pathway, ABC transporter pathway, lysosomes, complement and coagulation cascades, and glyoxylate and dicarboxylate metabolic signaling pathways. Most of these biological processes and pathways play very important roles in cell growth.

Importantly, almost one-fourthof the mutated genes were involved in the ECM-receptor interaction pathway, cell adhesion, cell motility, integrin-mediated signaling pathway regulation, and Rho protein signal transduction regulation. These processes and pathways are closelyassociated with cancer cell progression, migration, invasion, proliferation, and survival [24-27]. During malignant tumor progression, cells lose their original tissue contacts, move through the ECM, enter the blood and/or lymphatic system, and finally form new neoplasms.

Table 7. The 49 differentially mutated genes in CRCC C vs. CRCC S ($p < 0.05$)**

Chr.	SNP name	Alleles	Mutation(s)	Gene
1	exm53609	[A/G]	Missense_N156S	KLF17
1	exm64741	[A/C]	Missense_P549T	L1TD1
1	exm109069	[A/G]	Silent, Missense_G246R, Missense_G315R, Missense_G285R	RHBG
2	exm268940	[A/G]	Missense_R2790G	SPEG
2	exm221502	[T/C]	Missense_S239L, Missense_S295L	POLR1B
3	exm343170	[A/G]	Missense_G628R, Missense_G394R	PARP15
3	exm310819	[T/G]	Missense_K78N	SPINK8
3	exm336258	[T/C]	Missense_V72I	GUCA1C
4	exm407314	[T/C]	Missense_T268M, Missense_T366M	FAM47E
5	exm450340	[A/G]	Missense_N674S, Missense_N674S	NIPBL
5	exm2242697	[T/C]	Missense_S1654P, Missense_S1680P, Missense_S1341P	RGNEF
6	exm551752	[A/G]	Missense_G8R	SPATS1
6	exm554563	[T/C]	Missense_Q4048R	PKHD1
6	exm591241	[T/C]	Missense_R213Q	RSPH3
7	exm616266	[T/C]	Missense_T183A	GLI3
7	exm634903	[A/G]	Silent, Silent, Missense_S17P	CALCR
8	exm720028	[T/C]	Missense_T725A	MTSS1
8	exm695855	[A/G]	Silent, Missense_A651V	RAB11FIP1
9	exm768114	[T/C]	Missense_G487R	GRIN3A
9	exm755303	[T/C]	Missense_K1579E, Missense_K1584E	TRPM6
10	exm849133	[T/C]	Missense_C160Y	CWF19L1
10	exm812895	[A/G]	Missense_S396G	MRC1
11	exm968258	[T/C]	Missense_C31R	HYLS1
11	exm950576	[A/G]	Missense_G668S	KIAA1377
11	exm883596	[A/G]	Missense_H43Y	TRIM5
11	exm941972	[A/G]	Missense_M9V	MOGAT2
11	exm922352	[T/C]	Missense_P68L	C11orf20
11	exm897394	[T/G]	Missense_Q116K	CCDC34
11	exm971271	[T/C]	Missense_Q1331R	IGSF9B
11	exm961735	[A/G]	Missense_S889G	VPS11
12	exm1050700	[A/G]	Missense_H161R	TMEM132C
12	exm973046	[A/G]	Missense_R265Q	B4GALNT3
14	exm1083869	[A/G]	Missense_D2N	OR4L1
14	exm1124332	[A/C]	Missense_R310I, Missense_R210I	SERPINA9
14	exm1089709	[A/G]	Missense_S467P, Missense_S427P	ACIN1
14	exm1114782	[T/C]	Missense_V1686I	LTBP2
15	exm1167048	[T/C]	Missense_I1089V, Missense_I1132V	VPS13C
15	exm1185450	[T/C]	Missense_V845A	AKAP13
15	exm1185372	[T/C]	Missense_W494R	AKAP13
16	exm1202218	[A/G]	Missense_E7G	TELO2
19	exm1418682	[T/C]	Missense_G220R, Missense_G178R	CD320
19	exm1395595	[A/G]	Missense_H273Y, Missense_H297Y	THEG
19	exm1499653	[A/G]	Missense_I584V	ZNF578
19	exm1513142	[T/C]	Missense_L121P	ZNF304
19	exm1444070	[T/C]	Missense_L391P, Missense_L396P	ARRDC2
19	exm1421653	[A/G]	Missense_P4458L	MUC16
19	exm1438125	[A/C]	Missense_Q447P	CYP4F8
X	exm1633796	[A/G]	Missense_H161R	MAGEB16
X	exm1656466	[T/C]	Missense_M193T	CXorf64

**CRCC C vs. CRCC S, Fisher's exact test.

Table 8. Mutated genes located in different chromosomal regions in the CRCC C and CRCC S groups (detected by Human Exome BeadChip)

Chromosomal region (CGH)	Genes
-10p12-ter	MRC1
+1q21-23	RHBG
+3p21-ter	SPINK8
-8p21-23	MTSS1
-9q21	TRPM6

Abbreviations: +, Chromosomal gain; -, chromosomal loss; CRCC C, chromophobe renal cell carcinoma common type; CRCC S, chromophobe renal cell carcinoma with sarcomatoid change; CGH, comparative genomic hybridization.

Therefore, tumor cells inevitably experience alterations in cell-cell and cell-ECM adhesion, and tumor cell transformation is highly influenced by cell adhesion via adhesion receptors such as integrins, cadherins, cell surface proteoglycans, and tetraspanins [28]. In this process, some genes, which may play roles in the development of CRCC, are noteworthy. One such gene is TGFB1, which encodes a member of the transforming growth factor beta (TGF-β) cytokine family; these are multifunctional peptides that regulate proliferation, differentiation, adhesion, and migration [29]. In CRCC, TGF-β1 expression is predominantly membranous, and CRCCs with a capsule reportedly have significantly higher quantities of TGF-β1 expression in the tumor tissue and peritumoral renal parenchyma compared to tumors without [30]. Shimasaki et al. furtherreportedthat TGF-β1 may play a role in the formation of the tumor fibrous capsule in CRCC [31]. Another important gene is polo-like kinase (PLK)1. The PLK proteins are crucial regulators of cell cycle progression, centriole duplication, mitosis, cytokinesis, and the DNA damage response. PLKs undergo major changes in abundance, activity, localization, and structure at different stages of the cell cycle and interact with other proteins in a tightly controlled spatiotemporal manner as part of a network coordinating key cell cycle events. Their essential roles are highlighted by the fact that alterations in PLK function are associated with cancers and other diseases [32]. Zhang et al. reported that PLK1 is overexpressed in renal cancer and participates in the proliferation and invasion of renal cancer cells [33], and Dinget al. found that PLK1 may represent a rational therapeutic target for clear cell RCC [34]. Hence, it is worthfurther investigating the function of mutated TGF-β1 and PLK1 in CRCC; such studies will advance our understanding of CRCC biology and may lead to the development of novel therapeutic strategies.

Interestingly, we also found that the mutated genes were involved in the ATP metabolic process, carbohydrate biosynthetic process, and ABC transporter pathway. Microscopically, CRCC cells contain abundant mitochondria, which play an important role in cellular metabolism. Davis et al. implicated changes in mitochondrial function as a component of the CRCC biology, while suggesting alternative roles for mtDNA mutations in cancers relying on oxidative phosphorylation [22]. ABC transporters represent one of the largest and oldest families of membrane proteins and use energy derived from ATP hydrolysis to translocate, among various substrates, toxic compounds across the membrane. Recently, ABC transporters have been found to be able to actively efflux a multitude of structurally and mechanistically distinct cytotoxic compounds across membranes and are consequently believed to be a major contributor to multidrug resistance and chemotherapeutic failure in cancer patients [30]. Zoernig et al. reported that sequence mutations in the substrate-binding pocket of stem cell factor and the ABC subfamily G member 2 were potentially linked to RCC treatment resistance. Therefore, we speculate that ATP metabolic processes and the ABC transporter pathway may play roles in the development of CRCC.

Olfactory receptors are predominantly expressed in the olfactory epithelium and function in odorant detection; however, they are also expressed in other, unrelated, tissues. Recently, olfactory receptor stimulation has been shown to promote cancer cell invasion and metastasis [35]. Our results showed that there were 25 mutated genes involved in the olfactory receptor pathways (**Table 6**, **Figure 3**). However, the relationship between olfactory receptor signal transduction and RCC has not been investigated, andfurther studies are needed to clarify this point.

Using CGH and exome sequencing, we found thatthe mutation status of MTSS1, SPINK8, TRPM6, RHBG, and MRC1 differed between CRCC C and CRCC S. MTSS1 was first identi-

fied as a metastasis suppressor missing in metastatic bladder carcinoma cell lines. Loss of MTSS1 expression hasalso been observed in many other cancers, including lung, skin, ovarian, hepatocellular, gastric, prostate, and breast cancers [36-38]. There is strong evidence that MTSS1, as a multifunctional molecular player, plays an important role in cancer development, progression, and metastasis [39], and Du et al. reported that MTSS1 may suppress RCC growth via the Sonic Hedgehog pathway [40], indicating that MTSS1 mayrepresent a potential therapeutic target for CRCC S.

In conclusion, our study shows that multiple genetic abnormalitiesare present in CRCC C and CRCC S. These genetic alterations may provide clues regarding CRCC tumorigenesis and serve as a basis for future developments of targeted therapies against CRCC C and CRCC S.

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Disclosure of conflict of interest

None.

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