Gene expression profiling of clear cell papillary renal cell carcinoma: comparison with clear cell renal cell carcinoma and papillary renal cell carcinoma

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Clear cell papillary renal cell carcinoma is a distinct variant of renal cell carcinoma that shares some overlapping histological and immunohistochemical features of clear cell renal cell carcinoma and papillary renal cell carcinoma. Although the clear cell papillary renal cell carcinoma immunohistochemical profile is well described, clear cell papillary renal cell carcinoma mRNA expression has not been well characterized. We investigated the clear cell papillary renal cell carcinoma gene expression profile using previously identified candidate genes. We selected 17 clear cell papillary renal cell carcinoma, 15 clear cell renal cell carcinoma, and 13 papillary renal cell carcinoma cases for molecular analysis following histological review. cDNA from formalin-fixed paraffin-embedded tissue was prepared. Quantitative real-time PCR targeting alpha-methylacyl coenzyme-A racemase (*AMACR*), *BMP* and activin membrane-bound inhibitor homolog (*BAMBI*), carbonic anhydrase IX (*CA9*), ceruloplasmin (*CP*), nicotinamide *N*-methyltransferase (*NNMT*), schwannomin-interacting protein 1 (*SCHIP1*), solute carrier family 34 (sodium phosphate) member 2 (*SLC34A2*), and vimentin (*VIM*) was performed. Gene expression data were normalized relative to 28S ribosomal RNA. Clear cell papillary renal cell carcinoma expressed all eight genes at variable levels. Compared with papillary renal cell carcinoma, clear cell papillary renal cell carcinoma expressed more *CA9*, *CP*, *NNMT*, and *VIM*, less *AMACR*, *BAMBI*, and *SLC34A2*, and similar levels of *SCHIP1*. Compared with clear cell renal cell carcinoma, clear cell papillary renal cell carcinoma expressed slightly less *NNMT*, but similar levels of the other seven genes. Although clear cell papillary renal cell carcinoma exhibits a unique molecular signature, it expresses several genes at comparable levels to clear cell renal cell carcinoma relative to papillary renal cell carcinoma. Understanding the molecular pathogenesis of clear cell papillary renal cell carcinoma will have a key role in future sub-classifications of this unique tumor.

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Clear cell papillary renal cell carcinoma is a morphologically and immunohistochemically distinct renal epithelial neoplasm originally described as a ‘clear cell papillary renal cell carcinoma of end-stage kidneys’.¹ Since its initial description, it is

apparent that although clear cell papillary renal cell carcinoma has a propensity to arise in the setting of end-stage kidney disease, these tumors can occur *de novo* in normal kidneys. Histologically, clear cell papillary renal cell carcinoma is characterized by papillary infoldings composed almost entirely of cells with clear cytoplasm and polarized low-grade nuclei. Immunohistochemically, clear cell papillary renal cell carcinoma expresses cytokeratin 7 and carbonic anhydrase IX (*CA9*) but does not express alpha methyl-coenzyme-A racemase (*AMACR*), CD10, or transcription factor E3 (*TFE3*).² Clear cell

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papillary renal cell carcinoma does not harbor the chromosome 3p deletions seen in clear cell renal cell carcinoma or demonstrate trisomies 7 and 17, or loss of Y that is characteristic of papillary renal cell carcinoma.³ Clearly, clear cell papillary renal cell carcinoma is a distinct tumor that shares some morphological, immunohistochemical, and molecular features of both clear cell renal cell carcinoma and papillary renal cell carcinoma, but few studies have examined the molecular gene expression profile of these neoplasms. Although clear cell papillary renal cell carcinoma may have overlapping morphological features with clear cell renal cell carcinoma and papillary renal cell carcinoma (especially those with clear cell features) in areas, clear cell papillary renal cell carcinoma and papillary renal cell carcinoma are more aggressive tumors. Herein, using real-time (RT)-PCR, we targeted eight previously identified candidate genes (Table 1) to assess their expression levels in clear cell papillary renal cell carcinoma, clear cell renal cell carcinoma, and papillary renal cell carcinoma. We selected a panel of four candidate clear cell renal cell carcinoma markers and four papillary renal cell carcinoma markers, which had been identified in previous expression-profiling studies from our group. These markers were chosen to study whether the novel subtype clear cell papillary renal cell carcinoma was distinct from or similar to clear cell renal cell carcinoma or papillary renal cell carcinoma in terms of gene expression.

Materials and methods

Case Selection and Analysis

Seventeen cases of clear cell papillary renal cell carcinoma, 15 cases of clear cell renal cell carcinoma, and 13 cases of papillary renal cell carcinoma were selected for analysis. All cases were re-reviewed by a urological pathologist. The diagnosis of clear cell papillary renal cell carcinoma was based on classic morphological features and corresponding immunohistochemical profile (positive cytokeratin 7 expression and negative CD10, P504S/AMACR and TFE3 expression). Tumor grading and staging was based on

the standard Fuhrman nuclear-grading system and the American Joint Committee on Cancer, 7th edition tumor-node-metastasis staging system, respectively.^{4,5} This study was completed following the guidelines of and with approval from the institutional review board of our institution.

Quantitative RT-PCR

Quantitative RT-PCR experiments were performed in duplicate on total RNA isolated from formalin-fixed paraffin-embedded tissue from each specimen. Unstained histological sections were deparaffinized with ethanol and xylene. The tumor cells were microdissected with a sterile scalpel. Tissue was digested in buffer containing proteinase K at 60 °C overnight, and total RNA was isolated by phenol chloroform extraction and subjected to DNase treatment. RNA quality and quantity were assessed with Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Up to 3 µg of RNA was used for first-strand cDNA synthesis with Superscript III (Invitrogen/Life Technologies, Grand Island, NY, USA). PCR was carried out with sybrogreen master mix (Applied Biosystems/Life Technologies) in the 96-well format using ABI PRISM 7500 Fast RT-PCR system. PCR runs were analyzed by using Relative Quantification Manager (Applied Biosystems) software. Relative mRNA expression levels of eight genes (*AMACR*, *BAMBI*, *CA9*, *CP*, *NNMT*, *SCHIP1*, *SLC34A2*, and *VIM*, see Table 1) were assessed. These genes were selected from previous data generated by our laboratory as markers of clear cell renal cell carcinoma and papillary renal cell carcinoma.⁶

Data Analysis

Gene expression data were normalized relative to the geometric mean of the 28S ribosomal RNA house-keeping gene. mRNA extracted from normal kidney was used as a reference standard. For each tumor, the relative mean C_t of each gene was calculated as follows: (value well 1 + value well 2)/2. The tumor ΔC_t was calculated as follows: gene tested relative mean C_t – 28SRNA relative mean C_t . The $\Delta\Delta C_t$ was

Table 1 Genes overexpressed in clear cell renal cell carcinoma and papillary renal cell carcinoma

Symbol	Overexpressed in PRCC vs CCRCC	UniGene	GeneBank	Name
<i>AMACR</i>	PRCC	Hs.508343	NM_014324	<i>Alpha-methylacyl coenzyme-A racemase</i>
<i>BAMBI</i>	PRCC	Hs.533336	NM_012342	<i>BMP and activin membrane-bound inhibitor homolog</i>
<i>CA9</i>	CCRCC	Hs.63287	NM_001216	<i>Carbonic anhydrase IX</i>
<i>CP</i>	CCRCC	Hs.558314	NM_000096	<i>Ceruloplasmin</i>
<i>NNMT</i>	CCRCC	Hs.503911	NM_006169	<i>Nicotinamide N-methyltransferase</i>
<i>SCHIP1</i>	PRCC	Hs.134665	NM_014575	<i>Schwannomin-interacting protein 1</i>
<i>SLC34A2</i>	PRCC	Hs.479372	NM_006424	<i>Solute carrier family 34 (sodium phosphate) member 2</i>
<i>VIM</i>	CCRCC	Hs.455493	NM_003380	<i>Vimentin</i>

Abbreviations: CCPRCC, clear cell papillary renal cell carcinoma; PRCC, papillary renal cell carcinoma.

calculated as follows: tumor ΔC_t – normal kidney ΔC_t . Fold-change of mRNA was calculated as $2^{(-\Delta\Delta C_t)}$. Average fold-change was calculated by averaging the fold-change of the total cases for each tumor type.

Statistics

For differential mRNA expression data, values for each tumor type were compared using the Wilcoxon rank-sum test. For comparison of mean-normalized mRNA expression, a two-tailed Student's *t*-test assuming equal variances was performed. The mean mRNA fold-change in expression of each gene between each tumor histological subtype was analyzed. Statistical significance was set at $P < 0.05$.

Results

Case Selection and Analysis

Overall, 45 tumors from 28 male and 17 female patients with a mean age of 53 years (range 29–78 years) were included in the study. Eight male and 9 female patients, with a mean age of 53 years (range 29–73 years), had clear cell papillary renal cell carcinoma (Figures 1a–d), 9 male and 6 female patients, with a mean age of 58 years (range 40–78 years), had clear cell renal cell carcinoma, and 11 male and 2 female patients, with a mean age of 59 years (range 40–74 years), had papillary renal cell carcinoma. Fifteen of the 17 cases (88%) of clear cell papillary renal cell carcinoma were identified during screening of the patients for renal insufficiency. The average maximum tumor dimension of the 45 tumors was 5.8 cm (2.5 cm for clear cell papillary renal cell carcinoma, 8.7 cm for clear cell renal cell carcinoma, and 6.4 cm for papillary renal cell carcinoma). Thirty cases of renal cell carcinoma were pathological stage pT1 (17 clear cell papillary renal cell carcinoma, 2 clear cell renal cell carcinoma, and 11 papillary renal cell carcinoma), 3 clear cell renal cell carcinoma were in pT2, 11 clear cell carcinoma were in pT3 (11 clear cell renal cell carcinoma and 2 papillary renal cell carcinoma), and 1 clear cell renal cell carcinoma was in pT4. Multifocality was seen in nine clear cell papillary renal cell carcinoma and two papillary renal cell carcinoma. Lymph node metastases were seen in two clear cell renal cell carcinoma patients, and no patient had evidence of distant metastases at the time of surgical resection. Seventeen cases of clear cell papillary renal cell carcinoma, 4 clear cell renal cell carcinoma, and 7 papillary renal cell carcinoma showed nuclear features consistent with Fuhrman nuclear grade 2; 7 clear cell renal cell carcinoma and 5 papillary renal cell carcinoma were classified as Fuhrman nuclear grade 3. The clinicopathological characteristics of the 45 cases are summarized in Table 2.

Quantitative RT-PCR, Data Analysis and Statistics

The differential mRNA expression ($-\Delta\Delta C_t$) of the four papillary renal cell carcinoma genes (*AMACR*, *BAMBI*, *SCHIP1*, and *SLC34A2*) and the four clear cell renal cell carcinoma genes (*CA9*, *NNMT*, *CP*, and *VIM*) in each of the 45 individual renal cell carcinomas is shown in Figures 2a and b, respectively. The individual $-\Delta\Delta C_t$ values for the papillary renal cell carcinoma genes and the clear cell renal cell carcinoma genes were normalized using a $\log_2$ transformation ($2^{(-\Delta\Delta C_t)}$, Figures 3 and 4, respectively), and the results were averaged to give an overall mean-normalized mRNA expression value for the entire cohort of clear cell papillary renal cell carcinoma, papillary renal cell carcinoma and clear cell renal cell carcinoma (Figure 5). The data points were statistically analyzed using the Wilcoxon rank-sum test (Figure 2) or Student's *t*-test (Figure 5).

When comparing clear cell papillary renal cell carcinoma with papillary renal cell carcinoma, clear cell papillary renal cell carcinoma were characterized by overexpression of *CA9*, ceruloplasmin (*CP*), and vimentin (*VIM*; $P = 0.001$, 0.003 , and < 0.001 , respectively, by Wilcoxon rank-sum test; $P = 0.56$, 0.031 , and 0.020 , respectively, by Student's *t*-test) and relative overexpression of *CP* and nicotinamide N-methyltransferase (*NNMT*) when examined individually ($P = 0.001$ and < 0.001 , respectively, by Wilcoxon rank-sum test). The transformed and averaged values for *CP* and *NNMT* approached statistical significance ($P = 0.056$ and 0.068 , respectively, by Student's *t*-test). Clear cell papillary renal cell carcinoma showed relative underexpression of *AMACR*, *BAMBI*, and *SLC34A2* ($P < 0.001$, < 0.001 , and $= 0.014$, respectively, by Wilcoxon rank-sum test and $P = 0.005$, < 0.001 , and $= 0.001$, respectively, by Student's *t*-test). Clear cell papillary renal cell carcinoma and papillary renal cell carcinoma expressed comparable levels of schwannomin-interacting protein 1 (*SCHIP1*; approached statistical significance by Wilcoxon rank-sum test but not statistically significant by Student's *t*-test). The statistical analyses of the genes expressed in clear cell papillary renal cell carcinoma vs papillary renal cell carcinoma are summarized in Table 3.

When comparing clear cell papillary renal cell carcinoma with clear cell renal cell carcinoma, clear cell papillary renal cell carcinoma expressed slightly lower levels of *NNMT* (approached statistical significance by Wilcoxon rank-sum test ($P = 0.041$) but not significant by Student's *t*-test). No statistically significant differences (by Wilcoxon rank-sum test or Student's *t*-test) in *AMACR*, *BAMBI*, *SCHIP1*, *SLC34A2*, *CA9*, *CP*, or *VIM* expression were seen between clear cell papillary renal cell carcinoma and clear cell renal cell carcinoma. The statistical analyses of the genes expressed in clear cell papillary renal cell carcinoma vs clear cell renal cell carcinoma are summarized in Table 3.

Figure 1 Clear cell papillary renal cell carcinoma. (a) Clear cell papillary renal cell carcinoma (hematoxylin and eosin (H&E), low magnification). (b) Clear cell papillary renal cell carcinoma (H&E high magnification). (c) Positive cytokeratin 7 (CK7) expression in clear cell papillary renal cell carcinoma. (d) Negative AMACR/P504S expression in clear cell papillary renal cell carcinoma.

When comparing clear cell renal cell carcinoma with papillary renal cell carcinoma, clear cell renal cell carcinoma expressed relatively higher amounts of *CA9*, *CP*, *NNMT*, and *VIM* ($P=0.002$, 0.037 , <0.001 , and <0.001 , respectively, by Wilcoxon rank-sum test; $P=0.023$, 0.007 , <0.001 , and 0.009 , respectively, by Student's *t*-test). Papillary renal cell carcinoma expressed relatively higher amounts of *AMACR* ($P=0.001$ by Wilcoxon rank-sum test and $P=0.036$ by Student's *t*-test) and *BAMBI* (*BMP* and activin membrane-bound inhibitor homolog; $P<0.001$ by Wilcoxon rank-sum test and Student's *t*-test). Papillary renal cell carcinoma demonstrated relative overexpression of *SLC34A2* ($P=0.033$ by Wilcoxon rank-sum test), and the relative overexpression approached statistical significance when the values were transformed and averaged ($P=0.066$ by Student's *t*-test). Papillary renal cell carcinoma expressed slightly higher levels of *SCHIP1* compared with clear cell renal cell carcinoma (approached statistical significance by Wilcoxon rank-sum test ($P=0.065$) but not significant by

Student's *t*-test). These results are consistent with our previous work.⁶ Statistical analysis of the genes expressed in papillary renal cell carcinoma vs clear cell renal cell carcinoma is summarized in Table 4.

Discussion

Clear cell papillary renal cell carcinoma is a morphologically and immunohistochemically unique subset of renal cell carcinoma that do not harbor consistent chromosomal imbalances or *VHL* mutations according to the few molecular studies reported in the literature.^{7,8} To our knowledge, no studies have examined the mRNA expression of distinct papillary renal cell carcinoma or clear cell renal cell carcinoma genes in clear cell papillary renal cell carcinoma. Our data show that clear cell papillary renal cell carcinoma expresses abundant *CA9*, *CP*, *NNMT*, and *VIM* compared with papillary renal cell carcinoma, and somewhat similar but not

Table 2 Clinicopathological features of the selected renal cell carcinoma cases

No.	Age	Sex	Greatest dimension (cm)	pT	pN	pM	Fuhrman nuclear grade	Histologic subtype
1	52	F	5	(m) T1b	Nx	NA	2	CCPRCC
2	57	M	3.6	(m) T1a	Nx	NA	2	CCPRCC
3	47	M	0.9	T1a	Nx	NA	2	CCPRCC
4	58	F	1.2	T1a	Nx	NA	2	CCPRCC
5	32	M	3.5	(m) T1a	Nx	NA	2	CCPRCC
6	45	M	1.8	(m) T1a	Nx	NA	2	CCPRCC
7	66	M	2.5	(m) T1a	Nx	NA	2	CCPRCC
8	29	F	1.1	T1a	Nx	NA	2	CCPRCC
9	49	M	2	T1a	Nx	NA	2	CCPRCC
10	66	F	0.9	(m) T1a	Nx	NA	2	CCPRCC
11	44	M	2.5	T1a	Nx	NA	2	CCPRCC
12	64	F	2.1	(m) T1a	Nx	NA	2	CCPRCC
13	66	F	1	T1a	Nx	NA	2	CCPRCC
14	73	F	3.2	(m) T1a	Nx	NA	2	CCPRCC
15	60	F	1.3	T1a	Nx	NA	2	CCPRCC
16	39	M	5.4	T1b	Nx	NA	2	CCPRCC
17	59	F	4.8	(m) T1b	Nx	NA	2	CCPRCC
18	64	F	10.5	T2b	Nx	NA	2	CCRCC
19	60	M	16	T3c	Nx	NA	3	CCRCC
20	55	F	7	T1b	Nx	NA	2	CCRCC
21	46	M	7.9	T3b	N0	NA	3	CCRCC
22	48	M	5.1	T3a	N2	NA	3	CCRCC
23	51	M	7	T3b	Nx	NA	2	CCRCC
24	60	M	12	T4	N1	M1	4	CCRCC
25	54	F	10	T2a	N0	NA	4	CCRCC
26	66	M	14	T3b	N0	NA	3	CCRCC
27	60	F	11.5	T2b	N0	NA	3	CCRCC
28	40	F	6	T3b	N0	NA	4	CCRCC
29	78	M	5.2	T3b	Nx	NA	3	CCRCC
30	54	M	2.3	T1a	Nx	NA	3	CCRCC
31	62	F	4.5	T3b	Nx	NA	2	CCRCC
32	75	M	12.5	T3b	N0	NA	4	CCRCC
33	65	M	6.5	T3a	Nx	NA	3	PRCC
34	47	M	6	T1b	Nx	NA	3	PRCC
35	56	M	6.4	T1b	Nx	NA	3	PRCC
36	74	M	4.8	T1b	Nx	NA	2	PRCC
37	51	F	26	T3a	Nx	NA	4	PRCC
38	64	M	4.5	(m) T1b	Nx	NA	2	PRCC
39	56	M	3.2	T1a	Nx	NA	3	PRCC
40	69	M	6	T1b	Nx	NA	2	PRCC
41	65	M	2.4	T1a	Nx	NA	2	PRCC
42	40	F	3.7	T1a	Nx	NA	2	PRCC
43	60	M	2.2	T1a	Nx	NA	3	PRCC
44	64	M	6	T1b	Nx	NA	2	PRCC
45	57	M	5.9	(m) T1b	Nx	NA	2	PRCC

Abbreviations: CCRCC, clear cell renal cell carcinoma; CCPRCC, clear cell papillary renal cell carcinoma; M, multiple; NA, not available; PRCC, papillary renal cell carcinoma.

identical expressions of *AMACR*, *BAMBI*, *SCHIP1*, *SLC34A2*, *CA9*, and *CP* compared with clear cell renal cell carcinoma. *NNMT* levels are however decreased in clear cell papillary renal cell carcinoma, compared with clear cell renal cell carcinoma. These results suggest that although clear cell papillary renal cell carcinoma expresses a unique molecular signature when compared with clear cell renal cell carcinoma or papillary renal cell carcinoma, it clearly shows closer overlap with clear cell renal cell carcinoma compared with papillary renal cell carcinoma.

Previous studies have shown that clear cell renal cell carcinoma shows abundant expression of VIM, a diversely expressed intermediate filament that has a role in cell adhesion, migration, survival, and epithe-

lial-to-mesenchymal transition.⁹ A recent report suggests that VIM overexpression functions as a renal cell carcinoma oncogene and is regulated by miRNA-138.¹⁰ Furthermore, clear cell papillary renal cell carcinoma demonstrates increased VIM expression by immunohistochemistry, a feature that can be useful in the pathological diagnosis of these tumors.¹¹ VIM is dysregulated in clear cell renal cell carcinoma and clear cell papillary renal cell carcinoma, but the contribution of VIM to the pathogenesis of clear cell papillary renal cell carcinoma is unclear and merits further investigation.

Clear cell papillary renal cell carcinoma expressed low levels of *AMACR*, *BAMBI*, and *SLC34A2*. The low *AMACR* expression is consistent with the absence of *AMACR* immunohistochemical staining

Figure 2 (a, b) Differential mRNA expression ($-\Delta\Delta C_t$) in renal cell carcinoma. Total RNA was isolated from formalin-fixed paraffin-embedded tissue, and mRNA levels for *AMACR*, *BAMBI*, *SCHIP1*, *SLC34A2*, *CA9*, *CP*, *NNMT*, and *VIM* were assessed. Gene expression data were normalized relative to the geometric mean of the 28S ribosomal RNA (28SRNA) housekeeping gene. mRNA from normal kidney was used as a reference standard. Differential mRNA expression ($-\Delta\Delta C_t$) is shown. Each bar is an individual tumor. *P*-values are calculated by Wilcoxon rank-sum test.

described in this tumor.^{3,12,13} *AMACR*, *BAMBI*, and *SLC34A2* are genes that are expressed at higher levels in papillary renal cell carcinoma, suggesting that clear cell papillary renal cell carcinoma is molecularly distinct from papillary renal cell carcinoma. However, *SCHIP1* was expressed at a similar level between the two tumor types; therefore, some molecular overlap does exist. Future studies are needed to characterize additional papillary renal cell carcinoma genes that are differentially expressed compared with clear cell papillary renal cell carcinoma in order to better characterize the distinct molecular phenotype of clear cell papillary renal cell carcinoma compared with papillary renal cell carcinoma.

Clear cell papillary renal cell carcinoma also expressed high levels of *CP* and *CA9* similar to the levels seen in clear cell renal cell carcinoma. *CP* is an acute-phase reactant protein that is expressed in many inflammatory situations, and serum *CP* protein levels are elevated in patients with renal cell carcinoma.⁶ *CA9* is a hypoxia-inducible protein overexpressed in clear cell renal cell carcinoma

secondary to *VHL* dysregulation, but Rohan *et al.*⁸ showed that *CA9* upregulation in clear cell papillary renal cell carcinoma occurs via a *VHL*-independent mechanism.^{8,14} These observations suggest that although clear cell papillary renal cell carcinoma overexpresses *CA9*, the pathway for its overexpression is likely different than that of clear cell renal cell carcinoma. This supports the hypothesis that although clear cell papillary renal cell carcinoma share some molecular features with clear cell renal cell carcinoma, unique molecular pathway alterations exist.

Clear cell papillary renal cell carcinoma are typically described as low-grade malignancies.⁷ In our study, clear cell papillary renal cell carcinoma tended to be smaller, multifocal, and of lower pathological stage and grade, which was similar to the clinicopathological features seen in papillary renal cell carcinoma (Table 2). These clinicopathological features were not shared with clear cell renal cell carcinoma; clear cell renal cell carcinoma were larger, all unifocal, and most were in pathological

Figure 3 Normalized $2^{(-\Delta\Delta C_t)}$ mRNA expression of the papillary renal cell carcinoma genes. Total RNA was isolated from formalin-fixed paraffin-embedded tissue, and mRNA levels for *AMACR*, *BAMBI*, *SCHIP1*, and *SLC34A2* were assessed. Each bar represents the log₂ gene mRNA expression level of a single tumor.

Figure 4 Normalized $2^{(-\Delta\Delta C_t)}$ mRNA expression of the clear cell renal cell carcinoma genes. Total RNA was isolated from formalin-fixed paraffin-embedded tissue, and mRNA levels for *CA9*, *CP*, *NNMT*, and *VIM* were assessed. Each bar represents the log₂ gene mRNA expression level of a single tumor.

Figure 5 Overall mean-normalized mRNA expression of the eight candidate genes. Total RNA was isolated from formalin-fixed paraffin-embedded tissue, and mRNA levels for *AMACR*, *BAMBI*, *SCHIP1*, *SLC34A2*, *CA9*, *CP*, *NNMT*, and *VIM* were assessed. Each bar represents the average log2 gene mRNA expression level of the 17 individual clear cell papillary renal cell carcinomas, 15 individual clear cell renal cell carcinomas, and the 13 papillary renal cell carcinomas from Figures 4 and 5. *P*-values are calculated by Student's *t*-test.

Table 3 Summary of the gene expression for clear cell papillary renal cell carcinoma

mRNA		P-value			P-value	
		WRS	TT		WRS	TT
<i>AMACR</i>	Decreased	<0.001	0.005	Similar	0.143	0.611
<i>BAMBI</i>	Decreased	<0.001	<0.001	Similar	0.551	0.300
<i>SCHIP1</i>	Similar	0.508	0.446	Similar	0.227	0.633
<i>SLC34A2</i>	Decreased	0.014	0.001	Similar	0.937	0.165
<i>CA9</i>	Increased ^a	0.001	0.056	Similar	0.627	0.948
<i>CP</i>	Increased	0.003	0.031	Similar	0.627	0.613
<i>NNMT</i>	Increased ^a	<0.001	0.068	Decreased ^a	0.041	0.181
<i>VIM</i>	Increased	<0.001	0.020	Similar	0.189	0.107

Abbreviations: CCRCC, clear cell renal cell carcinoma; CCPRCC, clear cell papillary renal cell carcinoma; PRCC, papillary renal cell carcinoma; TT, Student's *t*-test; WRS, Wilcoxon rank-sum test.

^aSignificant by one metric.

Table 4 Gene expression of clear cell renal cell carcinoma relative to papillary renal cell carcinoma

mRNA	CCRCC expression relative to PRCC	P-value	
		WRS	TT
<i>AMACR</i>	Decreased	0.001	0.037
<i>BAMBI</i>	Decreased	<0.001	<0.001
<i>SCHIP1</i>	Decreased ^a	0.065	0.235
<i>SLC34A2</i>	Decreased ^b	0.033	0.066
<i>CA9</i>	Increased	0.002	0.023
<i>CP</i>	Increased	<0.001	0.007
<i>NNMT</i>	Increased	0.037	<0.001
<i>VIM</i>	Increased	<0.001	0.009

Abbreviations: CCRCC=clear cell renal cell carcinoma; PRCC= papillary renal cell carcinoma; TT = Student's *t*-test; WRS = Wilcoxon rank-sum test.

^aApproached statistical significance.

^bSignificant by one metric

stage T3 or greater (Table 2). However, the gene expression profile of the eight tested genes in clear cell papillary renal cell carcinoma was more similar to clear cell renal cell carcinoma (seven of the eight genes showed similar expression profiles) and not papillary renal cell carcinoma (only *SCHIP1* showed

similar expression). The fact that clear cell papillary renal cell carcinoma exhibit clinicopathological similarities to papillary renal cell carcinoma, but exhibit a gene expression profile that closely resembles clear cell renal cell carcinoma, underscores the need for additional translational studies

regarding the behavior of clear cell papillary renal cell carcinoma.

We analyzed the changes in mRNA expression by both nonparametric (Wilcoxon rank-sum test) and parametric (Student's *t*-test) means partly owing to the small number of samples, but also owing to the large intersample variation for each RNA expression value. Overall, there was good concordance between the statistical values obtained. When comparing clear cell papillary renal cell carcinoma with papillary renal cell carcinoma, discordant statistical significance was only seen with *NNMT* and *CA9* mRNA; both were significant by Wilcoxon rank-sum test and approached significance by Student's *t*-test (Figures 2 and 5, and Table 3). When comparing clear cell papillary renal cell carcinoma with clear cell renal cell carcinoma, there was a statistically significant difference in *NNMT* expression by Wilcoxon rank-sum test but not by Student's *t*-test.

The nonparametric Wilcoxon rank-sum test is much less sensitive to outliers than the parametric Student's *t*-test, but the Student's *t*-test is a more conventional method to assess log₂-transformed expression data. There are several potential outlying data points that may have skewed the Student's *t*-test results (Figures 3 and 4). However, we do not believe that this discordance in statistical significance between the Wilcoxon rank-sum test and Student's *t*-test compromises our primary conclusions, but rather reinforces the need to validate these data with additional tumor samples. Statistical discordance was also seen with *SCHIP1* (approached significance by Wilcoxon rank-sum test but not Student's *t*-test) and *SLC34A2* mRNA (significant by Wilcoxon rank-sum test but approached significance by Student's *t*-test) when comparing clear cell renal cell carcinoma with papillary renal cell carcinoma (Figures 3 and 4, and Table 4). However, previous studies with more samples clearly validated this association, further supporting the conclusion that a larger *n* is needed to mitigate the influence of outlying data points.⁶

In conclusion, our study demonstrates that clear cell papillary renal cell carcinoma exhibits a unique molecular signature when compared with clear cell renal cell carcinoma and papillary renal cell carcinoma, and although this signature is distinct, it expresses several genes at comparable levels to clear cell renal cell carcinoma relative to papillary renal cell carcinoma. Understanding the molecular pathogenesis of clear cell papillary renal cell carcinoma will have a key role in future subclassifications of this unique tumor.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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