Original Article

A preliminary study on the association between epithelial-mesenchymal transition and circulating tumor cells in renal cell carcinoma

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Background: Circulating tumor cells (CTCs) are considered useful prognostic factors for various cancers, and in 2014, our research group conducted a comparative experiment of CTC detection in patients with renal cell cancer (RCC). However, the reason for the low detection rate of CTCs in cancer patients using the CellSearch® system is still unknown, although it has been hypothesized to be attributed to the likelihood that CTCs undergoing epithelial-mesenchymal transition (EMT) do not express the CTC biomarkers cytokeratin (CK)8/18/19 or epithelial cell adhesion molecule (EpCAM). The overall aim of the current study was to investigate the expression levels of CK8/18/19 and EpCAM in relation to the EMT biomarkers vimentin and E-cadherin in patients with RCC.

Methods: Patients with RCC who had undergone radical nephrectomy or partial resection between May 2014 and December 2014 were initially recruited.

Results: Among 34 RCC patients, nine co-expressed EpCAM and CK8/18/19 in primary tumor tissues. The CellSearch® results showed that CK8/18/19 was expressed in 5 of 6 patients (5/6) and EpCAM was expressed in 6 patients (6/6). However, the isolation by size of tumor cells (ISET) technique showed these were co-expressed in only four of the 10. The expression of CK8/18/19, EpCAM, vimentin, and E-cadherin was distributed unequally in different enumeration groups of CTCs (all $P>0.05$), and the positive expression of CK8/18/19 was correlated with neutrophil number and tumor size ($P<0.05$). The positive expression of vimentin was correlated with the Karnofsky Performance Status (KPS) score and clinical stage of renal cancer patients ($P<0.05$).

Conclusions: Our results indirectly proved the occurrence of EMT in the formation of CTCs by comparing and analyzing the expression of CK8/18/19 and EpCAM in renal cancer tissues and the detection results of CTCs.

Keywords: Renal cell cancer (RCC); circulating tumor cells (CTCs); epithelial-mesenchymal transition

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Introduction

In a previous study, our team found a significant difference in circulating tumor cells (CTCs) detection results between the CellSearch® system and CTCBIOPSY, which we believed might be caused by the different principles of the two detection methods. Isolation by size of tumour cells (ISET) technology (CTCBIOPSY) is a method of separating peripheral blood CTCs by using physical characteristics of cell size. Its main principle is to intercept and enrich CTCs through an 8-micron aperture filter membrane, and its detection rate is not affected by epithelial cell adhesion molecule (EpCAM) and cytokeratin (CK) expression. CTCs cannot be found in healthy people's peripheral blood. CTCs and circulating tumor microemboli (CTM) undergoing epithelial-mesenchymal transition (EMT) can be detected using this technology in tumor patients. High CTCs in tumor patients is associated with poor prognosis. The CellSearch® system uses immunomagnetic enrichment to detect CTCs, and during the detection process, CTCs and white blood cells in the pretested blood are first enriched with magnetic fluid containing anti-EpCAM antibodies. If there is no or minimal expression of EpCAM antigen on the cell surface, a cell will not be recognized by the system, which will affect the enrichment of CTCs in renal cancer.

The basic principles of the above detection methods indicate that EpCAM-based immunoassay cannot achieve stable *in vitro* enrichment of all tumor types of CTC, while other CTC isolation methods based on ISET technology are mainly based on the size and deformation capacity of CTC, as tumor cells are generally larger than white blood cells (1,2). Therefore, CTCs can be physically isolated by means of physical methods such as microporous membrane filtration, fissure, and microchip.

Through a literature search and review, we found that decreased expression of EpCAM antigen in CTCs can be manifested in the following two situations: (I) CTCs with low expression of EpCAM; (II) non-epithelial CTCs.

(I) CTCs with low EpCAM expression: a study has shown EpCAM expression of CTCs in solid tumor patients was down-regulated after receiving adjuvant chemoradiotherapy (3). Further, CTCs subsets undergo EMT and become mesenchymal cells and no longer express epithelial properties (4).

(II) Non-epithelial CTCs (mixed tumor cells, EMT+ tumor cells, and CTSCs): continuous proliferation of cancer stem cells (CSCs) exists in malignant tumors. Circulating tumor stem cells (CTSCs) are a small portion of tumor cells (5-8). Assuming independent subclonal CSCs populations in tumors have different functional characteristics (7), only CTSCs subpopulations have the potential to metastasize to distant organs. EpCAM has been confirmed as a marker of CSCs (9,10), but no expression of EpCAM has been confirmed in CTSCs. Since CSCs continuously express transcription factors that induce EMT such as Snail, Twist and Slug (11,12), it is reasonable to expect epithelial markers such as EpCAM are down-regulated, and many mesenchymal markers are up-regulated during the formation of CTSCs like other EMT+ cells. Therefore, CTCs including epithelial tumor cells, tumor cells with EMT+, and CTSCs can coexist in peripheral blood (13-15). Recent studies have shown the presence of mixed phenotype (E/EMT+) CTCs in metastatic non-small cell lung cancer (16), early and metastatic breast cancer (17), and advanced prostate cancer (18). Compared with patients with early breast cancer, EMT related proteins such as vimentin and Twist1 are often induced in CTCs of patients with metastasis (19). *In vitro* and *in vivo* experiments have shown that CTCs epithelial cells enter the vascular system and once exposed to blood, will be transformed into EMT+ tumor cells by the stimulation of platelet-derived transforming growth factor-β (TGF-β) (20).

In 2015, several scholars (21-23) filtered and retested the waste liquid of blood samples tested by the CellSearch® system and the flushing fluid of the collector using an automatic sample collection device (ASCS). Immunofluorescence staining was used to analyze the microscreen results, and many CTCS with low EpCAM expression were found, which were characterized by the nucleus as 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride positive, CK positive, and cluster of differentiation (CD) 45 negative. Immunofluorescence staining suggested this was a CTC subgroup with low EpCAM expression. This indicates that the CellSearch® system can only detect part of CTC in the blood samples of tumor patients (24), and CTC with a low expression of EpCAM are excluded in the detection process (25). Another *in vitro* study (26) found the expression of EpCAM isomer induced by bevacizumab also led to the omission of CTC detection by the CellSearch® system, because different antigen states may cover the binding site and even reduce

the binding affinity of EpCAM when it is fixed on magnetic nuclear nanoparticles, negatively affecting the CTC detection process (27).

In addition, many studies (28-31) have provided evidence that the number of detections of CTCs without or with low EpCAM expression is increasing in specific tumor types, and that EpCAM-negative CTCS are associated with poorer prognosis (32). In 2011, EpCAM-negative CTCs not detected by CellSearch® were introduced as predictors of anti-angiogenesis therapy in patients with a poor prognosis of colorectal cancer (33) and in those with triple-negative breast cancer combined with brain metastases (34). In 2014, the results of multi-parameter flow cytometry CTC detection suggested EpCAM-negative CTC not detected by CellSearch® was associated with significantly reduced overall survival in breast cancer patients (35). In addition, there is evidence (36) that EpCAM-negative CTCs are preferentially associated with brain metastasis, while CTCs with high EpCAM expression have a higher tendency for bone metastasis in tumors (37). Several studies (38-40) have linked a pure mesenchymal CTC phenotype associated with poor prognosis in EpCAM-negative CTCS not detected by CellSearch®.

In this study, 34 patients enrolled in a previous study were used to compare the expressions of CK8/18/19 and EpCAM in CTCs of renal cancer patients and those in corresponding primary tumor tissues, and the causes analyzed. We aimed to investigate the mechanism of the low sensitivity of the CellSearch® system to detect peripheral blood CTCs in patients with renal cancer, and to reveal the changes of epithelial characteristics during the formation of CTCs in patients with the disease. The expression of epithelial markers [CK8/18/19 (+), EpCAM] and interstitial markers (vimentin, E-cadherin) in CTCs of patients with renal cancer was studied to further classify and analyze the relationship between different types and the clinicopathological features of patients and explore their prognostic value for renal cancer patients. We present the following article in accordance with the MDAR reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-22-142/rc).

Methods

Study patients and data collection

Patients with primary RCC who had undergone radical nephrectomy or partial resection between May 2014 and December 2014 were initially recruited. The inclusion criteria were as follows: aged 18 years or older; histologically confirmed RCC; being treated for the first time or had a minimum of a 6-month treatment-free period; and the Karnofsky Performance Status (KPS) score was ≥60, according to the World Health Organization definition. Patients with a history of other carcinomas in the past 5 years or dermatologic disease were excluded. Formalin-fixed, paraffin-embedded RCC tissue samples were collected from all eligible 40 patients for histological analysis. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Shandong Cancer Hospital and Institute (No. 201801001) and written informed consent was obtained from all recruited patients.

Immunohistochemistry

The paraffin-embedded RCC tissue microarray block was cut into serial 4-μm-thick sections, and the paraffin sections were baked in an oven at 70 ℃ for 1 h. The sections were dewaxed in xylene and graded ethanol solutions then heated at 100 ℃ and high pressure for 30 min under citrate or ethylene diamine tetraacetic acid (EDTA) solution (CK8/18/19, citrate pH 6; EpCAM, EDTA pH 8; E-cadherin, EDTA pH 9; vimentin, EDTA pH 8). Sections were cooled at room temperature for 20 min, washed with phosphate-buffered saline (PBS) three times for 3 min, immersed in the presence of 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity, and finally washed three times with PBS for 3 min. Sections were incubated with the following primary polyclonal antibodies: mouse anti-human CK8/18/19 (dilution: 1:100, Abcam, 2A4, Cambridge, UK), mouse anti-human EpCAM (dilution: 1:20, Abcam, Ber-EP4, Cambridge, UK), mouse anti-human vimentin (working fluid, Zhongshan Goldbridge Biotechnology Co., Ltd., Beijing, China), and rabbit anti-human E-cadherin monoclonal antibody (working fluid, Zhongshan Goldbridge Biotechnology Co., Ltd., Beijing, China), respectively, at 4 ℃ overnight. The sections were then incubated with horseradish peroxidase-labeled goat anti-mouse/rabbit secondary antibody at room temperature for 20 min, then stained with 3,3'-diaminobenzidine for 5 min, then hematoxylin for 30 s. Hydrochloric acid alcohol was used for hematoxylin differentiation and ammonia was used to turn the staining back to blue. The sections were then dehydrated, mounted, and sealed.

Evaluation of immunohistochemical staining results

The slides were independently examined by two senior pathologists who were unaware of the clinical parameters of the patients and the immunostaining intensities of CK8/18/19 and vimentin expression were assessed according to the method of DiMaio *et al.* (38). The staining intensity was further classified as follows: (I) positive intensity score: no color in 0 min, light yellow in 1 min, brown in 2 min, and brown black in 3 min; (II) proportion of all cells that were positively stained: <5%, 0 point; 6–25%, 1 point; 26–50%, 2 points; 51–75%, 3 points; and >75%, 4 points. The product of the two scores was used as the final score: <4 points indicated negative expression (–); 5–8 points indicated weakly positive expression (1+); and >9 points indicated strongly positive expression (2+). EpCAM expression was evaluated based on the method of Spizzo *et al.* (39), where a cell membrane with brown granules indicated positive staining. The percentage of positive cells was used to determine the staining intensity as follows: ≤10%, + (weak expression); 10–50%, ++ (moderate expression); ≥50%, +++ (strong expression). If no positive staining was shown, the result was considered negative. E-cadherin expression was evaluated based on the method of Kadowaki *et al.* (40). The cell membrane/cytoplasm appeared as brownish brown particles in positively stained samples, and the staining intensity was determined based on the percentage of positive cells: ≥90%, normal expression; <90%, abnormal expression.

Statistical analysis

Continuous variables were compared using the *t*-test and expressed as the mean ± standard deviation (SD). The χ^2 test or Fisher's exact test was used to compare the baseline characteristics of RCC patients in different groups of CTCs (positive *vs.* negative; ≤1 *vs.* >1; ≤2 *vs.* >2), CK8/18/19 (positive *vs.* negative), EpCAM (positive *vs.* negative), vimentin (positive *vs.* negative), and E-cadherin (reduced expression *vs.* normal expression). All statistical analyses were performed using the SPSS version 26.0.

Results

EMT- and CTC-related biomarkers in patients with CK8/18/19, EpCAM, vimentin, and E-cadherin expressed in tumor tissues

Statistical results showed CK8/18/19 positive expression (24 cases), EpCAM positive expression (11 cases), vimentin positive expression (19 cases), and E-cadherin positive expression (five cases) were found in 34 patients with RCC. Co-expression of EpCAM and CK8/18/19 in primary tumor tissues was seen in nine patients, and the CellSearch® results showed that CK8/18/19 was expressed in 5 of 6 patients (5/6) and EpCAM was expressed in 6 patients (6/6). However, the isolation by size of tumor cells (ISET) technique showed only four of the 10 patients co-expressed EpCAM and CK8/18/19 (*Table 1*).

Baseline characteristics of the study population

The positive expression of CK8/18/19 correlated with neutrophil number and tumor size in patients with renal cancer (P<0.05), while the positive expression of vimentin correlated with the KPS score and clinical stage (P<0.05) (*Tables 2,3*) (*Figures 1-8*).

Classifications of EMT phenotypes in relation to clinical features

The proportion of complete, incomplete, and wild-type phenotype patients was 44.12% (15/34), 52.94% (18/34) and 2.94% (1/34), respectively (*Table 4*). All types were correlated with International Metastatic Renal-Cell Carcinoma Database Consortium (IMDC) score, clinical stage, and CTCs (P<0.05).

Discussion

Epithelial-mesenchymal transformation is the transformation of epithelial cells into mesenchymal cells. It is involved not only in embryonic development and normal physiology, but also in many pathological processes. Tumor cells undergoing EMT have enhanced ability of invasion, metastasis and anti-apoptosis, and escape from the primary site into the blood to form CTCs. EMT can not only promote the production of CTCs, but also promote their survival, and ultimately promote the formation of metastatic foci of CTCs through mesenchymal-epithelial transition (MET).

In this study, the expression rates of four epithelial-mesenchymal transformation markers in primary renal cancer tissues were similar to those previously reported. The positive expression of CK8/18/19 correlated with the neutrophil number and tumor size of renal cancer patients, and the positive expression of vimentin correlated with their KPS score and clinical stage. However, single expression

Table 1 Expressed markers in the primary tumor tissues

Patient ID	Ep	CK	E-d	Vim	CS	ISET CTC	ISET CTM
SD000	+	++	–	–	2	1	0
SD014	+	++	–	++	0	1	7
SD020	+	++	–	+	0	0	0
SD023	–	++	+	+	0	0	1
SD033	–	–	–	–	0	0	0
SD074	–	+	–	++	0	0	0
SD084	+	+	–	–	1	0	0
SD092	+	++	–	–	0	0	0
SD093	+	++	+	++	2	0	0
SD121	+	++	–	+	1	0	0
SD130	–	++	–	++	0	0	0
SD132	+	–	–	–	2	0	0
SD145	–	–	–	–	0	0	0
SD155	–	++	–	+	0	0	0
SD161	–	+	–	–	0	0	0
SD164	–	–	–	+	0	0	0
SD167	–	+	–	++	0	2	0
SD178	–	++	+	+	0	0	0
SD184	–	+	–	+	0	3	0
SD202	–	+	–	–	0	0	0
SD209	+	+	–	–	1	7	0
SD269	+	++	–	++	0	6	0
SD273	–	–	–	+	0	0	0
SD291	–	–	–	++	0	0	0
SD303	–	–	+	+	0	0	1
SD326	–	–	–	–	0	0	0
SD348	–	–	–	–	0	0	0
SD351	–	+	–	+	0	0	0
SD365	–	+	–	–	0	1	0
SD376	–	+	+	–	0	1	4
SD377	–	+	–	++	0	2	0
SD382	–	++	–	++	0	0	0
SD413	–	+	–	–	0	0	0
SD414	+	–	–	–	1	2	0

Ep, EpCAM; CK, CK8/18; E-d, E-cadherin; Vim, Vimentin; CS, CellSearch®; ISET, isolation by size of tumor cell; CTC, circulating tumor cell; CTM, circulating tumor microemboli.

rates of each marker of EMT did not systematically correlate with patient clinicopathological factors, and further comprehensive analysis is needed.

The expression of EpCAM in the primary foci of the seven CTCs positive patients detected by the CellSearch® was positive, and the expression ratio of CK8/18/19 was high. This indicated the CellSearch® could only detect CTCs with high expression of EpCAM and CK, which was consistent with the primary results of this experiment. The expression of primary epithelial-stromal related markers in the 12 CTCs positive patients detected by CTC-biopsy system in this experiment had no significant correlation with the 12 CTCs positive patients. CK8/18/19 and EpCAM were highly expressed in the three patients with CTCs positive primary epithelial-stromal markers co-detected by the CellSearch® and were not correlated with the expression of E-cadherin and vimentin. This was consistent with the expression of primary epithelial-stromal related markers. The expression of CK8/18/19 and EpCAM in the primary epithelial-stromal markers in the nine patients with positive CTCs detected by CTC-biopsy system and negative CTCs detected by the CellSearch® was not necessarily high and was not related to the patients, but the diameter of CTCs was >8 μm in all patients. CK8/18/19 and EpCAM of primary epithelial-stromal markers in the four patients with negative CTCs detected by CTC-biopsy system and positive CTCs detected by the CellSearch® were all highly expressed, but their CTCs diameter may be less than 8 μm.

As mentioned earlier, by comparing the different results of CTCs detection by the two methods, we found that the expression of epithelial-stromal related markers in primary RCC patients correlated with the detection results of CTCs and was related to the principle of the detection method.

In this experiment, nine patients expressed both CK8/18/19 and EpCAM, accounting for 26.47% of all patients and the detection rate of CTCs with the CTC-biopsy system was 44.44% (4/9). Therefore, theoretically, the CellSearch system could also detect CTCs in the four patients with CK8/18/19 and EpCAM expression and CTCs detected by the CTC-biopsy system, while the CellSearch system could detect two cases with a detection rate of 50%. This suggests CK8/18/19 and/or EpCAM of CTCs were not expressed in these two patients. Combined with this study, the expression of CK8/18/19 and EpCAM in the kidney cancer tissues of the two patients and the detection results of CTCs indirectly proved that EMT occurred in the formation of CTCs. It is worth noting that, in 2021, Zhang *et al.* (41) developed a multiplex surface-enhanced

Table 2 Relationship between CK8/18/19 and clinicopathological factors

Variable	Positive (n=24)	Negative (n=10)	Total	P
Age (years), mean ± SD	56.21±8.807	57.30±8.001		0.830
Sex				
Male	20	7	27	0.394
Female	4	3	7	
KPS score	86.67±5.647	88.00±4.216	34	0.162
Platelet count, $\times10^9$/L	259.33±79.086	259.70±103.831	34	0.755
Neutrophil count, $\times10^9$/L	3.9142±1.232	4.979±2.338	34	0.046
Hemoglobin, g/L	139.17±18.055	138.30±15.377	34	0.597
IMDC score				0.296
1+2	20	10	30	
3+4	4	0	4	
TNM stage				1.000
I–II	12	5	17	
III–IV	12	5	17	
Fuhrman score				0.656
1	2	0	2	
2	9	4	13	
≥3	14	5	19	
Tumor size, cm				0.002
≤4	4	4	8	
>4 and ≤10	14	6	20	
>10	6	0	6	

SD, standard deviation; KPS, Karnofsky Performance Status; IMDC, International Metastatic Renal-Cell Carcinoma Database Consortium; TNM, tumor node metastasis.

Raman scattering nano-technology for comprehensive characterization of EMT-associated phenotypes in CTCs to monitor breast cancer metastasis. This method was able to directly differentiate the phenotypes of CTCs, further corroborating the results of this study.

Limitations

A deficiency of this study is that it is currently impossible to interpret the EMT phenotype based on the expression of all epithelial and stromal markers. Representative epithelial and stromal markers were selected for EMT phenotype interpretation and subsequent analysis. Admittedly, none of current methods for detecting CTCs are perfect. To increase the specificity of CTC detection, a method of negative exclusion can be utilized. Vascular endothelial cells and macrophages were excluded by immunohistochemical staining for CD45/CD31. In order to increase the sensitivity of CTC detection, various detection methods can be combined, such as the combination of immunoenrichment and ISET (Isolation by size of tumor cell). But this requires further exploration. Another disadvantage is that the small sample size resulted in a poor prognosis among patients in different groups of CK8/18/19, EpCAM, vimentin, E-cadherin, and EMT. Therefore, the next step is to further expand the sample size to clarify the clinical significance of

Table 3 Relationship between vimentin and clinicopathological factors

Variable	Positive (n=19)	Negative (n=15)	Total	P
Age (years), mean ± SD	57.05±8.740	55.87±8.374		0.422
Sex				
Male	16	11	27	0.672
Female	3	4	7	
KPS score	88.42±3.746	85.33±6.399	34	0.003
Platelet count, $\times10^9$/L	261.53±93.363	256.80±77.368	34	0.714
Neutrophil count, $\times10^9$/L	3.709±1.151	4.884±2.012	34	0.167
Hemoglobin, g/L	142.16±17.998	134.80±15.461	34	0.750
IMDC score				1.000
1+2	17	13	30	
3+4	2	2	4	
TNM stage				0.005
I–II	14	3	17	
III–IV	5	12	17	
Fuhrman score				0.265
1	2	0	2	
2	8	5	13	
≥3	9	10	19	
Tumor size, cm				0.890
≤4	5	3	8	
>4 and ≤10	11	9	20	
>10	3	3	6	

SD, standard deviation; KPS, Karnofsky Performance Status; IMDC, International Metastatic Renal-Cell Carcinoma Database Consortium; TNM, tumor node metastasis.

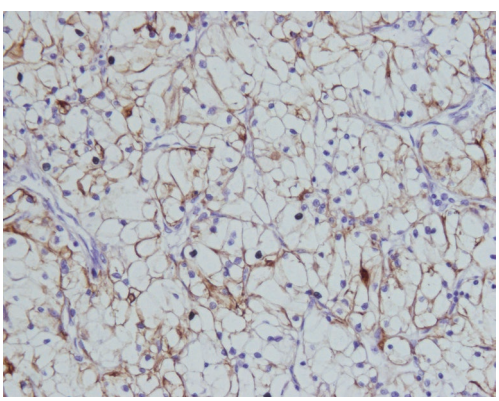

Figure 1 Negative expression of cytokeratin 8/18 in renal cell carcinoma of a patient (immunohistochemical staining, ×400).

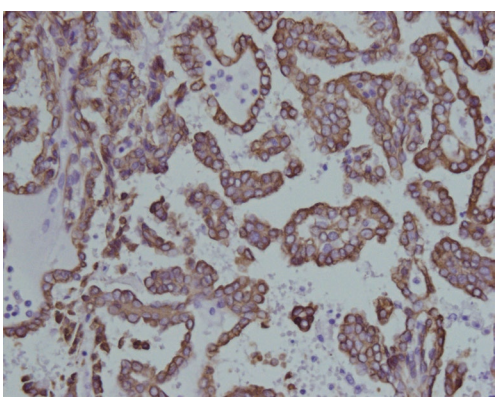

Figure 2 Positive expression of cytokeratin 8/18 in renal cell carcinoma of a patient (immunohistochemical staining, ×400).

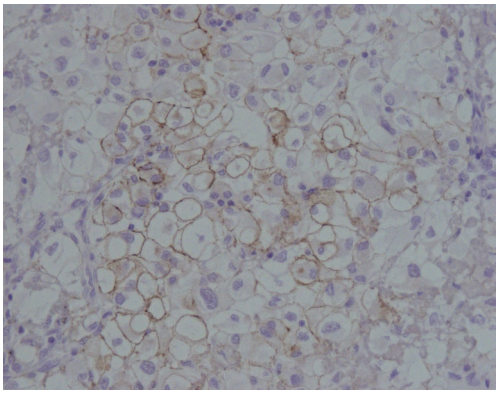

Figure 3 Negative expression of E-cadherin in renal cell carcinoma of a patient (immunohistochemical staining, ×400).

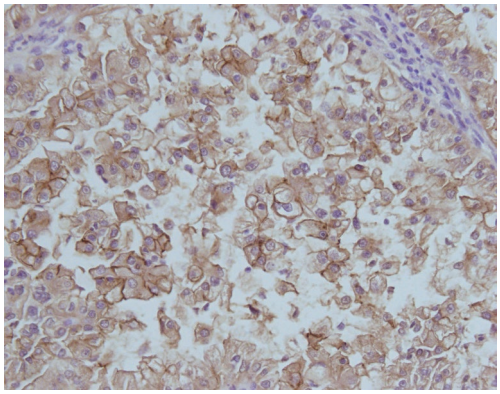

Figure 4 Positive expression of E-cadherin in renal cell carcinoma of a patient (immunohistochemical staining, ×400).

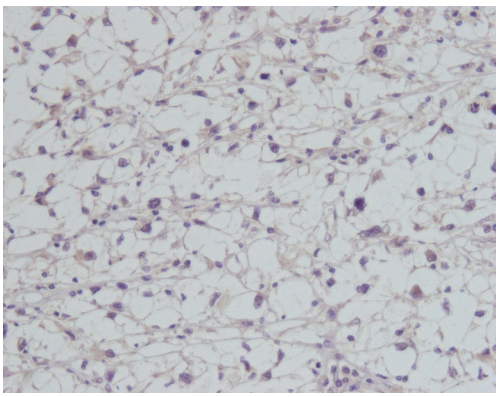

Figure 5 Negative expression of Epithelial cell adhesion molecule in renal cell carcinoma of a patient (immunohistochemical staining, ×400).

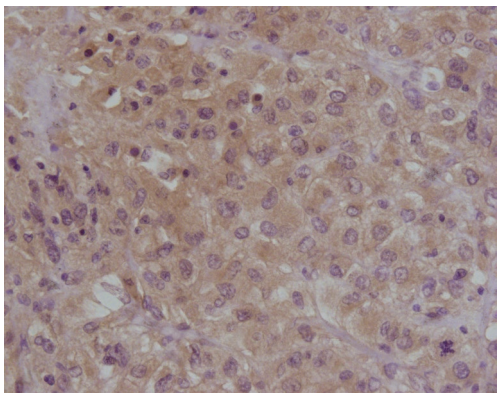

Figure 6 Positive expression of Epithelial cell adhesion molecule in renal cell carcinoma of a patient (immunohistochemical staining, ×400).

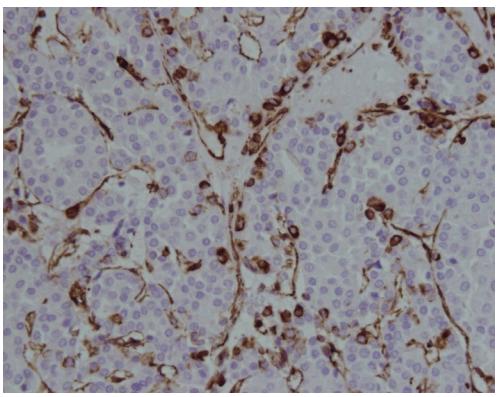

Figure 7 Negative expression of Vimentin in renal cell carcinoma of a patient (immunohistochemical staining, ×400).

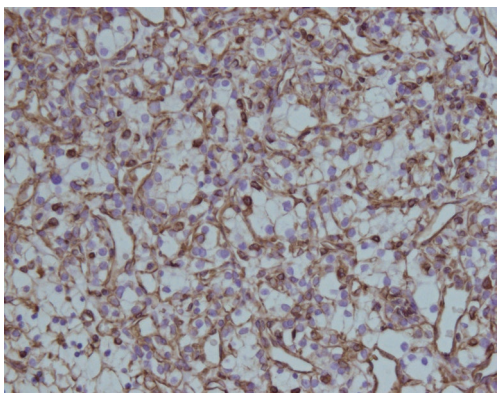

Figure 8 Positive expression of Vimentin in renal cell carcinoma of a patient (immunohistochemical staining, ×400).

Table 4 The expression of e-cadherin and Vimentin and its correlation with clinicopathological factors and CTCs

Variable	EMT phenotype			P value
	Complete (N=15)	Incomplete (N=18)	Wild-type (N=1)	
Age (years), mean ± SD	59.09±7.753	62.847±7.209	62	0.990
Sex				
Male	13	14	0	0.113
Female	2	4	1	
KPS score	90.21±8.0005	89.54±7.491	70	0.875
Platelet count, $\times10^9$/L	221.74±73.06	219.86±70.67	175	0.523
Neutrophil count, $\times10^9$/L	2.257±1.589	2.328±1.169	3.86	0.675
Hemoglobin, g/L	123.89±15.79	125.47±11.098	114	0.599
IMDC score				0.017
1+2	13	17	0	
3+4	2	1	1	
TNM stage				0.029
I–II	11	4	0	
III–IV	4	12	1	
Fuhrman score				0.469
1	2	0	0	
2	5	8	0	
≥3	8	10	1	
Tumor size, cm				0.464
≤4	3	5	0	
4–10	9	10	1	
≥10	3	3	0	
CTCs				0.012
Positive	6	9	1	
Negative	9	9	0	

CTC, circulating tumor cell; EMT, epithelial-mesenchymal transition; SD, standard deviation; KPS, Karnofsky Performance Status; IMDC, International Metastatic Renal-Cell Carcinoma Database Consortium; TNM, tumor node metastasis.

these markers.

Conclusions

This study preliminarily proved the existence of EMT in the occurrence and development of renal cell carcinoma through comparative analysis of the primary foci and CTCs. EMT classification of patients with renal cancer will help determine the degree of tumor differentiation and optimize the treatment strategy. On the other hand, this study suggests that CTCs with epithelial-mesenchymal transition are more easily detected in peripheral blood by ISET. In the future, the detection based on ISET and CTC positivity may be more accurate in describing the poor prognosis of

renal cancer patients.

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Footnote

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