Am J Transl Res 2023;15(1):502-510
www.ajtr.org /ISSN:1943-8141/AJTR0147632

Original Article

Correlation between mesenchymal circulating tumor cells and prognosis of urologic malignancies: a single-center retrospective analysis

Lingdian Wang, Degang Ding

Department of Urinary Surgery, Henan Provincial People's Hospital, Zhengzhou 450003, Henan, China

Received November 9, 2022; Accepted December 22, 2022; Epub January 15, 2023; Published January 30, 2023

Abstract: Objective: To evaluate the correlation of circulating tumor cells (CTCs) and mesenchymal CTCs (M-CTCs) with clinical characteristics and survival of patients with urologic malignancies. Methods: The clinical data of 52 patients with urinary system malignancy in Henan Provincial People's Hospital were retrospectively analyzed (40 cases of renal malignant tumor, 7 cases of prostate cancer, 3 cases of urothelial carcinoma, 1 case of testis cancer, and 1 case of penile cancer). The CTC counts of patients were collected, and the expression of epithelial-mesenchymal transition phenotype in CTCs was evaluated. The relationship of different types of CTC counts with tumor stage, location, size, metastasis, and differentiation, as well as their effect on progression-free survival (PFS) were analyzed. Results: We detected CTCs in all patients with urinary system malignancy. The positive rates of epithelial CTCs (E-CTC), M-CTCs, and epithelial/mesenchymal CTCs (E/M-CTCs) were 34.62%, 26.92% and 94.23%, respectively. Total CTCs (T-CTCs), M-CTCs and E/M-CTCs were correlated with distant metastasis (Z=-3.052, -3.574, -2.898; all P<0.005). M-CTC count was correlated with lymph node metastasis (Z=-3.125; P=0.002). Furthermore, the presence of T-CTCs ≥13.5, M-CTC ≥0.5 or E/M-CTCs ≥9.5 per 5 ml of blood was correlated with worse PFS in patients with urinary system malignancy. Conclusions: M-CTC and E/M-CTC counts correlate with the prognosis of patients with urinary system malignancy. Higher M-CTC and E/M-CTC counts are risk factors for worse prognosis in patients with urinary system malignancies. All in all, M-CTC count is a valuable tumor biomarker for urologic malignancies.

Keywords: Circulating tumor cells, epithelial-mesenchymal transition, urinary system malignant tumors, prognosis

Introduction

Circulating tumor cells (CTCs) are shed from primary or metastatic tumor lesions into the blood or lymphatic circulation [1]. After shedding from the primary lesions and entering the blood circulation, CTCs are subjected to the fluid shear stress of circulating blood and the killing effect of immune cells such as T cells and natural killer cells. Most CTCs die in this process, but a few CTCs achieve immune escape by modifying their surface antigens and changing the microenvironment around the cells, thereby surviving in the blood and changing the proportion of cells in the circulation [2]. Current studies have found that CTCs undergo epithelial-mesenchymal transition (EMT) in vivo. In one study, multiplex RNA in situ hybridization was used to classify CTCs enriched in the peripheral blood from breast carcinoma patients into epithelial (E-CTC), mesenchymal (M-CTC) and epithelial/mesenchymal (E/M-CTC) types according to the expression of different epithelial and mesenchymal markers. It was found that M-CTCs were more related to tumor progression and treatment tolerance [3]. M-CTCs have been confirmed to go hand in hand with the proliferation and metastasis of tumor cells, which can make tumor cells break through the basement membrane of vascular epithelium, thus showing stronger invasiveness and migration [4]. Studies have shown that analysis of the EMT phenotype of CTCs is of high value in assessing the prognosis of non-metastatic and metastatic malignancies [5-7].

Urinary system tumors mainly include bladder cancer, ureteral cancer, kidney tumor, renal pelvis cancer, and urethral cancer. Distal to the renal pelvis, the lumen is covered by urotheli-

Table 1. General data of the patients

	Cases	Kidney cancer	Prostate cancer	Urothelialcarcinoma	Testicular spermatogonia cancer	Penile cancer
The total number of patients	52	40	7	3	1	1
Age (years)						
<60	26	23	0	1	1	1
≥60	26	17	7	2	0	0
Sex						
Male	34	23	7	2	1	1
Female	18	17	0	1	0	0
T stage						
I-II	45	36	5	2	1	1
III-IV	7	4	2	1	0	0
Lymphatic metastasis						
Yes	7	5	1	1	0	0
No	45	35	6	2	1	1
Distant metastasis						
Yes	10	8	1	1	0	0
No	42	32	6	2	1	1

um. The internal environment contact is urine, and carcinogens present in urine often cause tumors in the urothelium. So, tumors are common in the renal pelvis, ureter, and bladder urothelium. Prostate cancer is the urinary system tumor with the highest incidence and mortality in men [8]. The diagnosis of the disease still depends on biopsy, which is often prone to cause complications such as hematuria and infection [9]. Due to the lack of reliable biological markers for urinary system tumors such as kidney cancer and urothelial malignant tumors [10], the current clinical diagnosis of such tumors still relies on pathological biopsy of living tissues, which often brings great pain to patients. The treatment and monitoring of the disease also rely on the assistance of imaging, which often has a certain lag. It is not found until the progression and distant metastasis of the lesion, which delays the treatment time. Therefore, it is essential to find reliable tumor markers to monitor tumor progression and treatment response in real time. Since the research on CTCs in urinary system tumors has not been thoroughly evaluated, this study aimed to detect CTCs in patients with urinary system tumors, hoping to find reliable biological markers in patients with urinary system tumors, so as to clarify the prognosis of patients and to monitor tumor recurrence in real time.

Materials and methods

Patients

The clinical data of 52 patients with urinary system tumors who underwent peripheral blood circulating tumor cytology testing in Henan Provincial People's Hospital, China from January 2019 to December 2020 were retrospectively collected. Of the 52 patients, there were 40 cases of renal malignant tumor, 7 cases of prostate cancer, 3 cases of urothelial carcinoma, 1 case of testicular spermatogonial carcinoma, and 1 case of penile cancer. The collected clinical data included sex, age, TNM stage, tumor node metastasis, and distant metastasis, as shown in **Table 1**. Inclusion criteria: (1) Patients who were diagnosed with primary urinary system tumors by clinicopathological diagnosis in the Henan Provincial People's Hospital or preliminarily diagnosed with urinary system tumors, according to the patient's medical history, urinary system color doppler ultrasound, CT, and other imaging data. (2) Patients who underwent surgical resection. Exclusion criteria: (1) Patients with a history of other primary malignant tumors or hematological diseases. (2) Patients with recent radiotherapy or chemotherapy. (3) Patients who were not initially diagnosed with urinary system tumors. (4) Patients who declined to participate

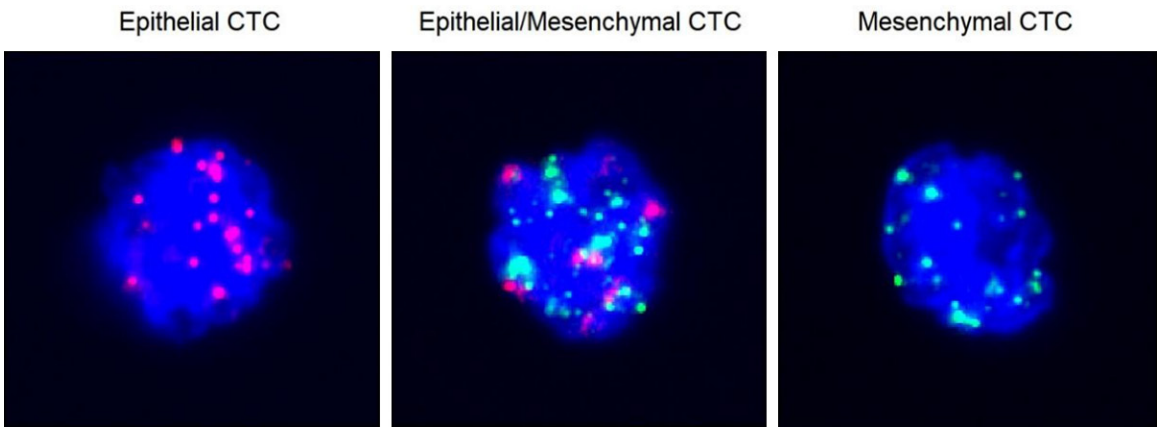

Figure 1. Fluorescence microscopy images of different subtypes of circulating tumor cells. Blue fluorescence: DAPI nucleus; Red fluorescence: epithelial marker expression signal point; Green fluorescence: mesenchymal cell marker expression signal point.

in the study. This study was approved by the Ethics Committee of Henan Provincial People's Hospital (2022-073-02).

CTC detection

CTCs were detected by CanPatrol® CTC enrichment technique. The method consists of two main steps, isolating CTCs and detecting EMT markers (EpCAM and Vimentin) using RNA In Situ Hybridization (ISH). Specific steps are as follows. First, 5 ml of fasting peripheral blood was collected, placed into Ethylene Diamine Tetraacetic Acid anticoagulant tube and stored at indoor temperature. Peripheral blood samples were treated with red blood cell lysis buffer (Solarbio, China) within 4 h of collection and filtered with 8 μm diameter pore calibration membranes (EMD Millipore) to enrich CTCs. Then, the CTCs were subjected to ISH with a combination of epithelial (EpCAM and CK8/CK18/CK19) and mesenchymal (Vimentin and TWIST1) markers. Finally, the samples were stained with DAPI (Solarbio, China) for 5 min and analyzed with an automated imaging fluorescent microscope (EVOS FL Auto, Thermo Fisher Scientific). Fluorescence microscope images of different subtypes of CTCs are shown in **Figure 1**. Then, the CTCs from each patient were grouped according to the identification of the markers. Patients were defined as CTC-positive if ≥1 CTCs were observed per 5 ml of blood.

Data collection and follow-up

The basic data of patients were collected, including sex, age, TNM stage, lymph node metastasis, distant metastasis, and tumor differentiation degree. The data of CTCs were collected, including the number and distribution of total CTCs (T-CTCs), epithelial CTCs (E-CTCs), mesenchymal CTCs (M-CTCs) and epithelial/mesenchymal CTCs (E/M-CTCs).

Patients were followed up after the first treatment. The follow up included the patient's general condition, function examination of blood, urine, liver and kidney, imaging examination data (CT, ultrasound or other) of the surgical area, lymph node metastasis or distant metastasis, and CTC counts. Patients were followed up every 3 months after the first diagnosis until we lost contact, death, or the end-point of December 2021. Patients were followed up regularly according to the data of each outpatient or inpatient period. Patients who failed to return were followed up by telephone. The primary outcome measures were progression-free survival (PFS) and overall survival.

Statistical methods

SPSS 26.0 statistical software was used to process the data. The t-test was used to compare the differences between the means of two groups. The rank-sum test was used to compare the differences of the medians of the two groups. The one-way analysis of variance was used to compare the differences of the means of three or more groups. Comparison of the differences between two groups: when the sample size was ≥5, the χ^2 test was used, when 1≤ sample size <5, the continuous corrected χ^2 test was used, and when the sample size <1,

Table 2. Distribution of CTC subtypes and baseline characteristics of patients (n, %)

	E-CTC			M-CTC			E/M-CTC		
	≥1 (n=18)	<1 (n=34)	P	≥1 (n=14)	<1 (n=38)	P	≥1 (n=49)	<1 (n=3)	P
Age (years)			0.244			0.211			0.552
<60	7 (38.89)	19 (55.88)		9 (64.29)	17 (44.74)		24 (48.98)	2 (66.67)	
≥60	11 (61.11)	15 (44.12)		5 (35.71)	21 (55.26)		25 (51.02)	1 (33.33)	
Sex			0.172			0.448			0.229
Male	14 (77.78)	20 (58.82)		8 (57.14)	26 (68.42)		33 (67.35)	1 (33.33)	
Female	4 (22.22)	14 (41.18)		6 (42.86)	12 (31.58)		16 (32.65)	2 (66.67)	
Tumor type			0.781			0.151			1.000
Kidney cancer	15 (83.33)	25 (73.53)		12 (85.72)	28 (73.68)		37 (75.51)	3 (100.0)	
Prostate cancer	3 (16.67)	4 (11.77)		0	7 (18.42)		7 (14.29)	0	
Urothelial carcinoma	0	3 (8.82)		1 (7.14)	2 (5.26)		3 (6.12)	0	
Testicular spermatogonia carcinoma	0	1 (2.94)		0	1 (2.63)		1 (2.04)	0	
Penile cancer	0	1 (2.94)		1 (7.14)	0		1 (2.04)	0	
T stage			0.948			0.139			0.867
I-II	15 (83.33)	30 (88.23)		10 (71.43)	35 (92.11)		43 (87.76)	2 (66.67)	
III-IV	3 (16.67)	4 (11.77)		4 (28.57)	3 (7.82)		6 (12.24)	1 (33.33)	
Lymphatic metastasis			1.000			0.004			0.867
Yes	2 (11.11)	5 (14.71)		5 (35.71)	2 (5.26)		6 (12.24)	1 (33.33)	
No	16 (88.89)	29 (85.29)		9 (64.29)	36 (94.74)		43 (87.76)	2 (66.67)	
Distant metastasis			0.255			0.003			1.000
Yes	5 (27.78)	5 (14.71)		7 (50.0)	3 (7.89)		10 (20.41)	0	
No	13 (72.22)	29 (85.29)		7 (50.0)	35 (92.11)		39 (79.59)	3 (100.0)	
Differentiation degree			0.087			0.424			0.571
Low	9 (50.0)	17 (50.0)		6 (42.86)	20 (52.63)		25 (51.02)	1 (33.33)	
Middle	0	7 (20.59)		1 (7.14)	6 (15.79)		6 (12.24)	1 (33.33)	
High	9 (50.0)	10 (29.41)		7 (50.0)	12 (31.58)		18 (36.74)	1 (33.33)	

Note: CTC: Circulating tumor cell, E-CTC: epithelial CTC, M-CTC: mesenchymal CTC, E/M-CTC: epithelial/mesenchymal CTC.

the Fisher's exact test was used. Receiver operator characteristic (ROC) curve analysis was used to determine the optimal cutoff values of CTC and M-CTC counts for dividing patients into favorable and unfavorable prognostic groups. The Kaplan-Meier method was used to establish the overall survival models, and the log-rank test was used to compare the survival rates between groups. $P<0.05$ was considered significant.

Results

Distribution of CTC subtypes and baseline characteristics of patients

CTCs were detected in all patients. CTCs were classified as epithelial type, mesenchymal type, or epithelial/mesenchymal type, and they were compared with the baseline characteristics of the patients. The positive rates of E-CTCs, M-CTCs and E/M-CTCs were 34.62%, 26.92% and 94.23%, respectively (**Table 2**). There was no significant difference in lymph node metastasis and distant metastasis between E-CTC <1 group and E-CTC ≥1 group ($P>0.05$). There were 5 patients with lymph node metastasis and 9 patients with non-lymph node metastasis in M-CTC ≥1 group, and 2 patients with lymph node metastasis and 36 patients with non-lymph node metastasis in M-CTC <1 group, and the difference between the two groups was significant ($\chi^2=8.114$, $P=0.004$). There were 7 patients with distant metastasis and 7 patients with non-distant metastasis in M-CTC ≥1 group, and 3 patients with distant metastasis and 35 patients with non-distant metastasis in M-CTC <1 group, and the difference between the two groups was significant ($\chi^2=9.124$, $P=0.003$). There was no significant difference in lymph node metastasis or distant metastasis between the E/M-CTC ≥1 group and E/M-CTC <1 group ($P>0.05$).

Correlation of T-CTCs and CTC subtypes with tumor metastasis

M-CTC count was statistically correlated with lymph node metastasis ($P<0.05$). T-CTC, M-CTC, and E/M-CTC counts were significantly corre-

Table 3. Correlation of total (T-CTCs) and CTC subtypes with tumor metastasis (M, IQR)

	T-CTC	E-CTC	M-CTC	E/M-CTC
Lymph node metastasis				
Yes	14 (8, 20)	0 (0, 1)	1 (0.5, 3)	13 (7.5, 15.5)
No	6 (3, 11)	0 (0, 1)	0 (0, 0)	6 (2, 10)
Z	-1.910	0.000	-3.125	1.572
P	0.056	1.000	0.002	0.116
Distant metastasis				
Yes	16 (11, 21)	0.5 (0, 1.75)	1 (0.25, 2.5)	13.5 (10.75, 17)
No	6 (2.25, 9.75)	0 (0, 1)	0 (0, 0)	5 (2, 8.75)
Z	-3.052	-1.476	-3.574	-2.898
P	0.002	0.140	<0.001	0.004

Note: CTC: Circulating tumor cell, T-CTC: total CTC, E-CTC: epithelial CTC, M-CTC: mesenchymal CTC, E/M-CTC: epithelial/mesenchymal CTC.

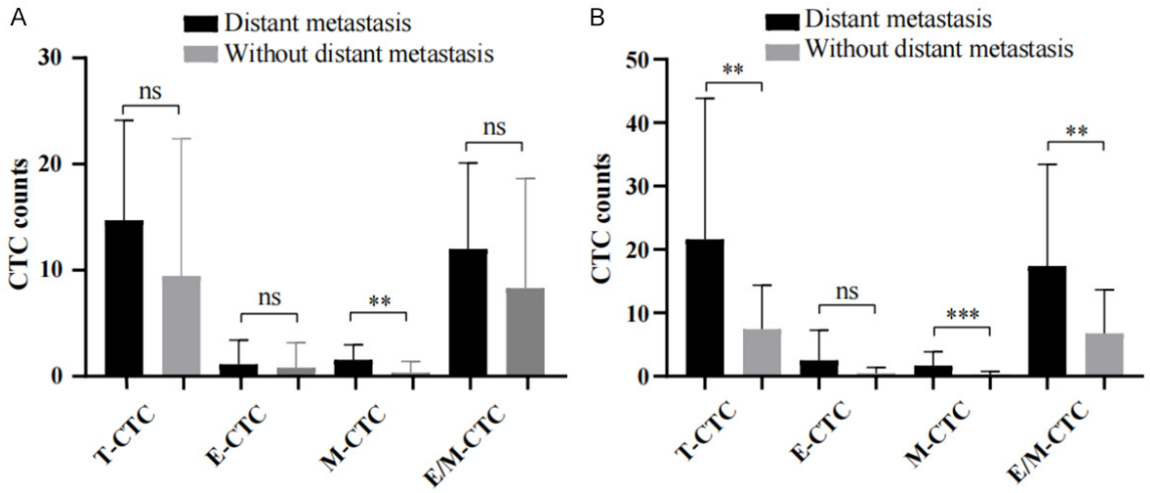

Figure 2. Relationship of the T-CTCs and CTC subtypes with tumor metastasis. A: Lymph node metastases and T-CTCs and CTC subtypes. B: Distant metastasis and T-CTCs and CTC subtypes. **P<0.01, ***P<0.001, ns P>0.05. CTC: circulating tumor cell, T-CTC: total CTC, E-CTC: epithelial CTC, M-CTC: mesenchymal CTC, E/M-CTC: epithelial/mesenchymal CTC.

lated with distant metastasis (P<0.05), as shown in **Table 3** and **Figure 2**.

ROC analysis showed (**Figure 3**) that when the number of T-CTC was 13.5, the highest sensitivity and specificity for predicting distant metastasis were 58.3% and 87.5%, respectively (area under the curve =0.761 (0.601-0.922), P=0.006). When the number of M-CTC was 0.5, the highest sensitivity and specificity for predicting distant metastasis were 66.7% and 85.0%, respectively (area under the curve =0.783 (0.610-0.957), P=0.003). When the number of E/M-CTC was 9.5, the sensitivity and specificity for predicting distant metastasis were 75.0% and 77.5%, respectively (area under the curve =0.730 (0.536-0.924), P= 0.016).

Relationship of T-CTC and CTC subtypes with patient survival

A total of 51 patients were followed up for 1 to 54 months (median: 20 months), with a follow-up rate of 98.07%. ROC analysis calculated that 13.5 T-CTCs, 0.5 M-CTC and 9.5 E/M-CTCs per 5 ml peripheral blood were the best threshold for survival analysis. Survival analysis showed that patients with T-CTCs ≥13.5 had poorer PFS than those with T-CTCs <13.5 (P=0.001, **Figure 4A**), with a 3-year PFS of 25.9% and 78.2%, respectively. Patients with

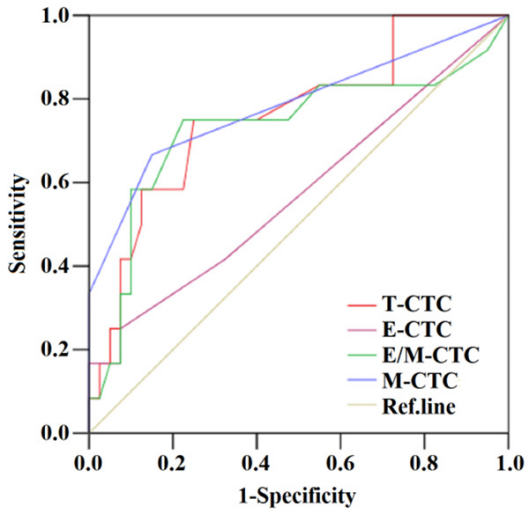

Figure 3. ROC curve of the total CTCs (T-CTCs) and CTC subtypes predicting tumor metastasis. The red line shows the T-CTC count; the purple line shows the E-CTC count; the green line shows the E/M-CTC count; the blue line shows the M-CTC count. ROC: receiver operating characteristic, CTC: circulating tumor cell, T-CTC: total CTC, E-CTC: epithelial CTC, M-CTC: mesenchymal CTC, E/M-CTC: epithelial/mesenchymal CTC.

M-CTC ≥0.5 had poorer PFS than those with M-CTC <0.5 (*P*=0.006, **Figure 4B**), with a 3-year PFS of 27.2% and 78.1%, respectively. Patients with E/M-CTCs ≥9.5 had poorer PFS than those with E/M-CTCs <9.5 (*P*=0.001, **Figure 4C**), with a 3-year PFS of 34.2% and 86.5%, respectively. Because no deaths occurred during follow-up, overall survival rates were not compared.

Discussion

At present, there is still a lack of reliable biomarkers for predicting the prognosis of urologic malignancies, and because of the high heterogeneity of urologic tumors, it is hard to find accurate staging criteria to predict the prognosis of urologic tumors. The treatment and prognosis monitoring of urologic tumors also rely on the assistance of imaging, which often has a certain lag. Most of the patients are not found until the progression and distant metastasis of the lesions, which delays the optimal treatment time. Therefore, it is of great significance to explore effective biomarkers of urinary system malignancies to improve the prognosis of this disease.

Over the years, along with the development of CTC detection technology, CTC count has been widely studied in tumor diagnosis, metastasis, and prognosis evaluation. In a study involving 263 metastatic castration-resistant prostate cancer patients, overall survival was found to be significantly worse in patients with increased CTCs in the third week of follow-up compared with patients with reduced and unchanged CTCs [11]. The purpose of this research was to compare and analyze the correlation of the positive rate of CTCs and the distribution of CTC subtypes with lymph node metastasis and distant metastasis of urinary system tumors. The presence of M-CTCs indicates a higher degree of malignancy, faster progression and poorer prognosis, which is consistent with previous studies in other types of tumors. This is especially for some patients with advanced cancer. For example, De Giorgi et al. [12] detected CTCs in women with metastatic breast cancer and concluded that CTC count ≥5 could be used as a predictor of overall tumor survival. Markiewicz et al. [13] studied the relationship between CTCs with different phenotypes and prognosis in women with breast cancer. Their results revealed that patients with M-CTCs had a poor prognosis than those with E-CTCs or no CTC, and CTCs with mesenchymal markers were closely related to poor prognosis, which also confirmed that the EMT process of CTCs played a vital role in tumor progression. At present, the detection methods and threshold values of CTCs are different in clinical studies, and there is no unified standard. Although the data obtained in the positive rate and subtype analysis of CTCs are different, they all reach a similar conclusion, that is, CTC count is related to the prognosis of patients with urological cancer [14-16].

The CanPatrol® system was used to analyze CTC counts and EMT subtypes in the 52 patients. It was revealed that the positive rate of CTCs was 100%, and the T-CTC count ranged from 1 to 81. The positive rates of E-CTCs, M-CTCs and E/M-CTCs were 34.62%, 26.92% and 94.23%, respectively. Chen et al. [17] adopted the same CanPatrol® system for detecting CTC count and EMT subtypes in 195 patients with liver cancer, and the CTC count in the 195 patients ranged from 0 to 86. The positive rate of CTCs was 95%, and the positive rates of E-CTCs, M-CTCs and E/M-CTCs were 53%, 57% and 83.0%, respectively. Their results suggest that CTC count and EMT classification are associated with clinical stage and

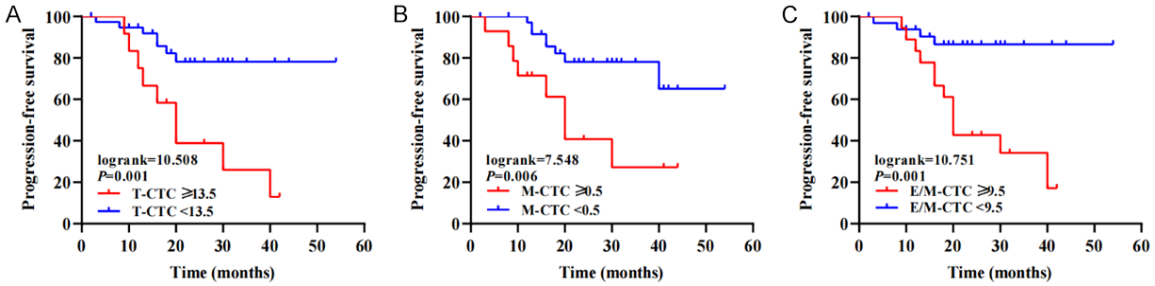

Figure 4. Survival Analysis. A: Association between the T-CTCs and progression-free survival of patients. B: Association between M-CTCs and patient progression-free survival. C: Association between E/M-CTC and patient progression-free survival. CTC: circulating tumor cells, T-CTC: total CTC, M-CTC: mesenchymal CTC, E/M-CTC: epithelial/mesenchymal CTC.

metastasis of primary liver cancer, and that CTC count and EMT classification may be markers for early diagnosis of metastatic progression of liver cancer. Their results are consistent with the results of this study. Since we did not detect the postoperative CTCs in patients in this research, the postoperative and preoperative CTC counts could not be compared. In order to observe the effect of CTC count and EMT classification on the prognosis of patients with urinary system malignancy, the ROC analysis calculated that the optimal threshold for survival analysis was 13.5 T-CTCs, 0.5 M-CTC and 9.5 E/M-CTCs per 5 ml peripheral blood. Survival analysis showed that patients with T-CTCs ≥13.5 or M-CTC ≥0.5 or E/M-CTCs ≥9.5 had poor PFS. This suggests that CTCs, M-CTCs and E/M-CTCs may be markers for early diagnosis of metastatic progression of urinary system malignancy. Related studies have shown that EMT often manifests as incomplete activation of the invasion and metastasis cascade, and as an intermediate stage of the invasion and metastasis cascade, this subtype is called mixed phenotype (E/M-CTC). In the presence of partially preserved intercellular adhesion (epithelium), the mixed phenotype can stimulate cells to aggregate and survive in the blood circulation (mesenchyme) [18]. Moreover, multicellular aggregation was observed in blood samples of prostate cancer patients, and it was found that E/M-CTC may promote cell cluster formation [19], and the ability of CTCs to form clusters is connected with increased metastatic potential [20, 21], which suggests that E/M-CTC goes hand in hand with the metastatic progression of urinary malignancies. Wang et al. [22] performed CTC analysis on 69 patients with renal cell carcinoma, and their results showed that there was no obvious difference in preoperative CTC count between the metastatic group and the non-metastatic group, but the E/M-CTC count was obviously different between the metastatic group and the non-metastatic group at 12 months after operation. It was demonstrated that the risk of recurrence or metastasis was correlated with the dynamic changes of CTC count, especially the trend of E/M-CTC count. These results show that E/M-CTCs can be used to dynamically monitor the disease progression in patients.

This research is a single-center clinical study with a small sample size and a certain degree of bias in the selection of patients, which may have affected the results. In this study, only a single CTC test was performed on the patients, without dynamic monitoring of CTC level changes and lack of follow-up data of the patients. Therefore, it is unfortunately not possible to show the CTC changes before and after treatment. Although metastasis invasiveness is shown during CTC diffusion, the underlying molecular mechanism remains unclear. In particular, the results show that the presence of M-CTCs most strongly suggest a poor prognosis.

Conclusion

The CTCs can be used as markers of urinary system tumors, and the classification of CTCs with EMT markers helps to identify more invasive subtypes, which are associated with lymph node metastasis or distant metastasis of tumors. M-CTCs and E/M-CTCs may predict more aggressive tumor and a worse prognosis.

Disclosure of conflict of interest

None.

Address correspondence to: Degang Ding, Department of Urinary Surgery, Henan Provincial People's Hospital, Zhengzhou 450003, Henan, China. Tel: +86-15068858093; E-mail: 13503848199@163.com

References

[1] Lin D, Shen L, Luo M, Zhang K, Li J, Yang Q, Zhu F, Zhou D, Zheng S, Chen Y and Zhou J. Circulating tumor cells: biology and clinical significance. Signal Transduct Target Ther 2021; 6: 404.

[2] Mohme M, Riethdorf S and Pantel K. Circulating and disseminated tumour cells - mechanisms of immune surveillance and escape. Nat Rev Clin Oncol 2017; 14: 155-167.

[3] Yu M, Bardia A, Wittner BS, Stott SL, Smas ME, Ting DT, Isakoff SJ, Ciciliano JC, Wells MN, Shah AM, Concannon KF, Donaldson MC, Sequist LV, Brachtel E, Sgroi D, Baselga J, Ramaswamy S, Toner M, Haber DA and Maheswaran S. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science 2013; 339: 580-584.

[4] Yu T, Wang C, Xie M, Zhu C, Shu Y, Tang J and Guan X. Heterogeneity of CTC contributes to the organotropism of breast cancer. Biomed Pharmacother 2021; 137: 111314.

[5] Ye Q, Ling S, Zheng S and Xu X. Liquid biopsy in hepatocellular carcinoma: circulating tumor cells and circulating tumor DNA. Mol Cancer 2019; 18: 114.

[6] Schuster E, Taftaf R, Reduzzi C, Albert MK, Romero-Calvo I and Liu H. Better together: circulating tumor cell clustering in metastatic cancer. Trends Cancer 2021; 7: 1020-1032.

[7] Yang J, Cheng S, Zhang N, Jin Y and Wang Y. Liquid biopsy for ovarian cancer using circulating tumor cells: recent advances on the path to precision medicine. Biochim Biophys Acta Rev Cancer 2022; 1877: 188660.

[8] Siegel RL, Miller KD, Fuchs HE and Jemal A. Cancer statistics, 2022. CA Cancer J Clin 2022; 72: 7-33.

[9] Lomas DJ and Ahmed HU. All change in the prostate cancer diagnostic pathway. Nat Rev Clin Oncol 2020; 17: 372-381.

[10] Nassar AH, Mouw KW, Jegede O, Shinagare AB, Kim J, Liu CJ, Pomerantz M, Harshman LC, Van Allen EM, Wei XX, McGregor B, Choudhury AD, Preston MA, Dong F, Signoretti S, Lindeman NI, Bellmunt J, Choueiri TK, Sonpavde G and Kwiatkowski DJ. A model combining clinical and genomic factors to predict response to PD-1/PD-L1 blockade in advanced urothelial carcinoma. Br J Cancer 2020; 122: 555-563.

[11] Goldkorn A, Ely B, Quinn DI, Tangen CM, Fink LM, Xu T, Twardowski P, Van Veldhuizen PJ, Agarwal N, Carducci MA, Monk JP 3rd, Datar RH, Garzotto M, Mack PC, Lara P Jr, Higano CS, Hussain M, Thompson IM Jr, Cote RJ and Vogelzang NJ. Circulating tumor cell counts are prognostic of overall survival in SWOG S0421: a phase III trial of docetaxel with or without atrasentan for metastatic castration-resistant prostate cancer. J Clin Oncol 2014; 32: 1136-1142.

[12] De Giorgi U, Mego M, Scarpi E, Giordano A, Giuliano M, Valero V, Alvarez RH, Ueno NT, Cristofanilli M and Reuben JM. Association between circulating tumor cells and peripheral blood monocytes in metastatic breast cancer. Ther Adv Med Oncol 2019; 11: 1758835919-866065.

[13] Markiewicz A, Nagel A, Szade J, Majewska H, Skokowski J, Seroczynska B, Stokowy T, Welnicka-Jaskiewicz M and Zaczek AJ. Aggressive phenotype of cells disseminated via hematogenous and lymphatic route in breast cancer patients. Transl Oncol 2018; 11: 722-731.

[14] Lorente D, Olmos D, Mateo J, Bianchini D, Seed G, Fleisher M, Danila DC, Flohr P, Crespo M, Figueiredo I, Miranda S, Baeten K, Molina A, Kheoh T, McCormack R, Terstappen LW, Scher HI and de Bono JS. Decline in circulating tumor cell count and treatment outcome in advanced prostate cancer. Eur Urol 2016; 70: 985-992.

[15] Scher HI, Armstrong AJ, Schonhoft JD, Gill A, Zhao JL, Barnett E, Carbone E, Lu J, Antonarakis ES, Luo J, Tagawa S, Dos Anjos CH, Yang Q, George D, Szmulewitz R, Danila DC, Wenstrup R, Gonen M and Halabi S. Development and validation of circulating tumour cell enumeration (Epic Sciences) as a prognostic biomarker in men with metastatic castration-resistant prostate cancer. Eur J Cancer 2021; 150: 83-94.

[16] Scher HI, Heller G, Molina A, Attard G, Danila DC, Jia X, Peng W, Sandhu SK, Olmos D, Riisnaes R, McCormack R, Burzykowski T, Kheoh T, Fleisher M, Buyse M and de Bono JS. Circulating tumor cell biomarker panel as an individual-level surrogate for survival in metastatic castration-resistant prostate cancer. J Clin Oncol 2015; 33: 1348-1355.

[17] Chen J, Cao SW, Cai Z, Zheng L and Wang Q. Epithelial-mesenchymal transition phenotypes of circulating tumor cells correlate with the clinical stages and cancer metastasis in hepatocellular carcinoma patients. Cancer Biomark 2017; 20: 487-498.

[18] Quan Q, Wang X, Lu C, Ma W, Wang Y, Xia G, Wang C and Yang G. Cancer stem-like cells with hybrid epithelial/mesenchymal phenotype leading the collective invasion. Cancer Sci 2020; 111: 467-476.

[19] Armstrong AJ, Marengo MS, Oltean S, Kemeny G, Bitting RL, Turnbull JD, Herold CI, Marcom PK, George DJ and Garcia-Blanco MA. Circulating tumor cells from patients with advanced prostate and breast cancer display both epithelial and mesenchymal markers. Mol Cancer Res 2011; 9: 997-1007.

[20] Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, Yu M, Pely A, Engstrom A, Zhu H, Brannigan BW, Kapur R, Stott SL, Shioda T, Ramaswamy S, Ting DT, Lin CP, Toner M, Haber DA and Maheswaran S. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell 2014; 158: 1110-1122.

[21] Gkountela S, Castro-Giner F, Szczerba BM, Vetter M, Landin J, Scherrer R, Krol I, Scheidmann MC, Beisel C, Stirnimann CU, Kurzeder C, Heinzelmann-Schwarz V, Rochlitz C, Weber WP and Aceto N. Circulating tumor cell clustering shapes DNA methylation to enable metastasis seeding. Cell 2019; 176: 98-112, e14.

[22] Wang ZL, Zhang P, Li HC, Yang XJ, Zhang YP, Li ZL, Xue L, Xue YQ, Li HL, Chen Q and Chong T. Dynamic changes of different phenotypic and genetic circulating tumor cells as a biomarker for evaluating the prognosis of RCC. Cancer Biol Ther 2019; 20: 505-512.