World Journal of Gastroenterology

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DOI: 10.3748/wjg.v21.i47.13259

World J Gastroenterol 2015 December 21; 21(47): 13259-13267
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Evaluation of epithelial-mesenchymal transitioned circulating tumor cells in patients with resectable gastric cancer: Relevance to therapy response

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Supported by Major Program of Science and Technology Program of Guangzhou, No. 201300000087; Research Fund of Public Welfare in Health Industry of National Health and Family Planning Commission of China, No. 201402015 and No. 201502039; National Key Technology R&D Program, No. 2013BAI05B05; and Key Clinical Specialty Discipline Construction Program.

Institutional review board statement: The study protocol was reviewed and approved by Institutional Review Board of Nanfang Hospital.

Institutional animal care and use committee statement: The present study did not involve animal experiments.

Conflict-of-interest statement: The authors do not have any conflict of interest to disclose.

Data sharing statement: No additional data are available.

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Received: July 31, 2015
Peer-review started: August 1, 2015
First decision: August 26, 2015
Revised: August 31, 2015
Accepted: October 17, 2015
Article in press: October 20, 2015
Published online: December 21, 2015

Abstract

AIM: To evaluate the epithelial-to-mesenchymal transition (EMT) of circulating tumor cells (CTCs) in gastric cancer patients.

METHODS: We detected tumor cells for expression of four epithelial (E^+) transcripts (keratins 8, 18, and 19 and epithelial cell adhesion molecule) and two mesenchymal (M^+) transcripts (Vimentin and Twist) by a quantifiable, dual-colorimetric RNA-*in situ* hybridization assay. Between July 2014 and October 2014, 44 patients with gastric cancer were recruited for CTC evaluation. Blood samples were obtained from selected patients during the treatment course [before surgery, after surgery and at the 6th cycle of XELOX based chemotherapy (about 6 mo postoperatively)].

RESULTS: We found the EMT phenomenon in which there were a few biphenotypic E^+/M^+ cells in primary

human gastric cancer specimens. Of the 44 patients, the presence of CTCs was reported in 35 (79.5%) patients at baseline. Five types of cells including from exclusively E^+ CTCs to intermediate CTCs and exclusively M^+ CTCs were identified (4 patients with M^+ CTCs and 10 patients with M^+ or $M^+ > E^+$ CTCs). Further, a chemotherapy patient having progressive disease showed a proportional increase of mesenchymal CTCs in the post-treatment blood specimens. We used NCI-N87 cells to analyze the linearity and sensitivity of CanPatrol™ system and the correlation coefficient (R^2) was 0.999.

CONCLUSION: The findings suggest that the EMT phenomenon was both in a few cells of primary tumors and abundantly in CTCs from the blood of gastric cancer patients, which might be used to monitor therapy response.

Key words: Gastric cancer; Epithelial-to-mesenchymal transition; Circulating tumor cells; Chemotherapy; Therapy response

Core tip: Epithelial-to-mesenchymal transition has been thought to play a critical role in tumor metastatic progression in preclinical models, however, characterizing the epithelial *vs* mesenchymal phenotypes of circulating tumor cells has been challenging. In this study, we aimed to evaluate epithelial-to-mesenchymal transition phenomenon in circulating gastric tumor cells by a combination of physical and biological methods. Our findings have provided evidence of the phenomenon both in rare cells within primary tumors and more abundantly in circulating tumor cells. Furthermore, we demonstrated that the evaluation of the mesenchymal circulating tumor cells in peripheral blood can be used to monitor therapy response in gastric cancer patients.

Li TT, Liu H, Li FP, Hu YF, Mou TY, Lin T, Yu J, Zheng L, Li GX. Evaluation of epithelial-mesenchymal transitioned circulating tumor cells in patients with resectable gastric cancer: Relevance to therapy response. *World J Gastroenterol* 2015; 21(47): 13259-13267 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i47/13259.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i47.13259

INTRODUCTION

Gastric cancer is a serious public health concern in East Asia, South America and Eastern Europe, accounting for more than 950000 new cases per year (China alone accounts for 42% of new cases worldwide), and it is the third cause of cancer death all around the world (GLOBOCAN 2012)[1]. Because mass screening is rarely practiced worldwide except Japan and Korea, gastric cancer is often diagnosed at an advanced stage. Like common cancers, most gastric cancer-related deaths result from metastasis[2], which is rarely predictable by standard imaging work-ups like positron emission tomography/computed tomography scans or tumor markers tests. Circulating tumor cells (CTCs) originating from solid tumors are related with the course of hematogenous metastatic spread to the distant sites[3], exemplifying the switch from localized to systemic disease. Therefore, evaluating CTCs has clinical relevance in the monitoring and the outcomes of metastatic tumors. The recent discoveries on CTCs demonstrate how these cells are related with hematogenous metastasis, with an opinion on the epithelial-mesenchymal transition (EMT) phenomenon[4]. The investigation by Yu *et al*[5] found dynamic changes in the number of epithelial and mesenchymal CTCs in breast cancer patients as well as the potential of monitoring therapy response.

It was thought that EMT phenomenon played a critical role in tumor metastatic progression in preclinical models[6,7], however, characterizing the epithelial *vs* mesenchymal phenotypes of CTCs has been challenging. Increasing evidence coming from clinical setting of CTCs supports the phenomenon of the EMT in human tumors. Accordingly, we are exploring the methods to identify the unique stem CTC subpopulation[7], and its significance is further emphasized by recent findings suggesting the occurrence of mesenchymal markers in tumor tissues as a poor prognostic factor in many cancers[5,8,9]. Furthermore, sequential analysis of CTCs, so called "liquid biopsy", may provide clinical significance on the effectiveness and progression of systemic therapies and consequently would facilitate "tailor-made" therapeutic strategies[10,11].

To date, the CellSearch System is the only FDA-cleared CTC enumeration assay, which defines a CTC according to its size, positivity for epithelial cell adhesion molecule (EpCAM) and CK, and negativity of CD45 expression[12]. The current techniques besides the CellSearch System are able to isolate CTCs by epithelial markers, however, these epithelial markers based methods most likely overlook a subpopulation of CTCs undergoing EMT[13,14]. Thus, the new CTC capture systems should be essential to isolate the cell subpopulation with mesenchymal phenotype. To our knowledge, there have been few reports regarding the detecting methods and clinical significance of mesenchymal CTCs in cancer patients, specifically gastric cancer.

In the present study, we adopted two mesenchymal transcripts, Vimentin and Twist, to detect mesenchymal phenotypes of CTCs and tumor tissues in advanced gastric cancer, which have been reported as sensitive markers to detect them[12]. Accordingly, the EMT phenomenon of CTCs in advanced gastric cancer and

its relationship with chemotherapy response were evaluated as well.

MATERIALS AND METHODS

Gastric cancer cell line

We used the NCI-N87 cell line for the analysis. NCI-N87 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, 100 U/mL of penicillin and 100 μg/mL of streptomycin at 37 ℃ in a humidified atmosphere containing 5% CO_2.

Patients and study design

Between July 2014 and October 2014, 44 patients with newly diagnosed gastric cancer in our institution, without receiving neoadjuvant chemo- or radiotherapy, were prospectively enrolled in the study. The blood samples from our selected patients were acquired during the treatment course [before surgery, after surgery and at the 6th cycle of XELOX based chemotherapy (about 6 mo postoperatively)[15]] and analyzed with CanPatrol™ System (Surexam Biotech, Guangzhou, China)[16]. The blood samples from ten healthy volunteers were used as controls.

Isolation and enumeration of CTCs (CanPatrol™ system)

For the gastric cancer patients, 5 mL peripheral blood samples were collected in EDTA tubes by venipuncture and filtered with a 8-μm diameter pores calibrated membrane (Millipore, Billerica, MA, United States)[16]. The required filtration system included a filtration tube containing the membrane (SurExam, Guangzhou, China), a manifold vacuum plate with valve settings (SurExam, Guangzhou, China), an E-Z 96 vacuum manifold (Omega, Norcross, GA, United States), and a vacuum pump (Auto Science, Tianjin, China). Before filtration, red blood cell lysis buffer was used to remove erythrocyte, then PBS with 4% formaldehyde (Sigma, St. Louis, MO, United States) was used to resuspend the remaining cells for 5 min. The pumping pressure is 0.08 MPa.

We established three groups of nucleic acid probes to identify and examine the expression levels of epithelial and mesenchymal genes in CTCs by a multiplex RNA-*in situ* hybridization (RNA-ISH) assay. Group 1 probes contained four pooled epithelial transcripts [keratins (KRT) 8, 18 and 19; EpCAM]. Group 2 probes had two mesenchymal transcripts (Vimentin and Twist). The last group only contained a CD45 transcript which was used to discriminate white blood cells and CTCs. The detail hybridization assay procedure followed the published literature[16]. Briefly, the cells retained on the filter were permeabilized and digested with protease. And then, the cells were subjected to a serial of hybridization reactions with a cocktail probe specific to the intended examined genes described above. Finally, we used 4',6-diamidino-2-phenylindole (DAPI) to stain the cell nucleus. The samples were analyzed with a fluorescent microscope. The red and green dots of fluorescent signal observed in the cells represented the epithelial and mesenchymal gene expression, respectively. The purple fluorescent dots represented the *CD45* gene expression (the markers of white blood cells). The assays were applied in both selected primary human gastric cancer specimens and all blood specimens.

Statistical analysis

Data are presented as median and range (or mean ± SD) for continuous variables or as frequencies and proportions for categorical variables. Mann-Whitney *U* test and the Kruskal-Wallis *H* test were used to analyze the relationship between CTC count at baseline and tumor stage. The relationship between CTC count and the molecular classification of primary tumor according to Her2 status was examined using Spearman's rank correlation coefficient. All statistical calculations were performed with SPSS for Windows release 18.0. A P value < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

Totally 44 patients with gastric cancer (median age 56 years, range 25-87 years) were enrolled in this study. These patients consisted of 6 (13.6%) patients with gastric signet-ring cell carcinoma (SRCC) and 38 (86.4%) patients with gastric adenocarcinoma. Of these, 11 patients (7 men and 4 women) had early gastric cancer diagnosed pathologically as American Joint Committee on Cancer stage IA and IB. All 37 patients underwent laparoscopic gastrectomy with D2 lymphadenectomy; the remaining 7 patients with unresectable tumors were treated by laparoscopic exploration or palliative surgery. Thirty-six patients had no detectable overt metastasis (stage M0), and 5 patients presented with existing synchronous metastases in peritoneal carcinosis (Table 1). CTCs were detected in 35 (79.5%) patients from the gastric cancer group and the average count was 7.25 CTCs. No CTCs were detected in all of the samples from healthy volunteers (Figure 1). With regard to clinicopathological features, 11 (37.1%) patients had lymphatic emboli and elevated Her2 levels (+++ or ++) were detected in 9 (22%) gastric cancer patients.

Detection of EMT markers in primary tumors and draining lymph nodes

We first applied the RNA-ISH assays to primary human gastric cancer specimens. There were a small number of biphenotypic E^+/M^+ cells among the majority of E^+ cancer cells with clear epithelial histology of primary tumors (Figure 2A). However, we only found E^+ cancer cells in draining lymph nodes (Figure 2B).

Table 1 Characteristics of gastric cancer patients *n* (%)

	All	CanPatrol	
		Positive, *n*/total	*P* value
Median age, yr (range)	56 (25-87)		
Gender			0.780
Male	31	25/31 (80.6)	
Female	13	10/13 (76.9)	
Post-TNM stage			
Tumor			0.799
T1	11	9/11 (81.2)	
T2	4	3/4 (75)	
T3	5	4/5 (80)	
T4	21	19/21 (90.5)	
Node			0.710
N0	22	17/22 (77.3)	
N+	19	18/19 (94.7)	
Metastasis			0.115
M0	36	30/36 (83.3)	
M1	5	5/5 (100)	
Site of metastases			
Peritoneal carcinosis	5	5/5 (100)	
Histology			0.400
Adenocarcinoma	38	31/38 (81.6)	
Signet-ring cell carcinoma	6	4/6 (66.7)	
Lymphatic emboli			0.461
No	21	17/21 (81)	
Yes	11	10/11 (90.9)	
Her2			0.303
+++	2	2/2 (100)	
++	7	7/7 (100)	
+	12	11/12 (91.7)	
0	20	15/20 (75)	

T1: Tumor invades lamina propria or submucosa; T2: Tumor invades muscularis propria or subserosa; T3: Tumor penetrates serosa (visceral peritoneum) without invasion of adjacent structures; T4: Tumor invades adjacent structures; N0: No regional lymph node metastasis; N+: Lymph node metastasis; M0: No distant metastasis; M1: Distant metastasis.

Correlation of CTC detection with clinico-histopathologic risk factors

We also analyzed blood samples from 44 gastric cancer patients. Using the RNA-ISH assays, we defined five types of cells including from exclusively epithelial (E^+) CTCs to intermediate ($E^+ > M^+$, $E^+ = M^+$, $M^+ > E^+$) CTCs and exclusively mesenchymal (M^+) CTCs from blood samples (Figure 3A). Of 35 patients, four patients with M^+ CTCs and ten patients with M^+ or M > E CTCs were observed. CTCs had been captured in 35 (79.5%) patients, with EMT features varying according to histological subtype (SRCC and adenocarcinoma) and molecular classification ($Her2^-$ and $Her2^+$) (Figure 3B). Among them, nine (25.7%) patients were detected with $Her2^+$ gastric cancer, and 26 (74.3%) patients with $Her2^-$ gastric cancer. The CTCs from patients with both SRCC and adenocarcinoma were predominantly mesenchymal phenotypes. Furthermore, we compared the CTC counts from patients with $Her2^+$ *vs* $Her2^-$ in primary tumors but observed no statistically significant correlations with the number of E^+/M^+ cells.

EMT of CTCs and therapeutic response

To test the possibility of the ratio of mesenchymal (M) to epithelial (E) content serving as an indicator of therapeutic response, we combined both the CTC counts and mesenchymal features of CTCs (Figure 4). Sequential blood samples were obtained from four patients undergoing adjuvant chemotherapy (XELOX regimen, capecitabine plus oxaliplatin). Three patients showed a decrease in CTC counts and/or a proportional decrease in mesenchymal feature in post-treatment samples at the 6th cycle adjuvant chemotherapy, compared with those in the post-surgery samples (Figure 4A-C). In contrast, one patient who had progressive disease evaluated by a CT scan showed an increased number or proportion of mesenchymal CTCs in the post-treatment specimen (Figure 4D).

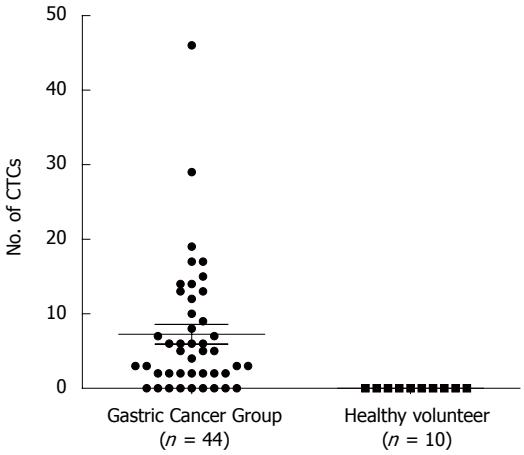

Figure 1 Circulating tumor cells were detected in 35 (79.5%) patients from gastric cancer group. The average number of circulating tumor cells (CTCs) was 7.25 in the group. CTCs were not observed in any samples from healthy volunteers.

Sensitivity of the CanPatrol™ system

We used NCI-N87 cells to analyze the sensitivity and linearity of the CanPatrol™ system. We spiked 10, 50, 100 and 200 NCI-N87 cells into 5 mL of blood to get the recovery of the cells (Figure 5). The correlation coefficient (R^2) was 0.999.

DISCUSSION

Because of the minimally invasive feature of obtaining sequential blood specimens from cancer patients as well as potential clinical implications of the CTCs, seeking for methods to isolate and analyze CTCs for diagnosis, prognosis evaluation, and monitoring of cancer patients has been highlighted in recent years[17]. There are several underlying benefits for successful CTCs/circulating tumor DNA-based diagnostics, due to the ability to obtain sequential blood samples from cancer patients throughout the treatment course[18]. That is, characterizing CTCs may potentially provide clinicians with: (1) biomarkers predictive of therapy resistance[19,20]; (2) an independent biomarker of prognosis[3,21-23]; and (3) an indicator for early relapse[24], as well as materials for the evaluation of

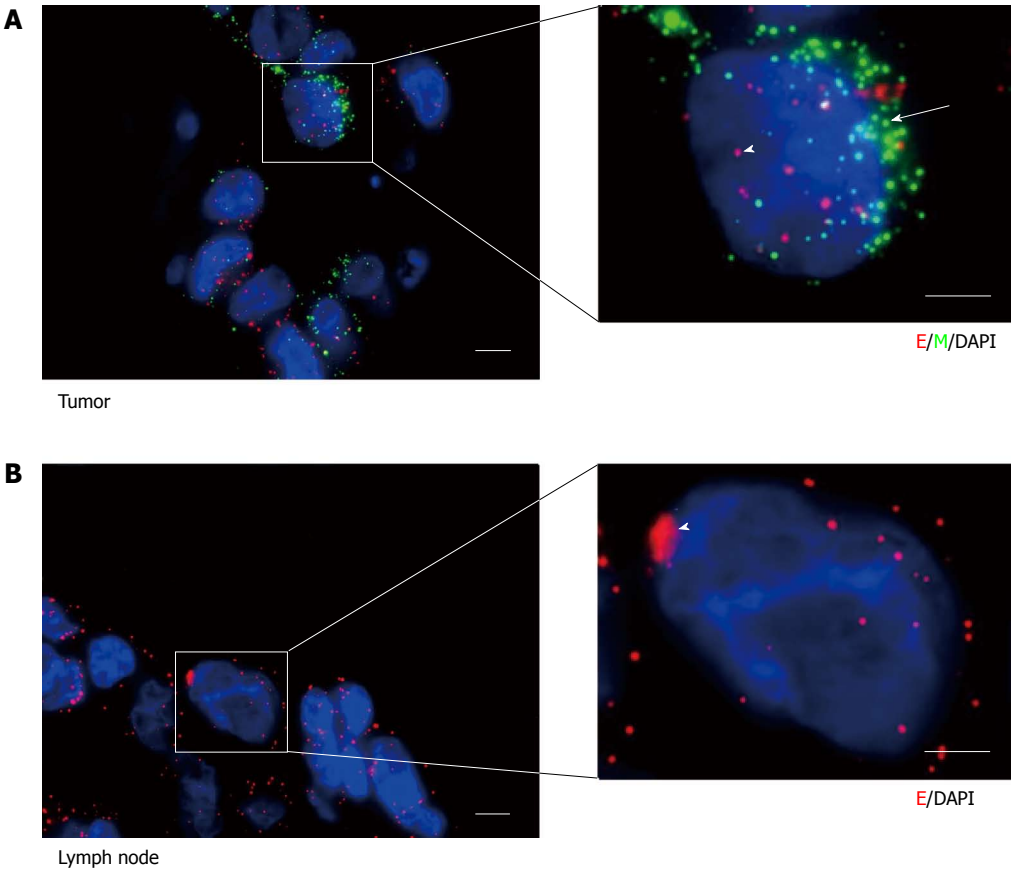

Figure 2 RNA-*in situ* hybridization analysis of epithelial-to-mesenchymal transition markers in human gastric tumors. The probes were constructed as described in the method section. Representative RNA-*in situ* hybridization analysis of pooled epithelial (E) (red dots, arrowheads) and mesenchymal (M) (green dots, arrows) markers in (A) primary tumor and (B) tumor-infiltrated lymph node of a patient with gastric cancer. Scale bars: (A) to (B), 20 mm; inserts, 10 mm.

therapy resistance[17,25,26]. Currently, CTC enumerations have been widely admitted as an independent prognostic biomarker for a few tumors, however, their tentative roles as predictive biomarkers that may influence treatment decisions have been highly elusive and challenging to carry out, partially attributing to the extreme exiguity of CTCs in the peripheral circulation.

CTC enumeration

CellSearch System was used in the majority of published studies, which depends on epithelial tumor cell expression of KRT 8, 18, and 19 and EpCAM, presence of an intact nucleus (DAPI), and absence of the leukocyte marker CD45. However, because of the heterogeneity of CTCs, most current platforms overlooked mesenchymal-like CTCs, on which mesenchymal markers, such as Vimentin and N-cadherin, are upregulated[4]. Like a recent published report[5], a novel technology with a combination of epithelial and mesenchymal markers was used in our study, resulting in an increase of ten percent (4 patients with mesenchymal CTCs) in gastric CTC enumeration as well as identifying 10 more patients with intermediate phenotypes (biphenotypic E^+/M^+ cells). The approaches of CTC isolation or enrichment can be mainly categorized into two groups: physical methods and biological methods[14]. Perhaps using more than one technique, limited isolation may be improved by the inclusion of physical method-based isolation of CTCs, which may help to specifically target CTCs. Besides the biological method (immunological antibody-based capture of CTCs), we simultaneously used a filtration-based approach (a physical method, an 8 μm filtration tube in the present study)[16] for CTC isolation, further increasing sensitivity and specificity.

Mesenchymal CTC detection and the EMT phenomenon

The vital technical challenge for CTC research comes from the rarity of tumor cells in blood. Most CTC technologies rely on the expression of epithelial markers (EpCAM-positive and keratins-positive cells) by tumor cells for their capture. But considerable disparity exists between the numbers of "epithelial" CTCs detected in different cancer types, perhaps because of a subpopulation of CTCs undergoing EMT, linked to their stemness[27]. Studies aiming to research the EMT phenomenon of CTCs have revealed high expression levels of mesenchymal markers such as AKT2, TWIST, PIK3α, N-cadherin and Vimentin[28]. Mesenchymal CTCs were correlated with cancer prognosis and therapy resistance in several cancer types[5,9,29]. While there exist few studies on the EMT

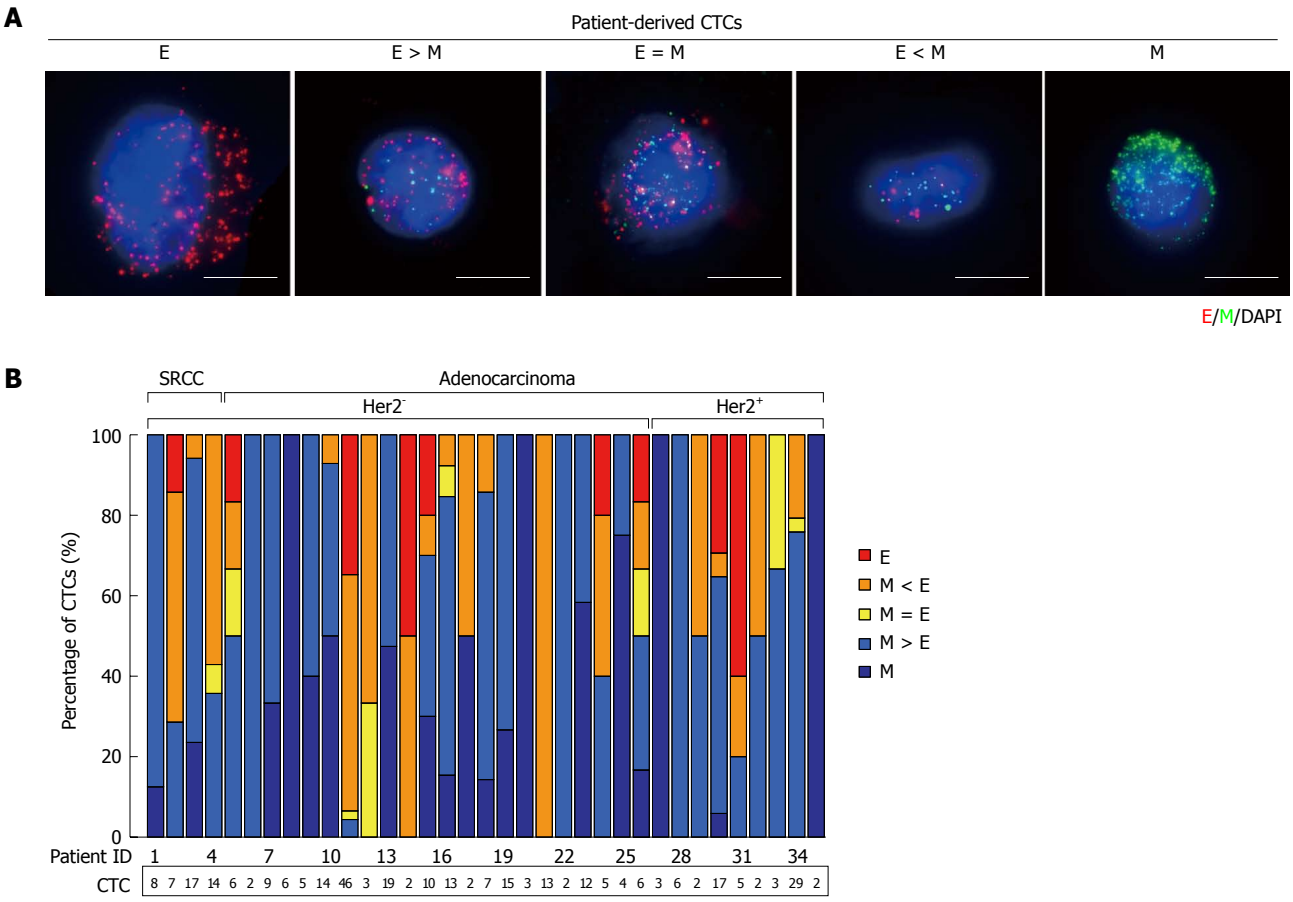

Figure 3 RNA-*in situ* hybridization analysis of epithelial-to-mesenchymal transition markers in circulating tumor cells from patients with gastric cancer. A: Representative images of five types of CTCs isolated from patients with gastric cancer, based on RNA-ISH staining of E (red dots) and M (green dots) markers. Scale bar, 5 μm; B: Quantitation of EMT features in CTCs based on E and M RNA-ISH staining of histological subtypes of gastric cancer (SRCC and Adenocarcinoma), along with molecular classification of primary tumors (Her2$^-$ and Her2$^+$). CTC numbers per 10 mL of blood are listed below. RNA-ISH: RNA-*in situ* hybridization; CTCs: Circulating tumor cells; E: Epithelial; M: Mesenchymal; EMT: Epithelial-to-mesenchymal transition; DAPI: 4',6-diamidino-2-phenylindole.

phenomenon in CTCs specifically in gastric cancer, it is currently a distinct research focus. In the present study, we attempted at detecting and characterizing the EMT phenomenon in CTCs and gastric tumor tissues in clinical settings by using mesenchymal CTC markers (Vimentin and Twist). We found that human primary gastric tumor tissues contain scarce tumor cells that express epithelial and mesenchymal markers, but not in lymph node metastasis. In addition, the presence of CTCs bearing a mesenchymal phenotype has also been detected in the present study, which highlights the heterogeneity present in the circulating gastric tumor cells. Although there was an obvious increase in the number of mesenchymal CTCs in late-stage gastric cancer (data now shown), these data did not show a statistically significant difference in our analysis, which might be due to the relative small sample size. A large scale trial with higher statistic power is warranted.

Mesenchymal CTCs and monitoring chemotherapy

CTCs may be the promise of serving as "liquid biopsies" for tumors with the potential for providing information predictive of response and chemotherapy resistance. Several reports have demonstrated the ratio of epithelial to mesenchymal markers on CTCs can be used to monitor the likeliness of therapy response. Yu and colleagues[5] found a subpopulation of CTCs with a mixed epithelial-mesenchymal phenotype at baseline and the mesenchymal phenotype was observed at stages of disease progression (suppressed at stages of treatment response), further implicating mesenchymal CTCs in the metastatic progression. Additionally, Satelli *et al*[9] suggested that CTC enumeration from a combination of EpCAM and Vimentin-based methods appeared to be a strong and reliable predictor for therapeutic outcomes in metastatic breast cancer with chemotherapy. Likewise, we compared CTC features in serial blood samples from four patients who underwent D2 gastrectomy (pre-operative, post-operative and post-adjuvant chemotherapy). One case who had progressive disease after 6 cycles of XELOX regimen showed the phenotypic changes in post-adjuvant chemotherapy specimen, compared with pre-treatment, showing an increased numbers of mesenchymal CTCs (Twist and Vimentin upregulated). The remaining three cases who responded to therapy showed a decrease in CTC counts and/or a

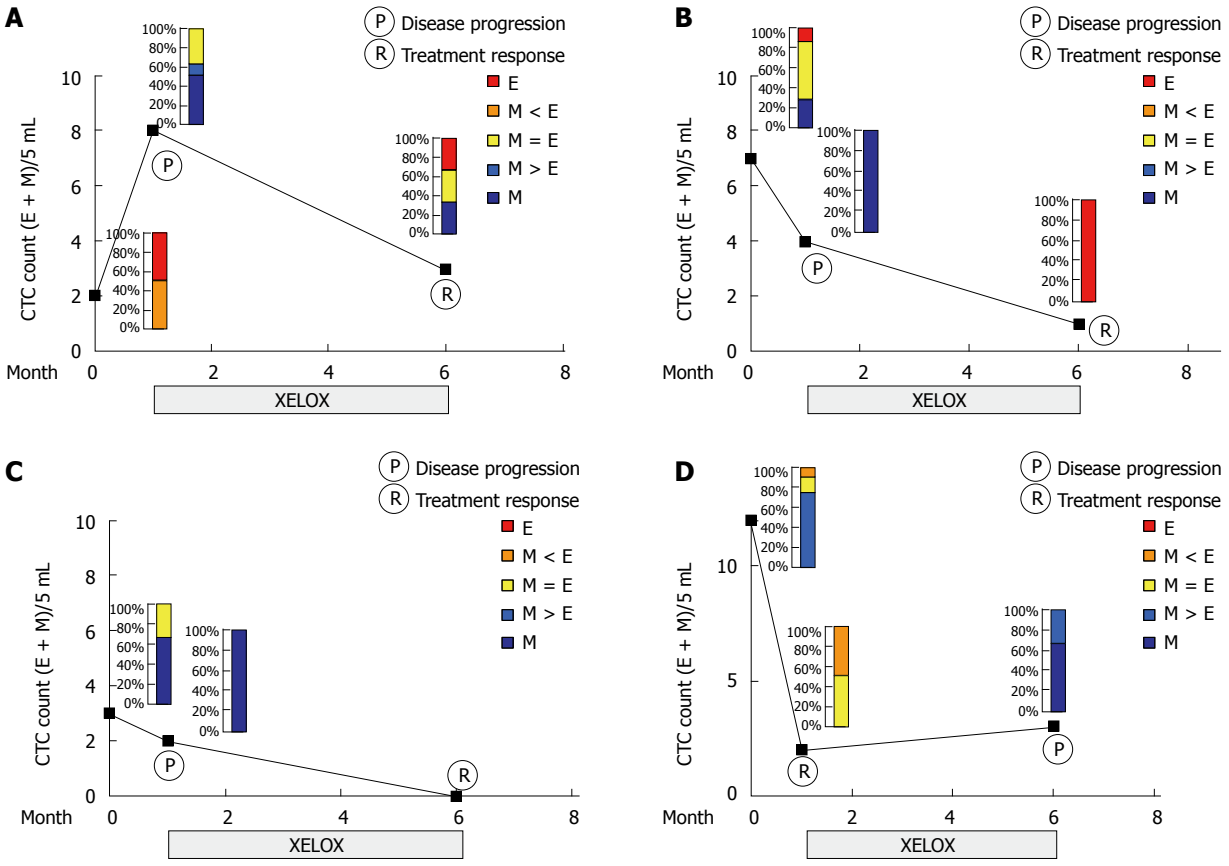

Figure 4 Longitudinal monitoring of epithelial-to-mesenchymal transition features in circulating tumor cells from a patient with resectable gastric cancer following surgery and adjuvant chemotherapy. CTC: Circulating tumor cell; E: Epithelial; M: Mesenchymal.

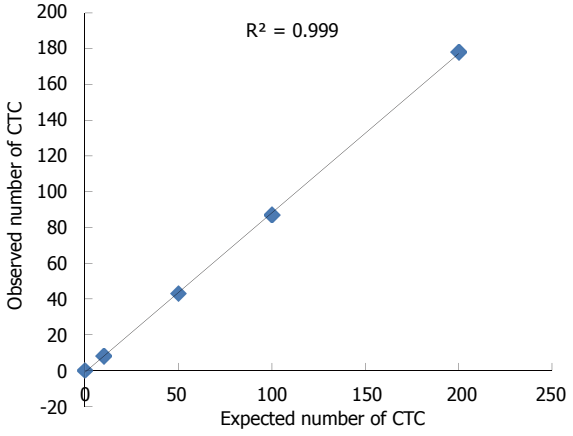

Figure 5 Regression analysis of the number of observed tumor cells *vs* the number of expected tumor cells produced a correlation coefficient (R^2) of 0.999. Even a single cell spiked into the samples was detected using this system. CTC: Circulating tumor cell.

proportional decrease in mesenchymal CTCs. Notably, one case who underwent curable resection surgery showed an increased number of mesenchymal CTCs in post-operative samples compared with pre-operative one, suggesting that surgical operation may play a critical role in the detachment of primary tumor cells to the peripheral circulation[30]. Therefore, adjuvant therapy should be highlighted to reduce the risk of hematogenous metastasis even after curable resection in selected patients.

Limitations

A caveat has to be noted for the present study as well as all other studies which do not confirm the tumor cell identity by genomic markers. Because markers for mesenchymal-like CTCs are mostly not tumor-specific[31]. Furthermore, small sample size is another drawback when evaluating therapy response through obtaining sequential blood specimens.

In conclusion, our findings have provided evidence of the EMT phenomenon in human gastric cancer specimens, both in rare cells within primary tumors and more abundantly in CTCs by a combination of physical and biological methods. Furthermore, we demonstrated that the evaluation of the mesenchymal CTCs in peripheral blood can be used to monitor therapy response in gastric cancer patients. Clinical relevance of mesenchymal CTCs as a potential biomarker of therapeutic resistance and as a potential drug target in gastric cancer warrants further investigation.

ACKNOWLEDGMENTS

The authors would like to thank Surexam Biotech,

Guangzhou, China for the technical support.

COMMENTS

Background

Like common cancers, most gastric cancer-related deaths result from metastasis, which is rarely predictable by standard imaging work-ups like positron emission tomography/computed tomography scans or tumor marker tests. Circulating tumor cells (CTCs) originating from solid tumors are related with the course of hematogenous metastatic spread to the distant sites, exemplifying the switch from localized to systemic disease. Therefore, evaluating CTCs has clinical relevance in the monitoring and the outcomes of metastatic tumors.

Research frontiers

The recent discoveries on CTCs demonstrate how these cells are involved in hematogenous metastasis, with a focus on the epithelial-mesenchymal transition (EMT). The investigation by Yu and colleagues found that dynamic changes in the number of epithelial and mesenchymal CTCs in breast cancer patients as well as the potential of monitoring therapy response.

Innovations and breakthroughs

So far, there have been few reports regarding the detecting methods and clinical significance of mesenchymal CTCs in cancer patients, specifically gastric cancer. This is the first study evaluating the mesenchymal CTCs in peripheral blood and therapy response in gastric cancer patients.

Applications

The EMT phenomenon in human gastric cancer specimens was found both in rare cells within primary tumors and more abundantly in CTCs by a combination of physical and biological methods. Furthermore, the evaluation of the mesenchymal CTCs in peripheral blood can be used to monitor therapy response in gastric cancer patients. Mesenchymal CTCs maybe is a potential biomarker of therapeutic resistance or a potential drug target in gastric cancer.

Terminology

CTCs in peripheral circulation originating from solid tumors are involved in the process of hematogenous metastatic spreading to distant sites, exemplifying the switch from localized to systemic disease. Mesenchymal-to-epithelial transition (MET) is a crucial physiologic event that converts mesenchymal cells to epithelial cells. There is increasing evidence suggesting that MET maybe also regulate epithelial carcinogenesis.

Peer-review

The paper is a good contribution in investigating the role of mesenchymal-to-epithelial transition in circulating tumor cells of gastric cancer. The issue is not new but every new contribution confirming the feasibility and efficacy of a possible new marker is welcome.

REFERENCES

1 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]

2 **Nguyen DX**, Bos PD, Massagué J. Metastasis: from dissemination to organ-specific colonization. *Nat Rev Cancer* 2009; **9**: 274-284 [PMID: 19308067 DOI: 10.1038/nrc2622]

3 **Cristofanilli M**, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, Reuben JM, Doyle GV, Allard WJ, Terstappen LW, Hayes DF. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 2004; **351**: 781-791 [PMID: 15317891 DOI: 10.1056/NEJMoa040766]

4 **Krebs MG**, Metcalf RL, Carter L, Brady G, Blackhall FH, Dive C. Molecular analysis of circulating tumour cells-biology and biomarkers. *Nat Rev Clin Oncol* 2014; **11**: 129-144 [PMID: 24445517 DOI: 10.1038/nrclinonc.2013.253]

5 **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, Maheswaran S. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. *Science* 2013; **339**: 580-584 [PMID: 23372014 DOI: 10.1126/science.1228522]

6 **Thiery JP**, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell* 2009; **139**: 871-890 [PMID: 19945376 DOI: 10.1016/j.cell.2009.11.007]

7 **Kang Y**, Pantel K. Tumor cell dissemination: emerging biological insights from animal models and cancer patients. *Cancer Cell* 2013; **23**: 573-581 [PMID: 23680145 DOI: 10.1016/j.ccr.2013.04.017]

8 **Okabe H**, Ishimoto T, Mima K, Nakagawa S, Hayashi H, Kuroki H, Imai K, Nitta H, Saito S, Hashimoto D, Chikamoto A, Ishiko T, Watanabe M, Nagano O, Beppu T, Saya H, Baba H. CD44s signals the acquisition of the mesenchymal phenotype required for anchorage-independent cell survival in hepatocellular carcinoma. *Br J Cancer* 2014; **110**: 958-966 [PMID: 24300972 DOI: 10.1038/bjc.2013.759]

9 **Satelli A**, Brownlee Z, Mitra A, Meng QH, Li S. Circulating tumor cell enumeration with a combination of epithelial cell adhesion molecule- and cell-surface vimentin-based methods for monitoring breast cancer therapeutic response. *Clin Chem* 2015; **61**: 259-266 [PMID: 25336717 DOI: 10.1373/clinchem.2014.228122]

10 **Tsujiura M**, Ichikawa D, Konishi H, Komatsu S, Shiozaki A, Otsuji E. Liquid biopsy of gastric cancer patients: circulating tumor cells and cell-free nucleic acids. *World J Gastroenterol* 2014; **20**: 3265-3286 [PMID: 24696609 DOI: 10.3748/wjg.v20.i12.3265]

11 **Alix-Panabières C**, Pantel K. Circulating tumor cells: liquid biopsy of cancer. *Clin Chem* 2013; **59**: 110-118 [PMID: 23014601 DOI: 10.1373/clinchem.2012.194258]

12 **Esmaeilsabzali H**, Beischlag TV, Cox ME, Parameswaran AM, Park EJ. Detection and isolation of circulating tumor cells: principles and methods. *Biotechnol Adv* 2013; **31**: 1063-1084 [PMID: 23999357 DOI: 10.1016/j.biotechadv.2013.08.016]

13 **Friedlander TW**, Premasekharan G, Paris PL. Looking back, to the future of circulating tumor cells. *Pharmacol Ther* 2014; **142**: 271-280 [PMID: 24362084 DOI: 10.1016/j.pharmthera.2013.12.011]

14 **Harouaka R**, Kang Z, Zheng SY, Cao L. Circulating tumor cells: advances in isolation and analysis, and challenges for clinical applications. *Pharmacol Ther* 2014; **141**: 209-221 [PMID: 24134902 DOI: 10.1016/j.pharmthera.2013.10.004]

15 **Noh SH**, Park SR, Yang HK, Chung HC, Chung IJ, Kim SW, Kim HH, Choi JH, Kim HK, Yu W, Lee JI, Shin DB, Ji J, Chen JS, Lim Y, Ha S, Bang YJ. Adjuvant capecitabine plus oxaliplatin for gastric cancer after D2 gastrectomy (CLASSIC): 5-year follow-up of an open-label, randomised phase 3 trial. *Lancet Oncol* 2014; **15**: 1389-1396 [PMID: 25439693 DOI: 10.1016/S1470-2045(14)70473-5]

16 **Wu S**, Liu Z, Liu S, Lin L, Yang W, Xu J. Enrichment and enumeration of circulating tumor cells by efficient depletion of leukocyte fractions. *Clin Chem Lab Med* 2014; **52**: 243-251 [PMID: 24021598 DOI: 10.1515/cclm-2013-0558]

17 **Pavese JM**, Bergan RC. Circulating tumor cells exhibit a biologically aggressive cancer phenotype accompanied by selective resistance to chemotherapy. *Cancer Lett* 2014; **352**: 179-186 [PMID: 25016063 DOI: 10.1016/j.canlet.2012.06.012]

18 **Heitzer E**, Ulz P, Geigl JB. Circulating tumor DNA as a liquid biopsy for cancer. *Clin Chem* 2015; **61**: 112-123 [PMID: 25388429 DOI: 10.1373/clinchem.2014.222679]

19 **Maheswaran S**, Sequist LV, Nagrath S, Ulkus L, Brannigan B, Collura CV, Inserra E, Diederichs S, Iafrate AJ, Bell DW, Digumarthy S, Muzikansky A, Irimia D, Settleman J, Tompkins RG, Lynch TJ, Toner M, Haber DA. Detection of mutations in EGFR in circulating lung-cancer cells. *N Engl J Med* 2008; **359**: 366-377 [PMID: 18596266 DOI: 10.1056/NEJMoa0800668]

20 **Miyamoto DT**, Lee RJ, Stott SL, Ting DT, Wittner BS, Ulman M,

Smas ME, Lord JB, Brannigan BW, Trautwein J, Bander NH, Wu CL, Sequist LV, Smith MR, Ramaswamy S, Toner M, Maheswaran S, Haber DA. Androgen receptor signaling in circulating tumor cells as a marker of hormonally responsive prostate cancer. *Cancer Discov* 2012; **2**: 995-1003 [PMID: 23093251 DOI: 10.1158/2159-8290.CD-12-0222]

21 **Hayes DF**, Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Miller MC, Matera J, Allard WJ, Doyle GV, Terstappen LW. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. *Clin Cancer Res* 2006; **12**: 4218-4224 [PMID: 16857794 DOI: 10.1158/1078-0432.CCR-05-2821]

22 **de Bono JS**, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, Doyle GV, Terstappen LW, Pienta KJ, Raghavan D. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. *Clin Cancer Res* 2008; **14**: 6302-6309 [PMID: 18829513 DOI: 10.1158/1078-0432.CCR-08-0872]

23 **Muinelo-Romay L**, Vieito M, Abalo A, Nocelo MA, Barón F, Anido U, Brozos E, Vázquez F, Aguín S, Abal M, López RL. Evaluation of Circulating Tumor Cells and Related Events as Prognostic Factors and Surrogate Biomarkers in Advanced NSCLC Patients Receiving First-Line Systemic Treatment. *Cancers* (Basel) 2014; **6**: 153-165 [PMID: 24452143 DOI: 10.3390/cancers6010153]

24 **Pachmann K**, Camara O, Kavallaris A, Krauspe S, Malarski N, Gajda M, Kroll T, Jörke C, Hammer U, Altendorf-Hofmann A, Rabenstein C, Pachmann U, Runnebaum I, Höffken K. Monitoring the response of circulating epithelial tumor cells to adjuvant chemotherapy in breast cancer allows detection of patients at risk of early relapse. *J Clin Oncol* 2008; **26**: 1208-1215 [PMID: 18323545 DOI: 10.1200/JCO.2007.13.6523]

25 **Scher HI**, Jia X, de Bono JS, Fleisher M, Pienta KJ, Raghavan D, Heller G. Circulating tumour cells as prognostic markers in progressive, castration-resistant prostate cancer: a reanalysis of IMMC38 trial data. *Lancet Oncol* 2009; **10**: 233-239 [PMID: 19213602 DOI: 10.1016/S1470-2045(08)70340-1]

26 **Friedlander TW**, Ngo VT, Dong H, Premasekharan G, Weinberg V, Doty S, Zhao Q, Gilbert EG, Ryan CJ, Chen WT, Paris PL. Detection and characterization of invasive circulating tumor cells derived from men with metastatic castration-resistant prostate cancer. *Int J Cancer* 2014; **134**: 2284-2293 [PMID: 24166007 DOI: 10.1002/ijc.28561]

27 **Mani SA**, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J, Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008; **133**: 704-715 [PMID: 18485877 DOI: 10.1016/j.cell.2008.03.027]

28 **Satelli A**, Mitra A, Brownlee Z, Xia X, Bellister S, Overman MJ, Kopetz S, Ellis LM, Meng QH, Li S. Epithelial-mesenchymal transitioned circulating tumor cells capture for detecting tumor progression. *Clin Cancer Res* 2015; **21**: 899-906 [PMID: 25516888 DOI: 10.1158/1078-0432.ccr-14-0894]

29 **Mego M**, Mani SA, Lee BN, Li C, Evans KW, Cohen EN, Gao H, Jackson SA, Giordano A, Hortobagyi GN, Cristofanilli M, Lucci A, Reuben JM. Expression of epithelial-mesenchymal transition-inducing transcription factors in primary breast cancer: The effect of neoadjuvant therapy. *Int J Cancer* 2012; **130**: 808-816 [PMID: 21387303 DOI: 10.1002/ijc.26037]

30 **Miyazono F**, Natsugoe S, Takao S, Tokuda K, Kijima F, Aridome K, Hokita S, Baba M, Eizuru Y, Aikou T. Surgical maneuvers enhance molecular detection of circulating tumor cells during gastric cancer surgery. *Ann Surg* 2001; **233**: 189-194 [PMID: 11176124 DOI: 10.1097/00000658-200102000-00007]

31 **Bednarz-Knoll N**, Alix-Panabières C, Pantel K. Plasticity of disseminating cancer cells in patients with epithelial malignancies. *Cancer Metastasis Rev* 2012; **31**: 673-687 [PMID: 22733306 DOI: 10.1007/s10555-012-9370-z]

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ISSN 1007-9327