

Tumor resection reduced circulating tumor cell dissemination

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Research

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Abstract

Background: Cancer is a major public health problem worldwide. Whether to perform tumor resection or non-resection remains controversial face different types. Circulating tumor cells (CTCs) which are classified into three subpopulations epithelial (E-CTC), mesenchymal (M-CTC), and epithelial mesenchymal (E/M-CTC) based on expression of specificity biomarker.

Methods: We identified CTCs using CanPatrol CTC enrichment technique from peripheral blood samples of 930 patients with tumor resection or non-resection. Patients were divided into two groups: Resection (n=615) and Non-resection (n=315). The relationship between tumor resection and count of CTCs were studied by using correlation analysis.

Results: The mean patient age was 57.52 years (range, 17–87 years). Univariate analysis tumor resection could significantly reduce the number of total CTC cells (hazard ratio (HR), 0.92, 95% confidence interval, CI= 0.903-0.937; $P < 0.001$), the E-CTC cells (HR, 0.889, 95% CI= 0.837-0.944; $P < 0.001$), the M-CTC cells (HR, 0.799, 95% CI= 0.735-0.869; $P < 0.001$), and the E/M-CTC cells (HR, 0.908, 95% CI= 0.888-0.928; $P < 0.001$). Furthermore, correlation analysis between total CTC and E-CTC, M-CTC, and E/M-CTC, the Pearson correlation coefficient was 0.467, 0.458, and 0.963, respectively.

Conclusions: In conclusion, the tumor resection can reduce the number of CTCs in peripheral blood.

Background

Cancer is the second leading cause of death in the United States and is a major public health problem worldwide. The latest study suggest that the continuous decline in cancer death rates since 1991 has resulted in an overall drop of 27%, and the cancer incidence rate (2006–2015) was stable in women and declined by nearly 2% per year in men during the past decade in the United States[1]. However, in large number of countries these incidences continue to rise [2]. Treatment of tumor metastases are an extremely important clinical issue worldwide since standardized early detection have developed and brought to an earlier detection and diagnosed cases in early stage of tumor[3]. While surgery resection can be effective curative method in carefully selected patients in tumor metastases[4]. The circulating tumor cells (CTCs) into the blood stream gradually proven to have great clinical potential for better prognosis in post-resection.

The CTCs originating from solid tumors, shed from primary and metastasized tumors, and other unidentified sites, into the blood stream[5]. CTCs can enhance tumor cell invasion and proliferation via undergoing epithelial-mesenchymal transition (EMT) [6]. According to the specific marker expression, the CTCs can now be divided into the epithelial (E-CTCs), mesenchymal (M-CTCs), and epithelial mesenchymal (E/M-CTCs) types. And the M-CTCs have been reported to positive correlation with more serious risk of recurrence in hepatocellular carcinoma[7, 8], non-small cell lung cancer[9], and prostate cancer[10] et al. Due to their abundant presence, and previous studies have shown that CTCs count is related to poor prognosis in many metastatic cancers. However, the relationship between CTCs count with tumor resection and non-resection remains unclear. The aim of this study was to determine the CTCs types and count in solid tumors after resection.

Methods

Treatment options for patient selection

This prospective study was conducted at the first hospital affiliated to army medical university (southwest hospital), Chongqing, China. Pre-treatment peripheral vein blood samples (10 mL) were collected from 930 patients who were enrolled between July 2018 and December 2019. The inclusion criteria are as follows: (1) histological evidence of a newly diagnosed tumor, (2) CTC examination conducted within 2 days before resection or non-resection. (3) Definitive pathological diagnosis of hepatocellular carcinoma (HCC), non-small cell lung cancer (NSCLC), colon carcinoma, gastric cancer, cholangiocarcinoma, pancreatic cancer, gallbladder carcinoma, nasopharyngeal carcinoma based on the World Health Organization criteria. Peripheral blood specific colorectal cancer, pancreatic cancer, tumor markers (4) willingness to provide written informed consent, (5) No previous anticancer treatment. The 930 patients were divided into two groups: the group of patients who underwent resection of the tumor (resection group, n=315) and the group of patients who did not undergo resection of the tumor (non-resection group, n=615). The Ethical Committee of southwest hospital approved the study protocol, and written informed consent was obtained from all participants.

Isolation and enumeration of CTCs

The accuracy and the procedure of the CanPatrol system for isolating CTCs have been described previously. Briefly, blood was collected 1 to 2 days before resection. Peripheral blood samples (10 mL, anticoagulated with EDTA). Subsequently, the red blood cell were removed with RBC lysis buffer, then filtered through a filtration system with 8µm pore-size nano membrane (Millipore, Billerica, USA). The trapped CTCs were incubated with specific probes targeting epithelial (EpCAM, CK8, CK18 and CK19), mesenchymal (Vimentin and Twist) markers and anti-leukocyte CD45, in an automatic nucleic acid hybridization apparatus (SurExam, Guangzhou, China). After counterstaining with DAPI the CTCs were counted.

Statistical analysis

Either the chi squared test or Fisher's exact test was used, as appropriate, to analyze any correlation between the two groups. Data were presented as mean \pm standard deviation (SD). The statistical analyses were performed using SPSS for windows (SPSS version 11.5, SPSS Inc., Chicago, IL) and GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA). Unpaired Student's t-Test was used to analyze the statistical significant between two groups, respectively. And P values less than 0.05 were considered statistically significant.

Results

Patients characteristics

A total of 930 patients (584 males and 346 females) with complete data during were enrolled in this study with different tumor types showing in Table 1. The mean patients age was 57.52 years (range, 17–87 years). All patients were divided into two groups the resection group (n=315, male 200, female 115) and the non-resection group (n=615, male 384, female 231). The baseline characteristics of the 930 patients are listed in Table 2.

All three subtypes of CTCs were isolated E-CTCs, M-CTCs, and E/M-CTCs were detected in peripheral blood samples of the patients which after resection or before non-resection, respectively. The clinicopathological characteristics of the all patients, demarcated by the CTCs subtypes, were counted. Table 3 shows that the peripheral blood total CTCs counts ranged from 0 to 181 (mean \pm standard deviation, 14.72 ± 18.239), E-CTCs counts ranged from 0 to 40 (1.82 ± 3.58), M-CTCs counts ranged from 0 to 26 (1.65 ± 2.663), E/M-CTCs counts ranged from 0 to 176 (11.26 ± 16.011).

CTC counts are associated with tumor resection

Univariate analysis of factors influencing all types of CTCs after tumor resection or non-resection. Patients who were underwent tumor resection could significantly reduce the number of total CTCs (HR, 0.92, 95% CI= 0.903-0.937; $p < 0.001$), the E-CTCs (HR, 0.889, 95% CI= 0.837-0.944; $p < 0.001$), the M-CTCs (HR, 0.799, 95% CI= 0.735-0.869; $p < 0.001$), and the E/M-CTCs (HR, 0.908, 95% CI= 0.888-0.928; $p < 0.001$). These results suggest that tumor resection was capable to decline the counts of all types of CTCs. So, there was a negative correlation between tumor resection and CTCs peripheral blood.

Furthermore, we observed that the total CTCs, E-CTCs, M-CTCs, and E/M-CTCs cells were obviously higher in non-resection group compared with resection group ($p < 0.001$) (Figure 1). In addition, we perform the correlation analysis between total CTCs and E-CTCs, M-CTCs, and E/M-CTCs, the pearson correlation coefficient was 0.467, 0.458, and 0.963, respectively. This meaning that the E/M-CTCs were the majority component of total CTCs.

Discussion

Currently, CTCs have attracted tremendous attention because of the minimally invasive approaches applied to obtain sequential blood specimens from cancer patients and their potential clinical implications[11]. Hence, CTC counts in peripheral blood as important clinical parameters in preoperative, perioperative, postoperative for new diagnostic and predict risk of cancer metastasis. In addition, CTCs are released from the primary tumor into the bloodstream and have the potential to spread to distant sites and develop into micrometastasis [12]. Although, previous studies have demonstrated that CTCs count is not correlation to tumor node metastasis staging and the high CTCs count was negative prognostic factor [15] and CTCs number in patients with non-small cell lung cancer has not find a correlation between CTCs count and disease stage[16].

On the other hand, in a study showed that CTCs count was higher in non-small cell lung cancer patients with later stage[18]. The CTCs positive in hepatocellular carcinoma (HCC) patients after liver resection has a significantly higher risk of recurrence compared to those who were CTCs negative, and the concept of CTCs being potential seeds of metastatic dissemination[17]. There are studies demonstrated that the surgical resection may increase the dissemination of tumor cells into the circulation[13, 14]. So, it is critical to know CTCs counts into the blood stream that dissemination from the primary tumor through surgical intervention to predict recurrence and metastasis, or judgement if chemotherapy is necessary after surgical treatment.

In this study, we compared with surgical tumor resection and non-resection to establish the relationship between CTCs counts between tumor surgery and not. We used the CanPatrol™ CTC enrichment technology and specific probes against the epithelia, mesenchymal, and the epithelia/mesenchymal CTCs. Firstly, the result revealed that the patients who received tumor resection has extremely significant reduction the E-CTCs, M-CTCs, and E/M CTCs compared with non-resection in peripheral blood. So, tumor resection is associated with CTCs count. And the E-CTCs, M-CTCs, and E/M CTCs pearson correlation coefficient was 0.467, 0.458, and 0.963, respectively. Secondly, we found that the E/M CTCs were the major components of total CTCs, and there was a positive correlation between total CTCs and E/M CTCs. These results might suggest that E/M CTCs might play more important roles than the other subpopulations in tumor resection or not. Furthermore, our study is different from other reports involving CTC detection and evaluation of the clinical significance of CTCs, we investigated the role of CTCs as well as the subpopulations in tumor resection or non-resection. Study demonstrates that M-CTC subpopulation more important in tumor metastasis after comparing the patients who are M-CTC positive had significantly lower

circulating lymphocyte counts with patients lacking M-CTC, [11]. Now, we find that E/M CTCs might be most important for prediction.

Conclusions

In conclusion, our results indicate that the E/M CTCs might play more important roles in tumor prognosis than other CTC subpopulations after tumor resection. In addition, the tumor resection can reduce the number of CTCs. Reducing CTCs may serve as marker for prediction of prognosis, including tumor recurrence, metastasis and survival.

Abbreviations

CTCs: circulating tumor cells; E/M-CTC: Circulating tumor cells shed from epithelial mesenchymal; HR: hazard ratio; CI: confidence interval

Declarations

Ethics approval and consent to participate

The study was approved by Ethics Committees/Institutional Review boards at

all participating southwest hospital, third military medical university. Written consent was obtained from all patients prior to participating in the study.

Consent for publication

Consent for publication was obtained from included participants.

Competing interests

The authors declare that they have no conflicts of interest

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Authors' contributions

CZ, XLZ designed and performed the study the manuscript. CZ and JY wrote the manuscript, XLZ and JY reviewed and revised the manuscript. All authors read and approved the final manuscript.

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Not applicable

Availability of data and material

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*, 2020 ;70:7-30.
2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;136:E359-86.
3. Patrlj L, Kopljar M, Kliček R, Patrlj MH, Kolovrat M, Rakić M, et al. The surgical treatment of patients with colorectal cancer and liver metastases in the setting of the "liver first" approach. *Hepatobiliary Surg Nutr*, 2014;3:324-9.
4. Hoshimoto S1, Faries MB, Morton DL, Shingai T, Kuo C, Wang HJ, et al. Assessment of prognostic circulating tumor cells in a phase III trial of adjuvant immunotherapy after complete resection of stage IV melanoma. *Ann Surg*, 2012;255:357-62
5. Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell*, 2014;158:1110-1122.
6. Kalluri, R. (2009). EMT: when epithelial cells decide to become mesenchymal-like cells. *J Clin Invest*, 2009 ;119:1417-9..
7. Wang Z, Luo L, Cheng Y, He G, Peng B, Gao Y, et al. Correlation Between Postoperative Early Recurrence of Hepatocellular Carcinoma and Mesenchymal Circulating Tumor Cells in Peripheral Blood, *J Gastrointest Surg* , 2018;22:633-639.
8. Ou H, Huang Y, Xiang L, Chen Z, Fang Y, Lin Y, et al. Circulating Tumor Cell Phenotype Indicates Poor Survival and Recurrence After Surgery for Hepatocellular Carcinoma. *Dig Dis Sci*. 2018;63:2373-23800.
9. Hashimoto M, Tanaka F, Yoneda K, Takuwa T, Matsumoto S, Okumura Y, et al. Positive correlation between postoperative tumor recurrence and changes in circulating tumor cell counts in pulmonary venous blood (pvCTC) during surgical manipulation in non-small cell lung cancer. *J Thorac Dis*. 2018 ;10:298-306..
10. Roviello G, Corona SP, Bonetta A, Cappelletti MR, Generali D. Circulating tumor cells correlate with patterns of recurrence in patients with hormone-sensitive prostate cancer. *Onco Targets Ther*. 2017;10:3811-3815.
11. Zhao XH, Wang ZR, Chen CL, Di L, Bi ZF, Li ZH, et al. Molecular detection of epithelial-mesenchymal transition markers in circulating tumor cells from pancreatic cancer patients: Potential role in clinical practice. *World J Gastroenterol*. 2019;25:138-150.
12. Sales JP, Wind P, Douard R, Cugnenc PH, Loric S. Blood dissemination of colonic epithelial cells during no-touch surgery for rectosigmoid cancer. *Lancet*. 1999;354:392.
13. Leroy S, Benzaquen J, Mazzetta A, Marchand-Adam S, Padovani B, Israel-Biet D, et al. Circulating tumour cells as a potential screening tool for lung cancer (the AIR study): protocol of a prospective multicentre cohort study in France. *BMJ Open*. 2017;7:e018884.

14. Yasukawa M, Sawabata N, Kawaguchi T, Taniguchi S. Effectiveness of Intraoperative Pulmonary Wedge Resection of Tumor Site Before Lobectomy for Early Lung Adenocarcinoma. *Anticancer Res.* 2019;39:6829-6834.
15. Zhang Z, Xiao Y, Zhao J, Chen M, Xu Y, Zhong W, et al. Relationship between circulating tumour cell count and prognosis following chemotherapy in patients with advanced non-small-cell lung cancer. *Respirology.* 2016;21:519-25.
16. Hofman V, Bonnetaud C, Ilie MI, Vielh P, Vignaud JM, Fléjou JF, et al. Preoperative circulating tumor cell detection using the isolation by size of epithelial tumor cell method for patients with lung cancer is a new prognostic biomarker. *Clin Cancer Res.* 2011;17:827-35.
17. Pantel, K., and Brakenhoff, R.H. Dissecting the metastatic cascade. *Nat Rev Cancer.* 2004;4:448-456.
18. Francesca Chemi, Dominic G. Rothwell, Nicholas McGranahan, Sakshi Gulati, Chris Abbosh, Simon P. Pearce, et al. Pulmonary venous circulating tumor cell dissemination before tumor resection and disease relapse. *Nature Medicine.* 2019; 25:1534–1539.

Tables

Table 1. Patient tumor types

| Tumor types | Colon cancer | Rectal cancer | Gastric cancer | Nasopharyngeal carcinoma | Cholangiocarcinoma | Esophageal cancer | Pancreatic cancer | Perianal tumor | Duodenal cancer | Small intestine cancer | Cardia cancer |
|-------------|--------------|---------------|----------------|--------------------------|--------------------|-------------------|-------------------|----------------|-----------------|------------------------|---------------|
| N | 201 | 307 | 222 | 6 | 59 | 23 | 79 | 7 | 12 | 4 | 10 |

Table 2. Patient demographics and clinical characteristics

| Characteristics | N | Proportion (%) |
|-----------------|-----|----------------|
| Gender | | |
| Male | 584 | 62.8 |
| Female | 346 | 37.2 |
| Age | | |
| ≥60 | 430 | 46.2 |
| <60 | 500 | 53.8 |
| Resection | 315 | 33.9 |
| Non-resection | 615 | 66.1 |

Table 3. Patient characteristics and different CTC cells subgroups

| CTC types | Minimum | Maximum | Mean | Std. Deviation |
|-----------|---------|---------|-------|----------------|
| Total CTC | 0 | 181 | 14.72 | 18.293 |
| E-CTC | 0 | 40 | 1.82 | 3.580 |
| M-CTC | 0 | 26 | 1.65 | 2.663 |
| E/M-CTC | 0 | 176 | 11.26 | 16.011 |

Figures

Figure 1.

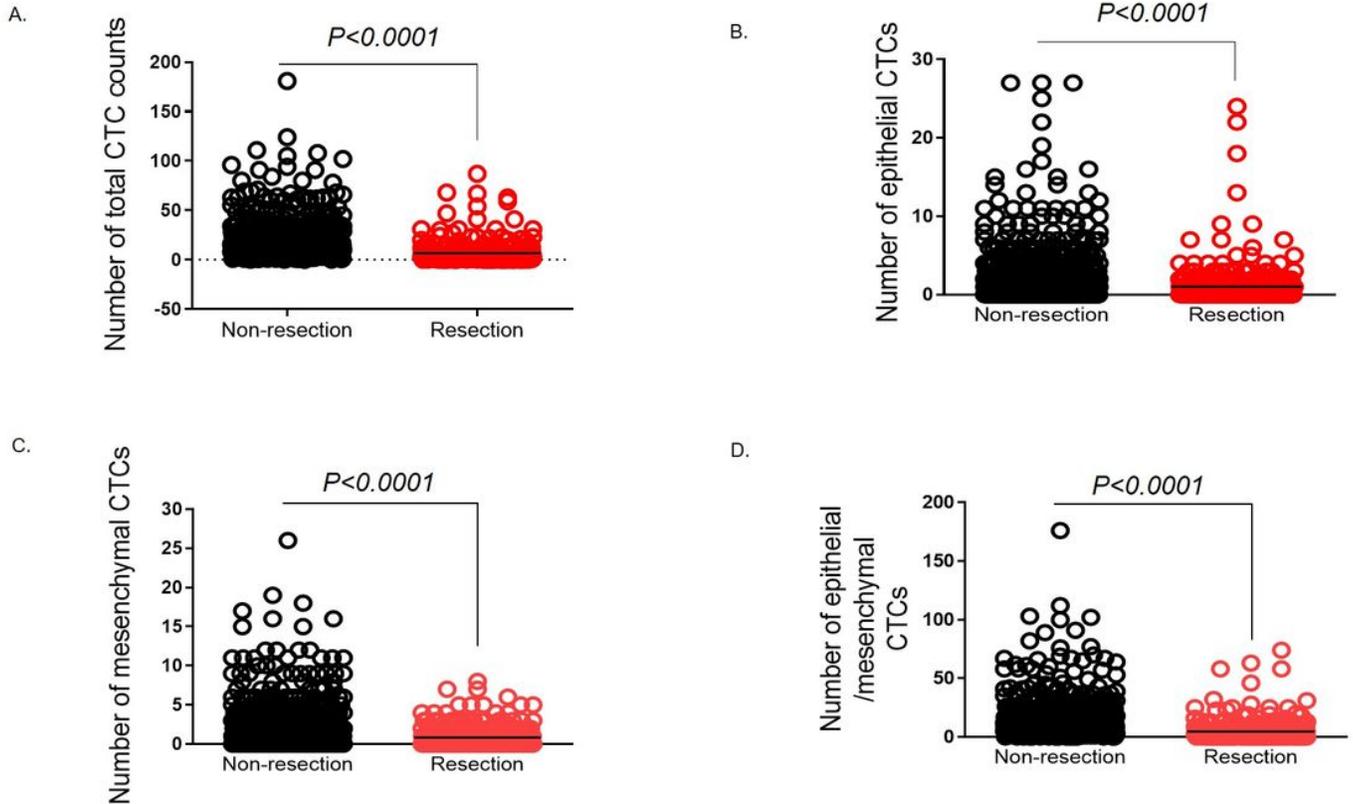


Figure 1

Comparison of CTCs counts in tumor patients with non-resection and resection. A. Frequency distribution of total CTCs in tumor patients with non-resection and resection. B. Frequency distribution of epithelial CTCs in tumor patients with non-resection and resection. C. Frequency distribution of mesenchymal CTCs in tumor patients with non-resection and resection. D. Frequency distribution of epithelial /mesenchymal CTCs in tumor patients with non-resection and resection. **** $P < 0.001$; Non-parametric T-test