Relationship between circulating tumor cells undergoing EMT and short-term efficacy following interventional treatment in patients with hepatocellular carcinoma

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ABSTRACT

Objective: A growing number of studies have indicated that epithelial-mesenchymal transition (EMT) phenotypes and the number of circulating tumor cells (CTCs) are significant indicators of tumor characteristics and treatment efficacy, and thus have a broad range of potential applications in the diagnosis and treatment of malignant tumors. The value of data on CTC phenotypes and CTC counts in the diagnosis of hepatocellular carcinoma (HCC) and assessment of efficacy after comprehensive interventional therapy remains unclear.

Methods: Data of 107 patients who exhibited space-occupying lesions in the liver on enhanced CT/MRI scans at the Guangdong Provincial People’s Hospital (a tertiary medical center) between August 2017 and October 2018, were retrospectively analyzed. All enrolled patients were treated with transcatheter arterial chemoembolization (TACE) combined with microwave ablation (MWA). An imFISH CTC assay was used to isolate and count CTCs with different EMT phenotypes in the patients’ peripheral blood, which facilitated an analysis of the value of CTC phenotype and CTC count data in the diagnosis or treatment of HCC.

Results: The CTC count and EMT phenotypes in HCC patients were not associated with patient characteristics such as age, sex, Hepatitis B Virus (HBV)-DNA status, alcohol consumption history, Aspartate Transaminase (AST) to Platelet Ratio Index (APRI) score, Eastern Cooperative Oncology Group (ECOG) score, Child-Pugh score, alpha-fetoprotein (AFP), number and size of tumors, vascular invasion, or metastasis (P > 0.05). The CTC count and EMT phenotypes in HCC patients before treatment were not predictive of short-term efficacy (P > 0.05).

Comprehensive interventional therapy reduced the total CTC count and mesenchymal CTC count (P = 0.034 and 0.022, respectively).

Conclusion: TACE in combination with ablation reduced the total CTC count and mesenchymal CTC count. The CTC count and EMT phenotypes may be associated with long-term efficacy.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide, and is the second leading cause of cancer-related deaths. This cancer type causes nearly 800,000 deaths annually, with >50% of new cases or deaths occurring in China.1 Despite improvements in monitoring and treatment techniques,2 due to latent liver disease and a high incidence of recurrence and metastasis after treatment, the prognosis is still poor. Pathologic biopsy is generally considered the gold standard for clinical diagnosis and decision-making by clinicians and researchers. Due to the invasion and boundaries of conventional pathologic biopsies, the biopsy specimens are unable to represent tumor heterogeneity and overall status, and do not allow for monitoring of dynamic tumor progression. Therefore, it is necessary to find a new minimally invasive or non-invasive diagnostic method to detect HCC at an early stage and to monitor the efficacy of HCC treatment. Over the recent years, a new diagnostic concept (liquid biopsy) which is non-invasive and allows for repeated analyses, has emerged and has gained substantial attention.3,4 Circulating tumor cell (CTC) monitoring in HCC has been clinically applied owing to the rapid development of molecular detection techniques; however, the diagnostic reliability of these data is unknown. In this study we analyzed 107 patients with hepatic malignancies, and used the imFISH method to detect different CTC phenotypes and evaluate CTC counts in patients. We then analyzed the...
intrinsic relationship between CTCs and the basic characteristics of HCC to evaluate the reliability of CTC monitoring for early diagnosis and prognosis determination in patients with HCC.

Materials and methods

Ethical statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study has been approved by the Ethics Committee of the Guangdong Provincial People’s Hospital, and all patients or their families have provided relevant informed consent. The ethical approval number is No. GDREC 2016437H.

Study design and clinical follow-up

This retrospective study was conducted at the Guangdong Provincial People’s Hospital as a single-arm and single-center trial. Blood samples were collected for analysis within three days before interventional treatment, and retesting was conducted after six months. A Cyttel System (Cyttel, Jiangsu, China) was used to isolate and count CTCs with different phenotypes. The patients had not received treatment before enrollment. All of the enrolled patients had a confirmed diagnosis of HCC based on histologic examination or imaging criteria of the European Association for the Study of the Liver (EASL). Patients who presented with a diagnosis of another malignant tumor were excluded from this study.

Patients were treated with transcatheter arterial chemoembolization (TACE) combined with microwave ablation (MWA). All patients underwent enhanced computed Tomography (CT)/Magnetic Resonance Imaging (MRI) 1–2 months after each surgery, and the efficacy was evaluated using the modified response evaluation in solid tumors (mRECIST). The Ethical Committee of our hospital approved the study protocol, and written informed consent was obtained from all participants.

Procedure

TACE treatment

The Seldinger puncture technique was used to build a vitro channel. Hepatic angiography was performed using a RH or YASHIRO catheter, and the nutritional artery of the tumor was entered using a Termao microcatheter. Pirarubicin (50 mg), iodized oil (Guerbet, France), and a non-ionic contrast agent were emulsified together. The ratio of iodinated oil to contrast agent was 1:1, and the amount of iodinated oil was determined according to tumor size and tumor blood supply, with the maximum amount not exceeding 20 mL. The iodized oil emulsion was then pulse-injected into the nutritional artery of the tumor, until embolization was complete (i.e., the flow rate of tumor blood slowed and the nutritional artery disappeared with administration of 2–5 cardiac contrast agents). An enhanced CT scan was performed within one week after TACE to evaluate the deposition of the iodized oil.

MWA treatment

Following CT completion, data acquisition, and reconstruction, the ablation needle (ECO-100AI10, ECO) was introduced into the predetermined site under CT guidance, and the target lesion was ablated based on intra-operative CT and pre-operative planning. When the optimal insertion angle and depth were achieved, the specific power and time settings were typically 5–10 min with a 65 W (W) ablation. The duration of ablation was directly related to the quality of the surrounding...
liver tissue, lesion depth, and demarcation line length. Finally, a CT scan was performed after ablation to determine the ablation range.

**Detection of different phenotypes and CTC count**

The Cyttel method was used to isolate and count CTCs with different phenotypes. Peripheral venous blood (3.2 mL) was collected in a BD vacutainer tube (Becton-Dickinson Company, Franklin, NJ, USA) within three days before treatment, and processed in CS1 and CS2 buffers (Cyttel) in succession. The resulting cell pellet was re-suspended in CS3 buffer, and then incubated with anti-CD45 antibody-conjugated immuno-magnetic beads (Cyttel). The white blood cells were separated by gradient centrifugation at 300g for 5 min in CS3 buffer (Cyttel). The resulting solution containing CTCs was smeared on a slide (Thermo Fisher Scientific, Franklin, NJ, USA), fixed, and dried for subsequent analysis.

The dried specimens were fixed and dehydrated, and a centromere of chromosome 8 (CEP8) probe (Cyttel) was added to the slides. The slides were placed in an automated hybridization instrument. Anti-human CD45 was added to the slides at room temperature, and the slides were mounted with mounting media containing 4, 6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA, USA).

**Measurements of other clinical indicators**

The best method by which to determine the severity of liver fibrosis clinically is a liver biopsy, but repeated monitoring during long-term treatment is difficult due to the invasive nature of the procedure, and poor reproducibility. The 2016 APASL consensus guideline and the EASL-Asociacion Latinoamericana para el Estudio del Higado (ALEH) clinical practice guideline announced that the aspartate aminotransferase-to-platelet ratio index (APRI) can provide high-quality evidence in the diagnosis or exclusion of significant liver fibrosis and cirrhosis.

APRI refers to the ratio index of Aspartate Transaminase (AST) and platelet count (PLT). APRI was calculated according to the following formula: APRI = (AST/upper limit of normal (ULN)) × 100/PLT (10^9/L). The ULN for AST is 40 U/L. It is generally accepted that an APRI score >2 points indicates that an adult patient has developed cirrhosis. According to previous studies, APRI has a significant correlation with the prognosis of HCC after treatment, and also has important implications for HCC surveillance in cured Hepatitis C Virus (HCV) patients.

**Statistical analysis**

SPSS 20.0 statistical analysis software was used. Measurement data are expressed as the mean ± standard deviation (SD), and independent sample t-tests were used for inter-group comparisons. Numerical data are described using the number of cases or percentages, and χ²-tests were used for comparison. The test level was set at an α = 0.05, and a P < 0.05 indicated statistical significance.

**Results**

**Patient characteristics**

From August 2017 to October 2018, a total of 173 patients exhibited space-occupying lesions in the liver as per the enhanced CT/MRI scan. These patients were considered for inclusion in this study. Thirty-nine patients were excluded due to the invasive nature of the procedure, and poor reproducibility. A total of 134 patients were reviewed. The total number of CTCs and the mesenchymal CTC counts were significantly lower than those before interventional therapy (P = 0.034 and P = 0.022, respectively); the details are shown in Table 5.

**CTC detection method**

Ashworth reported tumor cells similar to primary tumor in the

**Table 1**

| Characteristic | Parameter |
|---------------|-----------|
| Age (years)   | 58.96 ± 13.24 |
| Sex (Male/Female) | 102/5 |
| History of hepatitis B | 72 (67.3%) |
| HBV DNA (<100) | 41 (38.3%) |
| APRI (>2) | 14 (13.3%) |
| Child-Pugh score (A/B) | 91/16 |
| BCLC stage (A/B/C) | 19/53/35 |
| ECOG PS (0/1/2) | 56/43/8 |
| Lesion characteristic (Single/Multifocal) | 30/21/56 |
| Diameter of Lesion (mm) | 62.14 ± 39.28 |
| Portal vein invasion | 24 (22.4%) |
| AFP (ng/ml) | 4438.53 ± 11806.43 |
| Total bilirubin | 20.53 ± 12.34 |
| Albumin | 35.5 ± 5.21 |

HBV, Hepatitis B Virus; APRI, Aspartate aminotransferase to Platelet Ratio Index; ECOG, Eastern Cooperative Oncology Group; AFP, alpha fetoprotein.

**Table 2**

| Items | Total CTC | Epithelial CTC | Mixed CTC | Mesenchymal CTC |
|-------|-----------|---------------|-----------|-----------------|
| Age, years | | | | |
| <50 | 26 | 12 | 10 | 28 | 10 | 28 | 36 | 2 |
| ≥50 | 34 | 35 | 24 | 45 | 15 | 54 | 63 | 6 |
| P | 0.056 | 0.368 | 0.592 | 0.793 |
| Sex | | | | |
| Male | 57 | 45 | 33 | 69 | 23 | 79 | 95 | 7 |
| Female | 3 | 2 | 1 | 4 | 2 | 3 | 4 | 1 |
| P | 0.856 | 0.930 | 0.719 | 0.826 |
| HBV DNA | | | | |
| <100 | 37 | 29 | 20 | 46 | 17 | 49 | 62 | 4 |
| ≥100 | 23 | 18 | 14 | 27 | 8 | 33 | 37 | 4 |
| P | 0.997 | 0.678 | 0.458 | 0.742 |
| ECOG | | | | |
| <1 | 34 | 22 | 19 | 37 | 9 | 47 | 53 | 3 |
| ≥1 | 26 | 25 | 15 | 36 | 16 | 35 | 46 | 5 |
| P | 0.311 | 0.616 | 0.062 | 0.613 |
| child-Pugh score | | | | |
| A | 52 | 37 | 31 | 58 | 22 | 67 | 84 | 5 |
| B | 8 | 10 | 3 | 15 | 3 | 15 | 15 | 3 |
| P | 0.276 | 0.218 | 0.667 | 0.257 |
| Albumin | | | | |
| <30 | 8 | 11 | 3 | 16 | 3 | 16 | 16 | 3 |
| ≥30 | 52 | 36 | 31 | 57 | 22 | 66 | 83 | 5 |
| P | 0.176 | 0.099 | 0.574 | 0.299 |

CTC, Circulating tumor cells; HBV, Hepatitis B Virus; APRI, Aspartate aminotransferase to Platelet Ratio Index; ECOG, Eastern Cooperative Oncology Group.
Peripheral blood of patients with cancer in 1869, thus proposing the concept of CTCs for the first time. CTCs refer to tumor cells derived from the primary tumor or metastases, which enter into the blood circulation during tumor formation and progression. CTCs can be divided into epithelial, mixed, and mesenchymal types, based on whether or not epithelial-mesenchymal transition (EMT) has occurred. The detection of epithelial, mixed, and mesenchymal types, based on whether or not EMT has occurred is needed. The Cellsearch system, in which the detection of cancer cells is based mainly on the detection of the epithelial cell adhesion molecule (EpCAM) and cytokeratin (CK) molecule, is the only technical tool for clinical use in patients with HCC.

Table 3
Relationship of circulating tumor cells (CTCs) with tumor characteristics.

| Items                        | Total CTC | Epithelial CTC | Mixed CTC | Mesenchymal CTC |
|------------------------------|-----------|----------------|-----------|-----------------|
|                              | ≥3 <3     | ≥1 <1          | ≥1 <1     | ≥1 <1           |
| Largest nodule size           |           |                |           |                 |
| <5 cm                        | 33        | 20             | 18        | 35              |
|                              | 27        | 27             | 16        | 38              |
|                              | P         | 0.201          | 0.630     | 0.527           | 0.978           |
| Tumor distribution            |           |                |           |                 |
| Unilocular                   | 14        | 16             | 7         | 23              |
|                              | 11        | 10             | 9         | 12              |
|                              | P         | 0.344          | 0.336     | 0.677           | 0.107           |
| Multilocular                 | 35        | 21             | 18        | 38              |
|                              | 15        | 41             | 54        | 2               |
|                              | P         | 0.725          | 0.488     | 0.570           | 0.656           |
| Hepatic vein invasion        |           |                |           |                 |
| No                           | 56        | 45             | 30        | 71              |
|                              | 4         | 2              | 4         | 2               |
|                              | P         | 0.909          | 0.150     | 0.276           | 0.935           |
| Cheng’s Classification of Portal vein invasion | | | | |
| 0-I                          | 50        | 33             | 25        | 58              |
|                              | 10        | 14             | 9         | 15              |
|                              | P         | 0.106          | 0.494     | 0.446           | 0.856           |
| II-III                      | 45        | 37             | 24        | 58              |
|                              | 15        | 10             | 15        | 9               |
|                              | P         | 0.651          | 0.313     | 0.088           | 0.234           |
| Metastasis                   |           |                |           |                 |
| No                           | 45        | 37             | 24        | 58              |
|                              | 15        | 10             | 15        | 9               |
|                              | P         | 0.651          | 0.313     | 0.088           | 0.234           |
| BCLC stage                   |           |                |           |                 |
| A + B                        | 42        | 30             | 20        | 52              |
|                              | 18        | 17             | 14        | 21              |
|                              | P         | 0.500          | 0.203     | 0.063           | 0.927           |

CTC, Circulating tumor cells; BCLC, Barcelona Clinic Liver Cancer; AFP, alpha fetoprotein.

Numerous studies indicate that monitoring CTCs may have a wide range of potential applications in the early diagnosis of malignant tumors, assessment of conditions, selection of treatment methods, and monitoring of prognosis. Due to the high negative rate of AFP in HCC, the combined use of CTCs and AFP detection may enhance the sensitivity of early diagnosis of HCC. The CTC count or the EMT phenotypes and CTC count in HCC patients were not associated with patient characteristics such as age, sex, HBV-DNA status, history of alcohol consumption, APRI score, ECOG scope, Child-Pugh score, AFP, number and size of tumors, vascular invasion, or metastasis (P > 0.05; Tables 2 and 3).

The relationship between CTCs and therapeutic effects in HCC

Metastatic HCC is a leading cause of cancer deaths worldwide, and CTCs are critical components in the intra- or extra-hepatic metastatic process in HCC. CTCs are predominantly epithelial when released into the circulatory system, but switch to an EMT-activated phenotype due to the activation of several signaling pathways during hematogenous transit.

Table 4
Relationship of circulating tumor cells (CTCs) with short-term efficacy of TACE combined with MWA in patients.

| Items                        | Total CTC | Epithelial CTC | Mixed CTC | Mesenchymal CTC |
|------------------------------|-----------|----------------|-----------|-----------------|
|                              | ≥3 <3     | ≥1 <1          | ≥1 <1     | ≥1 <1           |
| 1st                          |           |                |           |                 |
| OR                           | 41        | 39             | 0.083     | 25              |
|                              | 33        | 36             | 0.718     | 31              |
|                              | 18        | 62             | 0.716     | 23              |
|                              | 75        | 5              | 0.684     | 92              |
| DC                           | 53        | 46             | 0.063     | 26              |
|                              | 36        | 69             | 0.250     | 23              |
|                              | 67        | 5              | 0.764     | 92              |
| 3rd                          |           |                |           |                 |
| OR                           | 39        | 35             | 0.293     | 12              |
|                              | 39        | 33             | 0.653     | 26              |
|                              | 14        | 58             | 0.167     | 20              |
|                              | 67        | 5              | 0.764     | 89              |
| 6th                          |           |                |           |                 |
| OR                           | 39        | 33             | 0.568     | 26              |
|                              | 39        | 41             | 0.653     | 33              |
|                              | 14        | 58             | 0.168     | 20              |
|                              | 67        | 5              | 0.764     | 89              |

CTC, Circulating tumor cell; OR, Objective Response Rate; DC, Disease control rate; CR, Complete Response; PR, Partial Response; SD, Stable Disease.

Evaluating the relationship between CTC counts and short-term efficacy in patients was performed by using the Chi-square test. P < 0.05 was considered significant. No difference was observed among patients in the 1st, 3rd, and 6th months. (OR: Objective Response Rate = CR + PR, DC: disease control rate = CR + PR + SD).
Huanman established a syngeneic mouse model of HCC and showed that CTCs exhibit distinct characteristics from primary tumor-derived cells; in the above mentioned study, a greater migration of CTCs compared to primary tumor-derived cells was observed, in addition to decreased E-cadherin and increased SLUG and fibronectin expression. Previous research has suggested that CTCs in hepatic veins and peripheral circulation prognosticate post-operative lung metastasis and intrahepatic recurrence, respectively. Mixed CTCs may be a vital factor for intrahepatic metastasis, and mesenchymal CTCs have the potential to be a predictor of extrahepatic metastasis.

In this study, neither the level of total CTCs nor the EMT phenotypes in HCC patients before treatment was predictive of short-term efficacy (P > 0.05). Comprehensive interventional therapy may reduce the total number of CTCs and mesenchymal CTCs (P = 0.034 and 0.022, respectively), thus affecting the long-term efficacy in patients (Tables 4 and 5). Based on previous research, HCC patients with positive peripheral mesenchymal CTCs have a higher risk of early recurrence, and thus peripheral mesenchymal CTC presence could be a potential biomarker in HCC prognosis monitoring. A reduction in the total number of CTCs and mesenchymal CTCs may indicate effective control of tumor progression. This finding could be one of the most effective ways to monitor therapeutic outcomes. Ye demonstrated that post-operative CTC counts (>2 and > 5) and changes in CTC counts may be independent prognostic indicators for progression-free survival (PFS) in patients with HBV-related HCC, with a change in the number of CTCs showing better predictive performance. Based on multivariate Cox regression analysis, CTC count was found to be an independent predictor of overall survival (P = 0.049) and PFS (P = 0.007) in patients treated with chemoembolization.

Surgical liver resection is associated with an increase in CTC count, and an increased post-operative CTC count is associated with a worse prognosis in patients with HCC. Considering the risk of tumor recurrence and metastasis, clinicians must develop appropriate treatment plans for patients. TACE combined with ablation is a widely accepted treatment for patients with HCC according to the National Comprehensive Cancer Network (NCCN), and has been shown to lead to better outcomes for patients.

This study has some limitations. First, it was a single-center retrospective study with the small sample size, which may have resulted in unexpected deviations during data extraction and analysis. Second, the potential ability of the CTC count and EMT phenotypes to predict long-term survival of HCC patients as yet remains unclear. Long-term follow-up is needed to assess the predictive performance of the CTC count and EMT phenotype parameters.

Patient consent

Written informed consent was obtained from patients for publication of these case reports and any accompanying images.

Declaration of competing interest

The authors declare that they have no conflicts of interests to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA A Cancer J Clin. 2018;68:394–424.
2. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. Lancet. 2018;391:1301–1314.
3. Xu HH, Wei W, Krawczyk M, et al. Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. Nat Mater. 2017;16:1155–1161.
4. Ogle LF, Orr JG, Willoughby CE, et al. Imagestream detection and characterisation of circulating tumour cells - a liquid biopsy for hepatocellular carcinoma? J Hepatol. 2016;65:305–313.
5. Shiha G, Ibrahim A, Helmy A, et al. Asian-Pacific Association for the Study of the Liver (APASL) consensus guidelines on invasive and non-invasive assessment of hepatic fibrosis: a 2016 update. Hepatol Int. 2017;11:1–30.
6. European Association for Study of Liver. Association Latinoamericana para el Estudio del Higado. EASL-ALEH Clinical Practice Guidelines: non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol. 2015;63:237–264.
7. Xiao G, Yang J, Yan L. Comparison of diagnostic accuracy of aspartate aminotransferase to platelet ratio index and fibrosis-4 index for detecting liver fibrosis in adult patients with chronic hepatitis B virus infection: a systemic review and meta-analysis. Hepatology. 2015;61:292–302.
8. Kanwal F, Kramer JR, Asch SM, et al. Long-term risk of hepatocellular carcinoma in HV patients treated with direct acting antiviral agents. Hepatology. 2020;71:44–55.
9. Dharan NJ, Neusaus J, Rockstroh J, et al. Benefit of early versus deferred antiretroviral therapy on progression of liver fibrosis among People with HIV in the START randomized trial. Hepatology. 2019;69:1135–1146.
10. Mansu T, Hayashi N, Iuchi T, et al. Clinical and biological significance of circulating tumor cells in cancer. Mol Oncol. 2016;10:408–417.
11. Liu X, Li J, Cadilha BL, et al. Epithelial-type systemic breast cancer cells with a restricted mesenchymal transition are a major source of metastasis. Sci Adv. 2019;5:eav0475.
12. Janning M, Kobus F, Babayan A, et al. Determination of PD-L1 expression in circulating tumor cells of NSCLC patients and correlation with response to PD-1/PD-L1 inhibitors. Cancers (Basel). 2019;11:835.
13. Sheng Y, Wang T, Li H, et al. Comparison of analytic performances of Cellsearch and iFISH approach in detecting circulating tumor cells. Oncotarget. 2017;8:8801–8806.
14. Xue F, Shi S, Zhang Z, et al. Application of a novel liquid biopsy in patients with hepatocellular carcinoma undergoing liver transplantation. Oncot Lett. 2018;15:5481–5488.
15. Wang S, Zheng Y, Liu J, et al. Analysis of circulating tumor cells in patients with hepatocellular carcinoma recurrence following liver transplantation. J Invest Med. 2018;66:1–46.
16. Cheng Y, Luo L, Zhang J, et al. Diagnostic value of different phenotype circulating tumor cells in hepatocellular carcinoma. J Gastrointest Surg. 2019;23:2354–2361.
17. Guo W, Sun YF, Shen MX, et al. Circulating tumor cells with stem-like phenotypes for diagnosis, prognostic, and therapeutic response evaluation in hepatocellular carcinoma. Clin Cancer Res. 2018;24:2093–2013.
18. Huanman J, Naidoo M, Zang X, et al. Fibronectin regulation of integrin β1 and SLUG in circulating tumor cells. Cells. 2019;8:618.
19. Sun YF, Guo W, Xu Y, et al. Circulating tumor cells from different vascular sites exhibit spatial heterogeneity in epithelial and mesenchymal composition and distinct clinical significance in hepatocellular carcinoma. Clin Canc Res. 2018;24:547–559.
20. Liu YK, Hu BS, Li ZL, et al. A novel improved strategy to detect the epithelial-mesenchymal transition process in circulating tumor cells in hepatocellular carcinoma patients. Hepatol Int. 2016;10:640–646.
21. Wang Z, Luo L, Cheng 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.
22. Ye X, Li G, Han C, et al. Circulating tumor cells as a potential biomarker for postoperative clinical outcome in HBV-related hepatocellular carcinoma. Canc Manag Res. 2018;10:5639–5647.
23. Shen J, Wang WS, Zha XL, et al. High epithelial cell adhesion molecule-positive circulating tumor cell count predicts poor survival of patients with unresectable hepatocellular carcinoma treated with transcatheter arterial chemoembolization. J Vasc Interv Radiol. 2018;29:1678–1684.
24. Yu JJ, Xiao W, Dong SL, et al. Effect of surgical liver resection on circulating tumor cells in patients with hepatocellular carcinoma. BMC Canc. 2018;18:835.
25. Biederman DM, Titano JJ, Bishay VL, et al. Radiation segmentectomy versus TACE combined with microwave ablation for unresectable solitary hepatocellular carcinoma up to 3 cm: a propensity score matching study. Radiology. 2017;283:895–905.
26. Cheng BQ, Jia CQ, Liu CT, et al. Chemoembolization combined with radiofrequency ablation for patients with hepatocellular carcinoma larger than 3 cm: a randomized controlled trial. J Am Med Assoc. 2008;299:1669–1677 [retracted in: DeAngelis CD, Fontanarosa PB. JAMA. 2009 May 13;301(18):1931].