Review Article

Specific study of biological tumor cytology: a narrative review

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Abstract: Tumor metastasis is a very complex invasion. The concept of circulating tumor cells (CTCs), which describes the tumor cells diffusing into the blood, is being increasingly recognized for its role in cancer metastasis as well as it is considered the key step of tumor blood metastasis. Epithelial-mesenchymal transition (EMT) is the reverse transition from mesenchymal phenotypes to an epithelial one. Tumor cells undergoing this process have stronger metastatic potential. Though the biological characteristics of CTCs after EMT can help explain the unknown phenomena in tumor metastasis, the biomarkers of CTCs after EMT are not accurate. Also the correlation between CTCs after EMT and the prognosis of patients and the dynamic transformation of cell groups during treatment are not clear. Tumor metastasis initiating cells (MIC), which eventually lead to metastasis in CTCs, are types of cancer stem cells, or at least tumor cells with many stem cell characteristics. Further investigation of the maintenance of tumor stem cell characteristics of CTCs is required. The circulating tumor microemboli (CTM) can prevent CTCs from losing their nests, and the presence of host cells facilitates special metastasis and helps tumor cells escape immune surveillance. In this paper, we reviewed research on the biological cytological characteristics of CTCs in recent years, and provided relevant supporting evidence for monitoring tumor recurrence, assessing patient prognosis, evaluating the sensitivity of anti-tumor biological drugs, and selecting an individualized treatment plan.

Keywords: Biological tumor cytology; epithelial mesenchymal transition (EMT); circulating tumor microemboli (CTM); biological therapy.

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Introduction

According to the statistics, about 90% of deaths among cancer patients are due to tumor metastasis rather than the malignant damage of tumor *in situ* (1-3). Tumor metastasis is a very complex invasion. Circulating tumor cells (CTCs) are the root cause of cancer metastasis. Tumor cells diffuse into the blood to form CTCs, which are a necessary prerequisite for recurrence and metastasis (4). CTCs are commonly found in the blood of patients with malignant tumors and are rare in the blood of healthy volunteers and patients with benign diseases. With the development of CTCs separation technology, CTCs can be studied at the molecular level, which is helpful to better understand the biology of metastasis (5,6). CTCs and CT DNA, as liquid biopsies, are used for early diagnosis of tumors, identification of cancer subtypes and prediction of disease prognosis in clinical practice, so as to achieve personalized

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treatment of cancer (7,8). Studying the biological characteristics of CTCs has provided new ideas and methods for researching the mechanism and treatment of tumor metastasis. Researchers have also monitored the recurrence and metastasis of tumor patients in the early stage, and provided relevant supporting evidence for judging the prognosis of patients, evaluating the sensitivity of anti-tumor drugs, and selecting an individualized treatment scheme (9). This paper aimed to summarize the related research. We present the following article in accordance with the Narrative Review reporting checklist (available at https://dx.doi.org/10.21037/tcr-21-237).

Biological specificity of tumor cells

With the development of separation and detection technology of CTCs, many studies have focused on elucidating the molecular mechanism of tumor metastasis caused by CTCs (10,11). Due to the heterogeneity of CTCs cell phenotypes and molecular characteristics, more efficient and accurate CTCs capture and identification technology are needed to accurately predict and prevent postoperative tumor metastasis. Therefore, it is more important to find malignant CTCs with the ability of proliferation, invasion and tumor colonization than to simply count the number of CTCs (12,13).

Epithelial stroma and transformation of CTCs

Epithelial mesenchymal transition (EMT) is a process in which tumor cells lose their original epithelioid cell polarity and intercellular association and become spindle-like with a variable cytoskeleton to complete the metastasis process. Tumor cells undergoing this process have stronger metastatic potential (14). At present, EMT is still controversial, but molecular biological analysis of CTCs shows that the biological characteristics are closely related to EMT phenomenon (15,16). The characteristics of EMT are shown by CTCs. The expression of EMT markers was found to be heterogeneous, which demonstrated the hypothesis that only some tumor cells had EMT instead of "all or none" (17). The results were confirmed by experiments in other tumor types like ovarian cancer (18). Studying the expression of different EMT markers in CTCs enables inference of the tumor progression. Kallergi *et al.* found that CTCs expressing stromal cell markers were more easily detected in the blood of patients with advanced cancer, which indicated the relationship between EMT and disease progression (19).

In conclusion, the biological characteristics of CTCs after EMT can help to explain the unknown phenomena in tumor metastasis. However, the biomarkers of CTCs after EMT are not accurate. The correlation between CTCs after EMT and the prognosis of patients and the dynamic transformation of cell groups during treatment are not clear (20). Single cell genome analysis of different phenotypes of cells may reveal the metastatic potential of single cells, but further functional research of the isolated cell populations is needed (21).

Tumor initiation cells and CTCs

Tumor metastasis is now considered as a very complex process of metastasis and invasion—its efficiency is very low (0.01%) when CTCs detach from tumor spreading into circulation and eventually form obvious metastasis (22). The diverse biological characteristics of tumor cells determine the complexity of tumor biological behavior. The heterogeneity of tumor biological behavior is related to the type, mode and amount of gene alteration. Genetic polymorphism not only determines biodiversity, but also causes the heterogeneity of tumor genes to change biological behavior (23).

Cancer stem cells have been used to investigate the process of metastasis (24). Many experiments have proved that cancer stem cells exist in solid tumors. Cancer stem cells, which have a strong self-renewal, proliferation and differentiation abilities, play a decisive role in the occurrence, recurrence and metastasis of tumors. At present, the research on cancer stem cells is still in its infancy. The specific biological characteristics of cancer stem cells are still unclear, and most of the research are limited to the isolation of cancer stem cells. Only by targeting and isolating cancer stem cells in a real sense can we carry out related research on killing cancer stem cells and cancer precursor cells, so as to achieve the ultimate goal of curing tumor (25).

In human pancreatic cancer cells, a group of $CXCR4^+$ and $CD133^+$ tumor initiation cells with strong invasion and self-renewal ability were found to be resistant to conventional chemotherapy (26,27). The number of CTCs and the high expression of epithelial cell adhesion molecule (EpCAM) were positively correlated with the low survival rate and the number of metastatic foci (28-30). Liu *et al.* found that detection the CTCs from the portal vein could be a powerful tool for the diagnosis intrahepatic metastases of the pancreatic cancer and provided new insight into

the biological feature of pancreatic cancer metastases and drug resistance (31). All groups of tumor metastasis initiating cells (MIC), which eventually lead to metastasis in CTCs, are types of cancer stem cells, or at least tumor cells with many stem cell characteristics. The process is not only a source of seed formation and transfer, but also an important cause of drug resistance. Deep research on the characteristics of cancer stem cells of CTCs has brought it into the advanced treatment stage. The use of CTC-chip technology can allow observation of CTC drug sensitivity after isolation and cultivation from patients with cancer, therefore it provides a more reasonable response plan for treating tumor metastasis (32,33). Further investigation of the molecular mechanism of generation, regulation, and maintenance of tumor stem cell characteristics of CTCs is required (34).

Tumor microemboli and CTCs

Circulating tumor microemboli (CTM) are cell masses composed of at least 3 CTCs or a CTC and a variety of other types of cells (including fibroblasts, endothelial cells, white blood cells, periderm cells, and platelets) (35).

The CTM play an important role in tumor metastasis (36,37). Preclinical studies have shown that compared with single CTC, the number of CTM is positively correlated with the prognosis of patients (38,39), and the formation of the mass is closely related to the expression of specific globin in tumor cell. The production of CTM can prevent CTCs from losing their nests, and the presence of stromal/host cells facilitates special metastasis and helps tumor cells escape immune surveillance (40). The Harvard team has shown that the presence of tumor matrix (especially fibroblasts) in CTM provides support for the formation of metastasis, and the removal of fibroblasts can significantly reduce the ability of metastasis; platelets, white blood cells, and periderm cells also promote metastasis. However, it is still unknown whether the cells in CTM have the same effect on metastasis. The study of CTM in tumor patients will further elucidate the composition and metastatic potential of CTM. Among patients with thyroid cancer, the cells in CTM have been shown to be in a state of cell cycle arrest, which may enhance their survival and resistance to chemotherapy (41). Both CTC and CTM show obvious heterogeneity, which could be explained by the heterogeneity of the tumor itself. At present, there are many new research findings on the metastasis mechanism of CTM.

Biological therapy and CTCs

Tumor biological therapy is a new and effective mode of cancer therapy, which aims to improve the quality of life. This therapy uses key molecular targets, nucleic acids, proteins and small molecule compounds as the therapeutic medium, combined with traditional treatment methods such as surgery, radiotherapy and chemotherapy, to achieve the effect of radical cure or inhibition of tumor development (42).

The emergence of targeted drugs has opened up a new therapeutic approach for tumor patients, provided an optimistic prospect for anti-tumor, and achieved good results in the clinical treatment of various types of cancer. Traditional targeted drugs tend to kill rapidly proliferating cancer cells after CTCs have metastasized, but the technical disadvantage is that they cannot reverse the metastatic fate that has formed and the development of drug resistance (43).

Tumor immunotherapy is a method of *in vitro* culture and amplification of immune cells which are collected from patients by using biotechnology and biological agents, and transplanting them back into patients' bodies. Due to the immunosuppression and other factors, the body's own immune cells cannot effectively play the role in killing tumors. Through *in vitro* culture, induction and activation, the antitumor activity of immune cells can be greatly enhanced. The enhanced immune cells will be injected back into the body to enhance the body's own immune function, so as to achieve the goal of treating tumors (44).

T cells as the main immune cells, recognize tumor cells' surface antigens and take tumor cells as targets for attack, which play an important role in the body's anti-tumor immune rejection. T cells specifically recognize the tumor antigen peptides, which was presented by MHC-I molecules on the surface of target cells, mainly through the T cell receptor (TCR) on its surface. TCR plays an important role in the process of recognizing and killing tumor cells. TCR achieve accurate recognition of neoantigen produced by gene mutation on tumor cells by triggering the conformational change of MHC-I, which make TCR and neoantigen bonding tighter, while releasing self antigen from the TCR molecule. TCR-based cancer immunotherapy is considered to hold great promise in some cases for curing patients with solid tumor (45,46).

Due to the inhomogeneity of CTCs surface antigens, epigenetic or protein variability and abundance, it is difficult for current techniques to effectively identify and capture CTCs *in vivo* to prevent tumor metastasis after surgery.

Therefore, developing biocompatible nanomaterials coated with targeted antibodies can hopefully achieve the highly specific capture of CTCs in bloodstream circulation. This method may inhibit the activity of CTCs and interfere with CTCs' adhesion to the vascular wall, so as to effectively prevent cancer metastasis (47).

Summary

In recent years, domestic separation and detection technology and single cell analysis technology have helped us understand the biological characteristics and related molecular mechanism of CTCs. The results of several preclinical studies are being translated into clinical practice. However, more questions still need to be answered, such as the specific mechanism and characteristics of CTCs and CTM, and the functional differences between single CTC and CTM. It is important to study the biological characteristics of CTCs and the molecular mechanism of tumor metastasis. The development of tumor biotherapy is rapid, and it is expected to make important contributions to improving the current situation of cancer treatment.

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Footnote

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