Research Progress of Single-cell RNA Sequencing in Esophageal Cancer

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Abstract: Esophageal cancer (EC) is a highly invasive malignant tumor with early onset, rapid progression, and high risk of recurrence and metastasis. Despite continuous advances in treatment methods, the five-year survival rate has not significantly improved. Single-cell RNA sequencing (scRNA-seq), a high-throughput sequencing technology that has received much attention in recent years, can identify gene expression information at the single-cell level, addressing the issue of cell heterogeneity in traditional sequencing technologies and greatly promoting the development of personalized diagnosis and precision medicine. This article summarizes its latest research progress in studying the tumor microenvironment, metabolic reprogramming, and neoadjuvant chemotherapy in the EC research, providing new insights and ideas for the study, clinical diagnosis, and precision treatment of EC.
***Keywords*:** single-cell RNA sequencing, esophageal cancer, tumor microenvironment, metabolic reprogramming, neoadjuvant chemotherapy

1. Introduction

Esophageal cancer (EC), as a malignant tumor with strong invasiveness and high concealment, has been consistently high in incidence and mortality due to its unknown pathogenesis, lack of typical early features, and easy recurrence and metastasis after surgery. The main histological types of EC include esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). ESCC is usually believed to originate from squamous epithelial cells of the esophagus and closely related to genetics and lifestyle factors such as alcohol consumption and smoking[1]. EAC originates from glandular cells near the stomach and is strongly associated with factors such as obesity, gastroesophageal reflux, and Barrett's esophagus[2].

Single-cell RNA sequencing (scRNA-seq) enables the profiling of transcriptomic expression at a single-cell level by isolating individual cells and extracting mRNA for library construction and sequencing and overcomes the limitations of traditional RNA sequencing in studying cellular heterogeneity. Applying scRNA-seq in the EC research can provide insights into tumor microenvironment (TME), metabolic reprogramming, and neoadjuvant chemotherapy (NACT). This article summarizes the recent research progress in the field of EC, aiming to provide new research ideas for precision medicine strategies in EC.

2. Application of scRNA-seq in TME

TME is a specialized environment necessary for tumor occurrence and development. Tumor cells can shape the TME functionally through the secretion of various chemokines and the release of extracellular signals. Exploring the continuous interaction between tumor cells and the TME plays a crucial role in studying tumor progression and treatment.

T cells, as the main immune cells in the TME, can inhibit tumor occurrence and development through various mechanisms, including recognizing tumor-specific antigens, directly killing tumor cells, and regulating immune responses[3]. Compared to normal tissues, ESCC tissues are rich in regulatory T cells (Tregs) and exhausted T cells, while lacking naïve T cells, effector T cells, and memory T cells, indicating an immunosuppressive state in the ESCC TME. T helper 17 cells (TH17s) accelerate tumor angiogenesis by secreting cytokines such as IL-17, which is associated with stromal cell activation. Follicular helper T cells (TFHs) can promote adaptive anti-tumor immune responses by forming tertiary lymphoid structures (TLSs) with germinal center B cells (GC Bs) and fibroblastic reticular cells (FRCs), or by activating tumor-infiltrating B lymphocytes (TIL-Bs) through CD40 signaling, nuclear factor κB (NF-κB) pathway, and antigen presentation, thus exerting an anti-tumor effect on the immunosuppressive TME[4-6]. Tumor-infiltrating myeloid cells (TIMs), including monocytes, macrophages, mast cells, dendritic cells (DCs) and neutrophils, play important roles in promoting tumor angiogenesis and invasion. Increased expression levels of cytokines and NF-κB-related genes (such as TNFSF13B, IL6 and NKFB1) in monocytes, an increased proportion of active gene VEGFA in mast cells and tolerogenic DCs (tDCs) that suppress the proliferation and activation of CD8+ T cells through the interaction of PD-1 and PD-L1 significantly enriched in tumors, suggesting their key roles in promoting EC development[4, 7, 8]. Monocytes, neutrophils and macrophages can also promote

tumor progression through the expression of angiogenesis genes (such as VEGFA, CXCL8, MMP9 and MMP12) and the secretion of angiogenic factors, thus exerting angiogenesis effects in the tumor progression process[9]. The effect of intra-tumoral cells in the EC TME is shown in Figure 1.

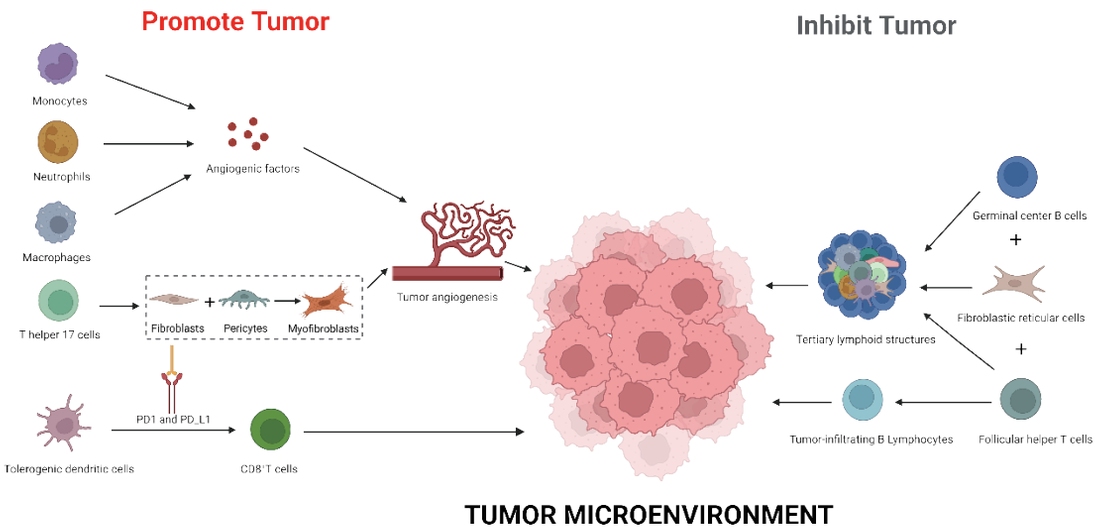

Figure 1. The effect of intra-tumoral cells in the EC TME

3. Application of scRNA-seq in metabolic reprogramming

Tumor cells can suppress anti-tumor immune responses by altering the metabolism of tumor-infiltrating immune cells. Studying the metabolic pathways of different cell types in EC can lay the theoretical foundation for a deeper exploration of its pathogenesis and the identification of potential therapeutic targets.

When the growth rate of tumor cells exceeds the supply capacity of its blood vessels, it leads to hypoxia in the TME, so as to inhibit the function of immune cells and detrimentally affect anti-tumor immune responses[10]. Upregulation of glycolysis and oxidative phosphorylation (OXPHOS) metabolic activity becomes an important mechanism for supporting tumor proliferation and development in hypoxic environments. The highly proliferative group of myofibroblasts shows upregulated glycolysis and OXPHOS pathways, and significant differences in OXPHOS activity between different patients validate their crucial role in the metabolic reprogramming of ESCC[5, 11]. The malignant epithelial cells of intramucosal ESCC in the E7 subset exhibit high levels of sphingolipid metabolism and polysaccharide synthesis and metabolism similar to progressive ESCC, demonstrating strong potential for tumor metastasis and angiogenesis. However, the E7 subset shows lower levels of glycolysis, ketone metabolism, OXPHOS, amino acid metabolism, and lipid metabolism compared to other epithelial subsets in progressive ESCC, indicating that intramucosal ESCC may not trigger extensive cell growth and proliferation or experience nutrient and oxygen deprivation[12].

4. Application of scRNA-seq in NACT

In recent years, the use of NACT has been shown to effectively slow down tumor progression, reduce the incidence of distant metastasis and improve the survival rate of EC patients. In-depth investigation into the mechanism of NACT on EC can provide new insights for its basic research and clinical treatment.

Compared to patients undergoing surgery alone (SA-ESCC), patients receiving NACT (NACT-ESCC) before surgery show a significant increase in exhausted CD8+ T cells, naïve CD8+ T cells, monocytes and plasma B cells. Conversely, the expression levels of epithelial cells, stromal cells, tumor endothelial cells, myofibroblasts, macrophages, initial B cells and inhibitory receptor genes are relatively low, indicating that NACT can enhance the anti-tumor immune response of patients and activate their acquired immune response[6, 7]. Furthermore, NACT can effectively alleviate the pathological deterioration of EAC patients through pathways such as adjusting T cell subsets, increasing the proportion of DCs, transforming into fibroblast subsets, and expanding the endothelial cell subsets, providing new immunotherapeutic development and application strategies for improving EAC treatment[8].

5. Discussion

Although various treatment modalities such as surgery, radiotherapy and chemotherapy have gradually been applied in the clinical management of EC, its overall five-year survival rate remains low. Therefore, it is urgent to explore new treatment approaches to improve patient prognosis. Applying scRNA-seq to EC research shifts the focus from previous studies that only focused on average levels to the single-cell level, which can provide more crucial information for the development of precise and personalized treatment approaches for EC. It is believed that with continuous optimization in scRNA-seq, a more comprehensive and in-depth understanding of EC can be achieved in the future, leading to improve survival rates for EC.

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