Dynamic Tumorigenic Trajectory and Transitional Signatures of Oncogenic Evolution by Single-Cell Transcriptomic Profiling of Esophageal Squamous Cell Carcinoma

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Esophageal cancer is the ninth most common type of cancer and the sixth leading cause of cancer deaths in the Chinese mainland.¹ One of the most prevalent subtypes of esophageal cancer is esophageal squamous cell carcinoma (ESCC). Epidemiological data indicate that more than half of the ESCC cases worldwide are in China.² Owing to the lack of diagnostic markers for early detection and effective treatment, the ESCC prognosis is generally poor, and the mortality rate of patients with ESCC is markedly high. ESCC initiation progresses from inflammation to hyperplasia, dysplasia, carcinoma in situ, and finally invasive carcinoma; however, the molecular mechanism of ESCC initiation and development remains unclear; this restricts the improvement of the prevention, early diagnosis, and clinical treatment of ESCC. Therefore, exploring the mechanism of ESCC initiation and identifying the pertinent diagnostic markers is important for the early diagnosis and treatment of ESCC.

Although many genomic variations critical to ESCC have been identified through whole-exome/genome sequencing,³⁴ the transformation and development of normal esophageal epithelial tissue into precancerous lesions through successive mutations and into invasive carcinoma, respectively, remain to be elucidated as all related previous studies are cross-sectional in nature. In addition, somatic mutations normally occur in esophageal tissues,⁵ implying that these mutations are not sufficient to cause the malignant transformation of esophageal epithelial cells. Other mechanisms such as microenvironmental alterations also play extremely important roles in the initiation and progression of ESCC.⁶⁷ Therefore, elucidating the dynamic transcriptomic alterations in the tumor microenvironment during ESCC tumorigenesis is necessary. Recently, single-cell transcriptomic profiling, a new technology that can be used to analyze cellular compositions and elucidate cell state transition in tissues, has been a promising approach.⁸¹²

Exploring the mechanisms of ESCC initiation requires capturing the tumorigenic lesions that continuously occur in the same patient; however, such a study is not feasible and...
would ideally be conducted using well-established mouse models with 4-nitroquinoline 1-oxide (4NQO)-induced ESCC. By using single-cell RNA sequencing, the process of ESCC initiation can be elucidated through the detection of 4NQO-induced ESCC tumor formation at multiple stages of malignant transformation. In a recent study, Yao et al. successfully created a mouse model wherein ESCC initiation was simulated and a developmental atlas was constructed for ESCC using single-cell transcriptomic profiling.

In this study, we examined the functional and expression changes in esophageal epithelial cells transitioning from normal to ESCC by using single-cell transcriptomic profiling. The cells were isolated from a mouse model with 4NQO-induced ESCC. A total of 1756 mouse esophageal epithelial cells classified into six subtypes (EpiC1–6) were examined. A single-cell diffusion map of these cells indicated clear differences during their transition across all pathological stages, and major changes along the progression of cells into hyperplasia and invasive carcinoma were identified. The distribution of marker genes such as Aldh3a1, Atp5a, S100a8, Igaf6, and Mmp14 differed among disease stages. In the hyperplasia stage, the abrupt upregulation of S100a8 in cells indicated that the esophageal tissues underwent a dramatic immune transition. In addition, two evolution fates of epithelial cells during 4NQO-induced ESCC tumorigenesis were identified using pseudotime and principal component analysis, and the key signaling pathways that resulted in the transition of esophageal epithelial cells from inflammation to hyperplasia were further investigated. The results suggest that some of the damaged esophageal epithelial cells may activate the inflammatory response and lead to cell transformation after exposure to 4NQO. Next, eight fibroblast clusters (FibC1–8) in the cellular microenvironment were identified by analyzing different gene expression patterns and the dynamic changes in the fibroblast clusters. The immune response was confirmed to be regulated by esophageal epithelial cells during 4NQO-induced ESCC tumorigenesis. Furthermore, the status of T cells during ESCC initiation was explored, and CD8+ T cells and CD4+ T cells were divided into seven clusters each. The T cells were found to have reduced anti-tumor effects and enhanced inflammatory responses during 4NQO-induced ESCC tumorigenesis. Interaction analysis indicated that the interactions between inflammatory immune cells (Th17 and neutrophils) and malignant epithelial cells increased. These results suggest that the inflammatory microenvironment may promote malignant transformation of esophageal epithelial cells. Finally, we validated the gene expression profiles in human ESCC specimens and confirmed that similar changes occur in human ESCC tissues.

This study describes the single-cell transcriptomic profiling of various cell types at different pathogenic stages during 4NQO-induced ESCC tumorigenesis. Based on these data, we constructed an atlas of the malignant transformation of epithelial cells exposed to 4NQO. The transition landscapes of immune cells and fibroblasts in tissue microenvironments at different tumorigenesis stages were also represented. These findings will help us to better understand the initiation and development of ESCC and lay the foundation for the development of molecular markers for early diagnosis and precise treatment strategies for ESCC; however, this study has several limitations. First, the esophageal tissues used for single-cell transcriptomic profiling were obtained from mice with 4NQO-induced ESCC. Although the initiation and progression of human ESCC can be simulated in this animal model, the results obtained from this mouse model cannot be directly applied in humans. Second, as the size of esophageal epithelial tissues of mice is small, the datasets for early esophageal lesions in this study were based on a relatively small amount of esophageal epithelial cells, which might have resulted in reduced accuracy of the transcriptomic analysis. Future studies should focus on the improvement of experimental methods, including the stripping of the entire esophageal epithelial layer. Third, the fates of 4NQO-induced epithelial cell and esophageal microenvironment transition were identified; however, the underlying molecular mechanisms were not explored. Despite these limitations, the transition status and transcriptomic alterations of various cell types in the esophagus during the development of ESCC were elucidated.

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Conflicts of interest
There are no conflicts of interest.

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