Extended Abstract
Volume 5:4, 2021

Hepatology and Pancreatic Science

Open Access

Toward understanding the molecular basis of esophageal squamous cell carcinoma

Ming Rong Wang

Cancer Institute and Hospital-CAMS & PUMC, China E-mail: wangmr2015@126.com

Abstract

Esophageal squamous cell carcinoma (ESCC) is among the most common human cancers, with an overall fiveyear survival rate of around 20%. To improve the diagnosis and prognosis of ESCC, we performed systematic studies on the molecular alterations in the disease. Frequent gains of chromosomal bands 3q26, 8q24, 11q13, losses of 3p14 and 9p21, amplifications of genes CCND1, EMS1 (CTTN), EGFR, PLK1, SKP2, PRKCI (PKCiota), deletions of CDKN2A/B, FHIT and rearrangements of NTRK3, DTL and PTPRD were found. The mutation profiling was characterized and potential therapeutic targets were identified. We further investigated intratumor heterogeneity (ITH) of the molecular alterations and constructed phylogenetic trees for genomic evolution, in which the mutations of ERBB4, FGFR2, BRCA2, ATM, TP53 and copy number changes of 11q13 and 9p21 were early events and those of PI3K/MTOR pathway, KIT, AURKA, CCND2 and 3q26 were late. By proteomic techniques and immunohistochemistry, multiple proteins were observed with high expression in tumor tissues but negative/low expression in morphologically normal operative margins.

Especially, copy number alterations of ANO1, CDKN2A, and high expression of p63 and ANO1 were also present in precancerous lesions (dysplasia). We further explored the mechanisms underlying the development and progression of ESCC and revealed that CRT, CTTN, PKCiota, SKP2 and PLK1 enhanced cell motility and resistance to apoptosis and promoted tumor growth and metastasis via activating the PI3K-AKT pathway, inhibiting beta-catenin degradation and up-regulating the apoptosis suppressor Survivin. These findings extend our understanding of ESCC, providing theoretical foundation for elucidating the mechanisms underlying the tumorigenesis of the esophagus and progression of ESCC and for developing classification biomarkers and therapy targets for ESCC treatment.

To enable a systematic analysis of immune cell populations, we normalized and pooled single-cell data from all samples and conducted unsupervised clustering to identify distinguishable populations. The whole procedure was performed using Seurat v3.0 with default parameters. We annotated these populations using their canonical markers and successfully identified the major types of tumor-infiltrating immune cells as shown in other cancers, including T cells, NK cells, monocytes/macrophages, dendritic cells (DCs), B cells, plasma cells, and mast cells, as well as a very small fraction (1.31%) of other non-immune cells that were mixed in with the sorted cells. The expression of classic markers of these cell types was consistent with the annotation . We then analyzed “other” cluster form tumors, and found that most cells had copy number variations (CNVs), including both amplifications and deletions, suggesting that this cluster included tumor cells.

By comparing the percentages of each cell type in CD45+ cells between tumor and adjacent tissues, we found an increase of T cells and monocytes/macrophages in tumors. In contrast, the percentages of B and NK cells were decreased (Fig. 1e and Supplementary Fig. 3a). In agreement with recent studies18, we found a large degree of variation in the immune composition among tumors . T lineage cells were the most abundant immune cell type in most tumors, making up 30–71% of the total CD45+ cell. However, considering the ratios of each immune cell type to all cells analyzed by flow cytometry during CD45+ cell isolation, there was high variation between matched tumor and adjacent tissues, as well as among individuals (Supplementary Data 1). Seven pairs of samples were roughly divided into two groups.

There were only minor differences between the matched adjacent and tumor tissues in three tumor-adjacent tissue pair. T cells made up to fewer than the 2% of total cells in these tumors. In contrast, the immune profiles of four other tumor-adjacent pairs presented a significant shift in a PCA, in which 6–12% of total cells were T cells in tumors (Fig. 1h, i). These tumors also showed increased numbers of monocytes/macrophages, compared with other tumors and adjacent tissues (Supplementary Fig. 3c). In addition, we found inter-patient variation in biologic signatures, including hypoxia, inflammation response, and TNFA-via NFKB pathways in lymphocytes. Interestingly, S135 and S158 showed similar gene signatures enrichment, and S133 and S134 showed similar gene signatures enrichment in these pathways.Next, we further validated our results for the major immune cell types with additional samples by flow cytometry and immunohistochemistry (IHC). We found an increase in T cells and macrophages decrease in NK and B cells in tumors, compared to adjacent tissues, which is consistent with the scRNA-

seq data . Notably, neutrophils were not identified in scRNA-seq as a population like others reported, but they were detected in low abundance by flow cytometry and IHC. The failure to detect neutrophils in scRNA-seq may be caused by the combination of the low abundance of neutrophils in ESCC and the limitation of the current 10× scRNA-seq technique. Neutrophils' low RNA content and abundance of RNases may lead to increased sensitivity to prolonged processes of scRNA-seq, which could potentially result in fewer transcripts being detected, resulting in these cells not passing quality control. Next, we compared the major compartments of infiltrating immune cells in ESCC to other cancer types with available data.

We found that ESCC was among the tumor types with a higher number of infiltrating T cells and monocytes/macrophages and a lower number of infiltrated B cells. This is consistent with our observation that ESCC had increased T cells and monocytes/macrophages and decreased B cell ratios, compared to their adjacent tissues . Notably, recent studies suggested positive effects of tumor-infiltrating B cells, especially those in tertiary lymphoid structures, on increasing response to immunotherapies. Whether it is responsible for the response of checkpoint blockade in ESCC needs an additional investigation.

This work is partly presented at 14th Euro-Global Gastroenterology Conference