Introduction

Esophageal squamous cell carcinoma (ESCC) accounts for over 80% of esophageal cancer, with a poor prognosis mainly due to a lack of symptoms at early stages. Although an early diagnosis of ESCC may lead to better outcomes, it is challenging to detect early ESCC as it originates from the basal cell layer and invades into the lamina propria.

Therefore, it is imperative to understand the mechanisms of ESCC initiation and establish model systems recapitulating ESCC neoplasia. Several studies have identified the frequent genetic alterations in ESCC patients. However, key genetic interactions initiating ESCC remain elusive.

Herein, we sought to investigate the essential genes of which mutations induce ESCC initiation by using CRISPR/Cas9 and mouse esophageal organoid model system.

Methods

32 different KO organoid lines were established by using CRISPR/Cas9 system based on the previous studies.

The proliferative property of each organoid was evaluated by its growing size and HIC staining.

Transcriptomes of 4 different organoids were analyzed by scRNA sequencing, followed by the comparison to human ESCC patients and cell proportion test.

Cell lineages of 4 organoids were calculated by RNA velocity, and the key transcription factors are predicted by Regulon analysis.

Allograft transplantation experiments were conducted and immune-related responses were evaluated.

Results

TP53, CDKN2A, KMT2D, KMT2C, FAT1, FAT4, AJUBA, NOTCH1, NOTCH3 are frequently mutated in the ESCC patients.

Notch1 KO with Trp53 KO (PN) and Cdkn2a KO (PCN) EOs displayed the loss of cell differentiation and hyperplasia. Increased number of proliferating cells was confirmed by Mki67 staining and BrdU assay of PN and PCN.

Single-cell transcriptomics revealed that PC (52.2%), PN (50.5%), and PCN (52.2%) organoids show similarity with the human ESCC patients, and PCN (21.9%) exhibited the highest similarity with the poor survival-associated ESCC patients compared to the other organoids. Gene set enrichment analysis (GSEA) further confirmed that the gene set highly expressed in PN and PCN organoids. The cell lineage inferred by RNA velocity showed that PN and PCN have multiple root cell clusters while WT or PC show relatively simple cell lineage.

Intriguingly, only PCN cells were growing in the allograft experiment with a 60% success rate while PN did not grow at all in the mice.

In the transcriptomic comparison analysis of PCN and PN, PCN showed suppressed gene expression of antigen processing and presentation and increased gene expression of cytokine signaling of the immune system.

PCN highly expresses Cd2 via NF-κB, inducing immune evasion.

Conclusions

Our results suggest that loss of TP53, NOTCH1, and CDKN2A are the minimum drivers for the ESCC not only by regulating cell-autonomous neoplasia but also by remodeling the immune landscape.

References

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2. Gao et al., 2014, Nature Genetics 46, 1097-1102
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