Dissecting Genetic Interactions Initiating Esophageal Squamous Cell Cancer and Remodeling Immune Landscape

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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[1]. 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[2,3,4]. 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[5].

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

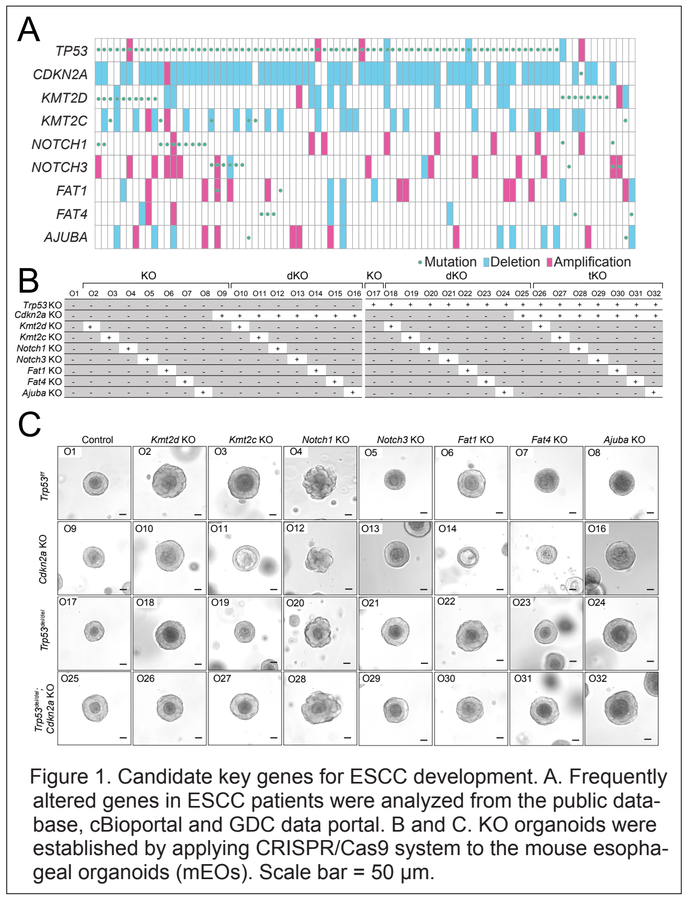

Figure 1. Candidate key genes for ESCC development. A. Frequently altered genes in ESCC patients were analyzed from the public database, cBioportal and GDC data portal. B and C. KO organoids were established by applying CRISPR/Cas9 system to the mouse esophageal organoids (mEOs). Scale bar = 50 μm.

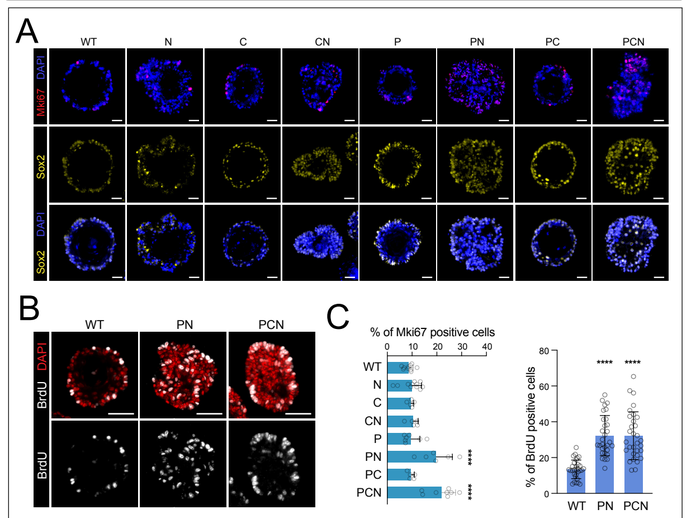

Figure 2. Trp53;Notch1 dKO (PN) and Trp53;Notch1;Cdkn2a tKO (PCN) showed the neoplastic growth in organoid system. A. Mki67 and Sox2 staining in each organoid. Scale bar = 50 μm. B. BrdU incorporation assay showing prolifating cells in WT, PN, and PCN. Scale bar = 20 μm. C. Mki67 and BrdU expression in the organoids were evaluated by calculating the proportion of positive cells to the total cell numbers in each organoid.

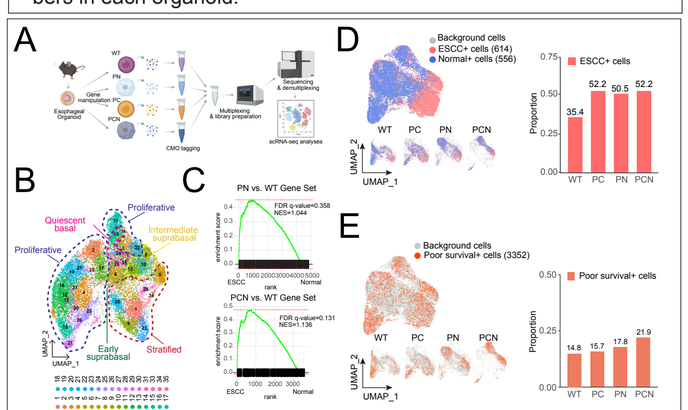

Figure 3. scRNA sequencing of organoid. A. Schematic representation of scRNA-seq. B. Cell clusters and cell type annotation in UMAP. C. GSEA analysis showing ESCC highly expressed genes are enriched in PN and PCN specific genesets. D. Gene expression features of PN and PCN are similar to those of ESCC patients. E. Gene expression feature of PCN showing the most similarity to those of ESCC patients with poor survival.

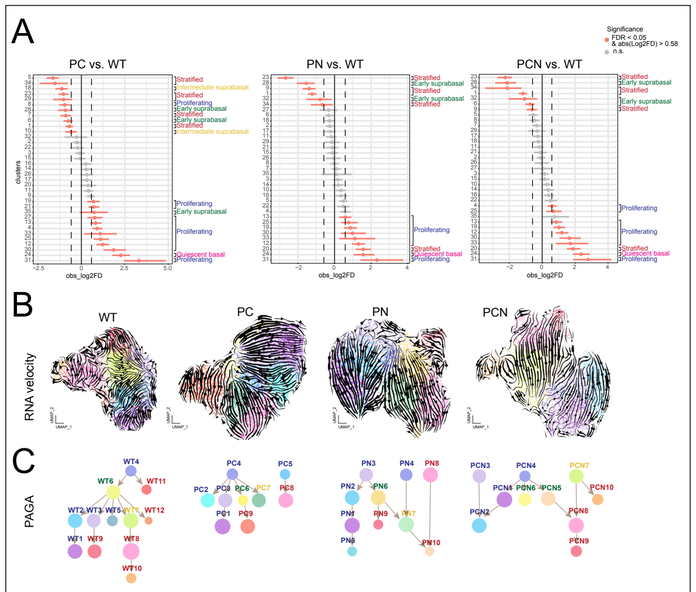

Figure 4. PN and PCN showed more proliferating cells and complex cell lineage compared to WT. A. Cell proportion analysis of each cluster of dataset. B and C. RNA velocity-based cell lineage inference and PAGA analysis showing cell trajectory of each dataset.

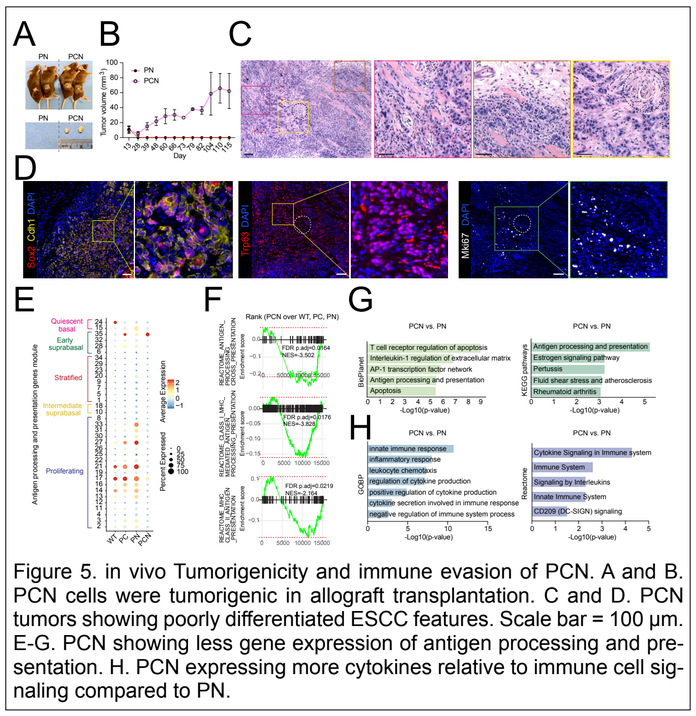

Figure 5. in vivo Tumorigenicity and immune evasion of PCN. A and B. PCN cells were tumorigenic in allograft transplantation. C and D. PCN tumors showing poorly differentiated ESCC features. Scale bar = 100 μm. E-G. PCN showing less gene expression of antigen processing and presentation. H. PCN expressing more cytokines relative to immune cell signaling compared to PN.

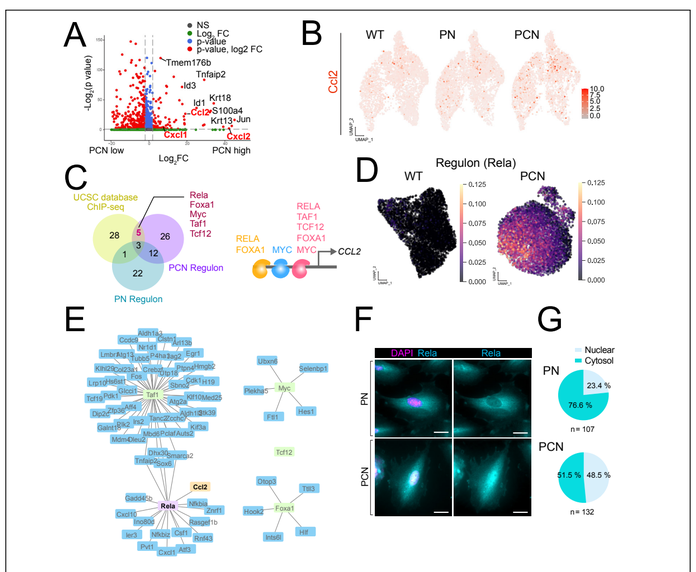

Figure 6. PCN-enriched Rela-Ccl2 axis. A. Volcano plot from DEG of PCN and PN showing chemokines and cytokines highly expressed in PCN. B. Feature plot showing Ccl2 in each dataset from scRNA-seq. C and D. Comparison of Regulon analysis with human Ccl2 promoter ChIP-seq database showing 5 candidate transcription factors. E. iRegulon module analysis showed the Rela-Ccl2 axis. F and G. Cellular localization of Rela is more intensified in the PCN nucleus.

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 % of 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 Ccl2 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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