Identifying Gene Combinations that Drive Epithelial to Mesenchymal Transition using an Inducible Cas12a Knockout Screen

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Metastasis is the spread of cancer to different organs. Metastatic tumors are often fatal and difficult to treat with conventional surgery or drugs[1]. A critical precursor to metastasis is Epithelial-to-mesenchymal transition (EMT). Epithelial cells contain tight junctions and adhere to each other. Mesenchymal cells are mobile cells that can migrate to different places of the body through the circulatory system or other body systems. EMT in cancer is the transition of epithelial cancer cells into a mesenchymal-like state, which leads to the delocalization of cancer to spread throughout the body[2]. These mesenchymal cancer cells may localize to a distant organ where it can undergo mesenchymal to epithelial transition (MET) to form tumors. EMT is often the result of several genes instead of one, the intricacy between them is not well understood[3]. Deeper investigations into the gene network surrounding EMT activation would allow improved knowledge of its mechanism and potential therapies. Thus, more research must be done to identify gene combinations that can cause EMT.

To identify the combinatorial effects of genes that are potentially involved in EMT, we will apply an optical pooled CRISPR-Cas12a system to OE19 cells, an epithelial cancer cell line. Since EMT is a dynamic process, we are creating a novel inducible system for CRISPR-Cas12a by using Cre-Ert2 recombinase to control the timing of genomic editing. This system can be induced with tamoxifen which activates Cre-Ert2 to flip the gene for Cas12a to knockout. We have curated a list of potential genes involved with EMT for knockout. Upon induction we can identify cells undergoing EMT by labeling cells with specific protein markers that differentiate epithelial cells from mesenchymal cells. This inducible system will also be valuable for studying gene expression kinetics and other applications in health and disease research.

References

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