Genome-wide CRISPR Knockout Screen to Identify Genes Whose Loss of Function Can Confer IL-3 Independence

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The process of cell transformation involves the acquisition of biological capabilities which include sustaining proliferative signaling, evading growth suppressors, resisting cell death. Identifying mechanisms by which proto-oncogenes and tumor suppressor genes are deregulated is critical to developing targeted therapy for cancers. The purpose of this study is to identify genes whose loss of function can confer growth factor independence, specifically to the cytokine Interleukin-3 (IL-3). To address this question, we utilized Ba/F3, which is an immortal hematopoietic pre-B cell-line, that relies on the presence of exogenous IL-3 for survival and proliferation. We applied a genome-wide pooled CRISPR knockout library and subjected the transfected cells to IL-3 deprivation. We analyzed the populations of surviving cells using Next-Generation sequencing. These hits were then compared to publicly available human Diffuse Large B-Cell Lymphoma (DLBCL) datasets to identify patient-relevant genetic lesions. As a validation of this screen, we identified PTEN and TP53, which are well-known tumor suppressor genes that have been previously reported to confer IL-3 independence in Ba/F3 cells. Additionally, we found several genes that are key negative regulators for growth promoting pathways downstream of IL-3 such as TSC2, NFAT5, and KSR2. Other genes, such as MTAP, UBE3D, OLIG3, and LOXL2, are frequently deleted in patients, however their functions remain poorly understood. These serve as novel pursuits for future investigation into their potential tumor suppressor activity in lymphomas as well as other cancer types. The identification of these genes and the discovery of their mechanism of action can provide insights to develop precise and effective cancer treatments. Funded by: NIH ES020721 to Taylor Andrews, NCI-R01 to Wei-Xing Zong.