Sequential CRISPR knockout screening reveals cellular genes compensating for Epstein-Barr Virus in Burkitt Lymphoma cell lines

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Epstein Barr Virus (EBV) infects B cells, transforms them into proliferating lymphoblastic cells, and can promote their evolution into lymphomas. While B cell malignancies caused by EBV have been well characterized for their mutational landscapes, how the virus supports the evolution of these lymphomas remains unclear. EBV is not sufficient to cause lymphomas, and viral genes necessary to transform B cells *in vitro* are often no longer expressed in tumor cells. We hypothesize that EBV-positive lymphomas evolve to express fewer viral genes to avoid immune detection. They do so by acquiring cellular mutations that compensate for the loss of viral gene expression. Two sequential approaches have been used to investigate this tumor evolution. First, two early passage Burkitt Lymphoma (BL) cell lines, Oku1 and Sav1, were screened using a CRISPR/Cas9 knockout library to identify cellular genes important for EBV-positive lymphomas. Second, using the remaining pool of CRISPR library screened cells generated in the first approach, we developed a method to evict EBV so that we can identify cellular genes whose loss can compensate for the loss of the virus in tumor cells.

CRISPR/Cas9 knockout screening was performed using the Toronto Knockout Library (TKOv3) [1]. This library contains four distinct, single guide RNAs (sgRNAs) per gene to target 18,053 protein-coding genes using lentiviral vectors. Cells were transduced with lentivirus pools at an MOI of 0.3 to generate single gene knockouts. The relative representations of each sgRNA were determined by collecting genomic DNA and amplifying the sgRNA portions immediately following transduction and at 15- and 30-days post transduction. This assay was performed twice with each of two different EBV-positive cell lines, Oku1, a Wp-restricted BL cell line, and the canonical BL cell line Sav1. Analysis of the conventional CRISPR knockout screen identified many gene knockouts that inhibited tumor cell growth and/or survival; Oku1 had 27 hits and Sav1 had 513 hits that were found to be significantly depleted (Bayes factor, BF>10) by BAGEL2 analysis [2] at 15 days post transduction. Approximately 10% of these hits are unique to these early passage BLs as compared to other BL cell lines such as Raji, Jijoye, Ramos, and BJAB.

Immediately following the CRISPR screen, sgRNAs targeting EBV were delivered using a novel lentiviral vector we designed so that its backbone would be undetected by PCR when preparing samples for sgRNA abundance analysis. This approach allows us to identify cellular genes that when knocked out remove the reliance of BL cells on EBV. The Wp-restricted BL, Oku1, contained more genes that when knocked out enhanced the growth of cells that lost EBV when compared to the canonical BL Sav1, likely because of the greater dependency of Oku1 on EBV for survival [3]. Of particular interest, we discovered a set of genes that when knocked out improves Oku1's growth and/or survival after EBV eviction (FDR<0.05). This set contains several genes involved in epigenetic regulation including SETDB1, WDR82, FAM208A, MPHOSPH8, and PPHLN1. We are validating these, and other candidate genes and their mechanisms to identify cellular genes EBV regulates to drive oncogenesis.

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