CRISPR Pooled Screening Identifies Differential Dependencies on Epigenetic Pathways

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Abstract

It has become clear in the past decade that dysregulation of epigenetic pathways is fundamental to many if not all tumors. Importantly, a number of epigenetic targeted therapies are now being tested in the clinic and are beginning to show promising efficacy. Identification of new targets for oncology therapeutics is critical, and the ideal target should: 1) be effective in a specific indication or genetically defined patient population; and 2) lead to a minimal amount of deleterious side effects in patients. Thus, target identification must be performed in a large number of cell lines to address both objectives and to ensure specific target dependence. However, it remains challenging to identify specific dependencies on epigenetic genes in preclinical models, which may result in part from the need for near complete loss of protein function for this class of enzymes to observe proliferation phenotypes, and this can be difficult to achieve with RNAi reagents. The advent of clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 technology enables the specific and complete knockout of the target protein and allows for observation of proliferation phenotypes that RNAi studies may not be able to achieve. Additionally, the CRISPR-Cas9 system is amenable to pooled cell line screening which permits the quick and accurate examination of proliferation effects across many genes and many cell lines.

To examine the specific dependencies of cell lines on epigenetic pathways, we have used pooled CRISPR-Cas9 screening to interrogate over 600 epigenetically-related genes in a panel of 100 cell lines. The performance of the screen is highly consistent and able to reproduce findings that have been previously reported. We observe that CRISPR pooled screening is highly effective at identifying targets which are known to be required for cell proliferation either in all cell lines or in a genetically-defined subset of cell lines. We also identify new epigenetic-related genes required for the proliferation of almost all the cell lines tested, and have termed these “pan-essential” epigenetic genes. Intriguingly, these pan-essential genes represent members of almost all classes of epigenetic pathways, including histone methyltransferases, histone acetylases and deacetylases, chromatin remodeling factors, regulators of mRNA splicing, DNA helicases and others. This is an important set of genes to identify, as they represent targets which are likely to induce broad clinical toxicity if inhibited in patients, yet may be identified as potential targets in pre-clinical interrogation which does not fully examine proliferation dependencies in a sufficiently broad panel of cell lines.

Importantly, we also identify a variety of epigenetic targets which induce altered proliferation in a subset of cell lines tested. These include epigenetic-related genes from many classes, including histone methyltransferases. Notably, for certain genes, trends are emerging that indicate a specific genetic marker(s) which may predict dependence on these epigenetic pathways. These genes represent highly promising targets for epigenetic therapeutics in a variety of oncology indications.

Results

A. Introduction to Pooled Cell Line Screening

B. Overview of pooled CRISPR and shRNA cell line screening.

C. Sequencing of the barcodes

D. CRISPR pooled screening identifies selective dependencies. A) Sensitivity to knockdown of KRas correlates with mutations in the KRas oncogene. B) Insensitivity to EGLN1 knockdown correlates with VHL mutations in renal cell carcinoma cell lines. C & D) Sensitivity to knockdown of SMARCA2 correlates with low expression of SMARCA4, but not SMARCA4 copy number (CN) or mutations (C). However low expression of both SMARCA2 and SMARCA4 predicts sensitivity to EZH2 KO (C). COXIV is an ovarian cancer cell line with low SMARCA2 and SMARCA4 expression which is sensitive to knockdown of EZH2 (D) but not SMARCA2 (B). NCIH1299 is a lung cell line with low SMARCA2 and SMARCA4 expression which is not sensitive to knockdown of EZH2 (C) but is sensitive to knockdown of SMARCA2 (B), suggesting that although SMARCA2 expression is low it is not absent in this cell line.

Conclusions

The results from our CRISPR pooled screening effort show that CRISPR pooled screening is highly reproducible, but requires multiple sgRNA reagents per gene to ensure accurate results. Screening of many cell lines allows for the identification of pan-essential genes which are required for the growth of most/all cell lines, but also the identification of genes with selective sensitivity which may represent promising drug targets in genetically defined indications. Some classes of genes have many members which are pan-essential, including the helicases and the MLL/Compass complex members, whereas other classes like the HDAC’s have only one or a few members which are pan-essential. Additionally, the CRISPR pooled screen allows for identification of many genes which show selective sensitivity, including some in which sensitivity correlates with mutations or expression.

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