Identification of potential cancer drug targets in prostate, blood, and breast cancer cells using HT RNAi screening with pooled shRNA libraries

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Materials & Methods

A pooled shRNA library consisting of 27,000 shRNA targeting 66,543 human protein-coding genes (DECIIPHER Human Library 1) was generated by cloning a pooled oligonucleotide library (Agilent Technologies) into a lentiviral vector expressing GFP and Puromycin resistance gene. The pooled shRNA library was packaged into individual lentiviral particles for use in the screen.

The following cells of hematopoietic origin were used: K562 (DAK), Jurkat (human T lymphoblast), and Raji (Burkitt's lymphoma). K562 (human promyelocytic leukemia) and Raji (Burkitt's lymphoma) cells were used as controls. Cells were transfected by the transduction method with a minimum of 2×10^7 PFU per transduction per replication. After selection using puromycin, transfected cells were collected at 'early' (18 hrs or 2 days) and one or more 'late' time points (Fig. 2). A preliminary transduction experiment, with the early time point of DECIIPHER human library serving as baseline control. Genetic material was extracted from cell pellets and oligonucleotide sequences amplified by PCR employing a light-cycling machine (USB Laboratories, USA).

The shRNA library was designed to be sequenced via 2x Illumina HiSeq2000, with a resulting read length of 100 bp.

The shRNA library was sequenced as a set of three 27K modules (DECIIPHER Human and Mouse Modules 1 and 2). The DECIIPHER Mouse Module contains 3,418 human and mouse protein-coding genes.

Pathway Analysis and Comparison with Other Published Screens

The primary goal of our study was to identify lethal genes for hematopoietic lineages not critical for survival of other cell types of the human body, and neoplasias arising from these cells.  We sought to identify genes required for survival of hematopoietic cell lines and to develop novel and more specific cancer targets. The “targeted therapy” approach has resulted in a paradigmatic shift in modern cancer treatment.

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