**Pooled RNAi Genetic Screening to Identify Functional Genes and Novel Drug Targets**

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**Abstract**

Results are shown from two RNAi genetic screens with open-source DECODER pooled lentiviral shRNA libraries: one "drop-out" screen to identify genes essential for viability in a panel of leukemic cells, and a second "rescue" screen to identify genes required for FAS induced apoptosis. Both screens found a combination of known and novel signaling pathway and regulatory genes whose functions were confirmed to be required to produce the biological responses. From the viability screen in the panel of leukemic cell lines, subsequent validation using single shRNA-expressing constructs showed that selected synthetic mouse shRNA constructs did in fact lead to cell death when transduced in cells. Analysis of the identified essential genes for leukemic biological interactions revealed non-random clusters of interacting proteins that provide a useful strategy for prioritization of potential targets. Analysis of the lethal combinations indicates redundant, complementatory, and compulsory responses in cancer cells. In the case of the FAS-induced apoptosis, in vitro screening data also enabled us to select targets that provided protection from FAS-induced hepatotoxicity. These results demonstrate that complex pooled shRNA libraries provide a highly efficient, flexible, and cost-effective alternative to array-based RNA expression screening methods for identifying genes regulating biological responses and possible new therapeutic targets.

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**Deciphering the genetic screen:**

The focused pooled lentiviral-based DECODER 27k shRNA library was constructed by cloning a pool of 27,000 shRNA-expressing oligonucleotide inserts (Agilent) into a lentiviral vector expressing TetFP and Puro. Each shRNA vector, expressed from a U6 wild-type promoter, contains an HT sequencing primer binding site and barcode-sequence downstream of the haemin and termination site (T6) for identification ofshRNA constructs via the Illumina G41s and HiSeq 3000. The complete pooled library was packaged into pseudoviruses particles by co-transfection into HDK 2000, with plasmids expressing the lentiviral packaging proteins.

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**Results and Conclusion**

Genome-wide RNAi screening provided an efficient and effective approach to identify unknown modulators of apoptosis. While interesting in itself, these may provide additional insight into the mechanisms of the apoptotic response; this study also demonstrates the potential of this approach for therapeutic development. A single comprehensive pooled shRNA screen was able to identify a number of unknown or underappreciated modulators of apoptosis. Follow-up work on several of the candidates not only validated their importance in FAS-induced apoptosis, but showed that in vitro pool screening results can be successfully translated to an in vivo model and used as a tool to predict in vivo chemical and biological effects for the modeled response.

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