Comparing RNAi and CRISPR Technology for Loss-of-Function Genetic Screens

Paul Diehl, Donato Tedesco, Sylvain Baron, and Alex Chenchik

Over the last decade, RNAi-based loss-of-function screens have proven to be an effective tool to identify essential genes and critical genetic drivers for a host of responses as well as potential drug candidates (see review: Diehl, et al., Drug Discovery Today: Technologies 11: 11-18, 2014). More recently, similar screens using CRISPR technology to knock out, rather than knock down, genetic targets have been shown to be an alternative to RNAi-based screens (Wang, et al., Science 343, 80-84, 2014; Shalem, et al., Science 343, 84–87, 2014). How do these two complementary screening approaches compare?

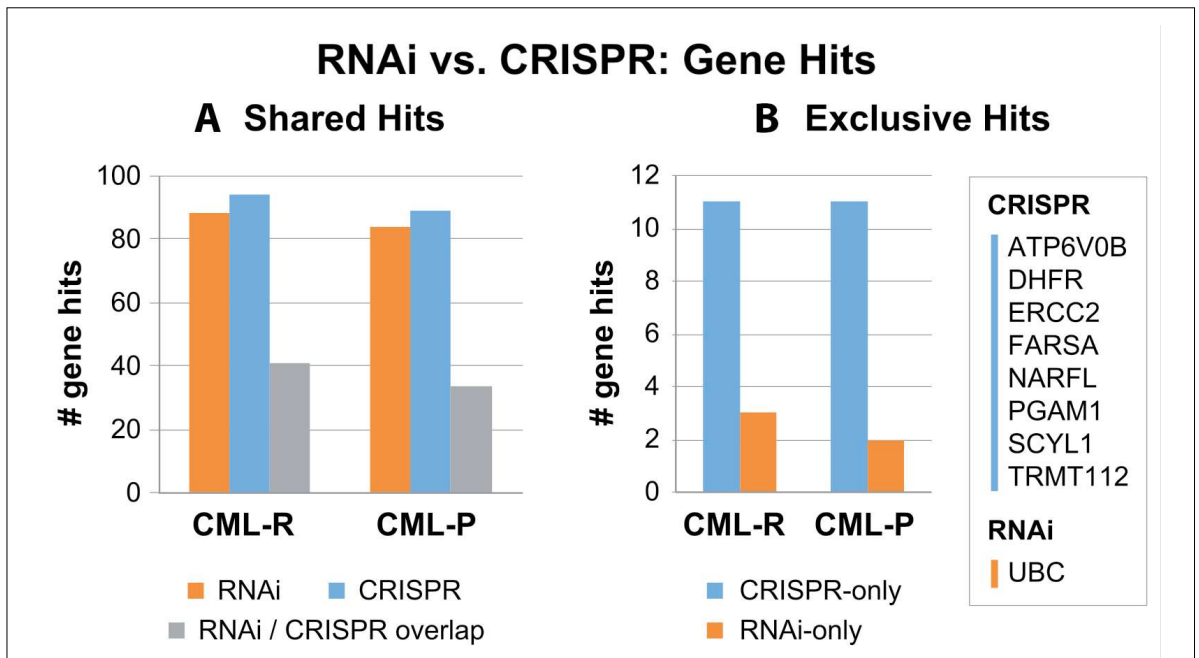

Figure 1. (A) Gene overlap of RNAi and CRISPR screens (grey bar) for the two cell lines. (B) Exclusive hits with gene names listed on the right.

Pooled RNAi and CRISPR Libraries

Cellecta has developed a platform to generate high-quality pooled libraries of lentiviral constructs expressing shRNAs. In partnership with Agilent Technologies, we generate complex pools of superior oligonucleotides that we clone en masse into a lentiviral vector to create libraries of thousands of shRNA expression constructs. These heterogeneous pools of shRNA expressers can then be packaged as VSV-g pseudotyped lentiviral particles, which efficiently transduce virtually any mammalian cell. Using essentially the same approach, we can also easily construct very similar libraries with CRISPR gRNAs.

For our comparison screen, we made a gRNA library that was analogous to Module 1 of our Human Genome-Wide (hGW) shRNA Library, which expresses approximately 50,000 shRNA that target ca. 6,300 human genes. We designed approximately 50,000 gRNA to target the same set of 6,300 genes. Both libraries were packaged as VSV-g pseudotyped particles.

RNAi and CRISPR Screens

CRISPR disrupts gene function in a very different manner than RNAi. CRISPR disrupts a gene when a mistake occurs in the repair of a DNA break it introduces in the targeted gene. RNAi, on the other hand, interferes with the translation of genes by inducing rapid degradation of a gene target's transcript. As a result, shRNA knocks down the expression of a gene's protein product whereas CRISPR gRNAs can completely knock out the gene. Our interest was to look at how these differences might affect the results of genetic screens.

The most straightforward loss-of-function screen identifies essential genes by means of transducing a population of cells with a pooled library of shRNA or gRNA constructs. After a growth period, genomic DNA from the whole population is isolated and the frequency of each integrated shRNA or gRNA lentiviral construct in the population is assessed by next-generation sequencing (NGS). Constructs expressing shRNA or gRNA that are lethal appear underrepresented after growth as compared to their initial representation in the pre-transduced library.

This sort of "dropout viability" screen to identify essential genes is often used to look for genetic susceptibilities in cancer cells. For this reason, we chose to run our test screens on isogenioc tumor cell lines derived from a patient with chronic mylogenous leukemia. One resistant to first and second generation TKi treatment (CML-R) and the other not resistant to TKi treatment (CML-P).

Screening Method

For the screens, each of the cell lines was transduced with 20 million viral particles from each library so that 400 cells on average received each shRNA or gRNA. After three days of growth, cells were selected with puromycin then growth maintained with replating as necessary. Fifty million cells were harvested for each screen at two weeks, three weeks, and four weeks. Barcode identifiers unique to each shRNA or gRNA were amplified by PCR from the genomic DNA and sequenced at greater than 50 million reads per sample.

Results of the Screens

The negative controls in both libraries—shRNA or gRNA that do not target any genes in mammalian cells—did not show any significant changes in representation in either screen in either cell line. The positive controls—shRNA or gRNA that target well-known essential genes—displayed significant depletion in all four screens. We did note higher variability in the shRNA positive controls relative to the gRNA ones, especially at the later time points. Based on these observations, we analyzed the two week time point for the shRNA screens and the three week time point for the gRNA screens.

The clearest difference between two screens was the total number of significantly depleted shRNA as compared to gRNA. About 10% of the shRNA had significant depletion levels, as compared to only about 2% of gRNA. Importantly, the number of genes identified by only one depleted shRNA wasv higher than the genes identified by only one gRNA (~35% vs 5%).

However, when we looked at genes identified by multiple depleted shRNAs or gRNAs, the results of two screens were more similar (*Figure 1A*). The overlap between the top genes identified as essential in each screen was close to 50% with both cell lines. Further, many of the genes that were only found in one or the other screen with the first pass could be identified in the complementary screen with slightly relaxed statistical criteria. Thus, the concordance between the two approaches was very high.

In order to answer the question whether CRISPR or shRNA exclusively identifies certain genes, we looked to see if any targets identified in both cell lines using one technology were statistically indistinguishable from the baseline in the complementary screen. We observed that a set of genes was identified by the CRISPR screens that showed no discernable signal in the shRNA screens (*Figure 1B*). Interestingly, though, only one target in the shRNA screens showed no signal in the gRNA screen.

For the genes found only in the CRISPR screens, most likely, small levels of their products are sufficient for cell viability while complete elimination of them is lethal. The one ubiquitin (UBC) gene found only in the shRNA screens is expressed in multiple tandem copies and has a paralog UBB. Thus, it may be difficult to disrupt production of all forms of the transcript with a single targeted gRNA. This genetic screen suggests that RNAi and CRISPR are complementary technologies with good overlap and that they can be used to confirm each other's results. ■

Cellecta

Paul Diehl
Director, Business Development
pauld@cellecta.com
www.cellecta.com

Contact Cellecta at info@cellecta.com to get more information about these screens.