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CRISPRâ€“Cas9: a new hope for drug discovery

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The repurposing of a primitive adaptive immune response in bacteria, known as CRISPR (clustered regularly interspaced short palindromic repeat), has revolutionised gene editing and provides a new and powerful tool to interrogate gene function on a genome-wide level. Indeed, the contribution of this technology to drug discovery looks, from our current vantage point, to be substantial. The application CRISPR-Cas9 technology to whole genome screening is transforming our ability to perform target identification experiments and to understand complex biological processes, such as drug resistance. Thought to be devoid of the caveats associated with siRNA and shRNA reagents, the hope is that novel targets can be uncovered and rigorously validated using CRISPR-Cas9, and that a pipeline of innovative and validated targets will enter drug discovery programs.

Indels are crucial

CRISPR-Cas9 screens make use of short guide RNAs (sgRNAs) and Cas9 nuclease, two components of the CRISPR machinery in bacteria. The sgRNA, which Cas9 binds to, contains a protospacer adjacent motif (PAM) site and this enables the targeting of Cas9 to the RNA:DNA hybrid. Once bound, the Cas9 nuclease generates DNA double strand breaks precisely 3 base pairs away from the PAM site. These double strand breaks are repaired by non-homologous end joining (NHEJ), which frequently results in small nucleotide insertions and or deletions (indels) that can lead to the interruption of the normal reading frame, thereby disrupting gene function.

Pooling resources

Using lentiviral transduction to deliver both the Cas9 nuclease and sgRNAs into cells, Horizon Discovery has adapted a pool-based screening protocol to examine the effect on cell survival of knocking out thousands of individual genes. The sgRNAs are designed to direct Cas9 to exons at the start of the open reading frame, such that any out-of-frame indels will result in gene disruption. The screen analysis is based on data from cells collected at the start of the screen (3-5 days after transduction) compared with cells collected at the end of a screen that have been exposed to a drug of interest, for example. The next generation sequencing (NGS) data from the initial time point acts as a comparator for the NGS data collected at the

end of the screen, such that sgRNA loss and gain over the time of the screen can be established. We use a dedicated CRISPR-Cas9 screening analysis platform, adapted from the MAGeCK workflow (Li et al., 2014), which enables individual sgRNAs and gene hits to be ranked.

Resistance discovery ahead of the clinic

Many of the initial screens that Horizon Discovery has undertaken for a number of clients from the Pharmaceutical industry have examined mechanisms of drug sensitivity and resistance. Indeed, for our initial proof of concept studies we, like others, examined mechanisms of resistance to vemurafenib, a drug that targets the BRAFV600E activating mutation. The GeCKOv2 genome wide library, which contains 6 guide RNAs against 19,050 genes (Sanjana et al., 2014), was transduced into BRAFV600E mutant A375 melanoma cells and the cells were treated with vemurafenib. A comparison of the abundance of each guide RNA at the start and end of the screen was used to assess whether drop out or enrichment of guide RNAs had occurred over the course of the screen. 228 guides were shown to be >100-fold enriched, with several sgRNAs targeting the same genes. Using our analysis platform, the highest ranking targeted genes (MED12, NF1, CUL3, NF2, TADA2B and TADA1) were those whose loss is known to confer resistance. These findings repeated previously published data (Shalem et al., 2013, Huang et al., 2012, Whittaker et al., 2013).

Interestingly, when the sgRNAs for each of these hits were evaluated individually, it was clear that not all guides performed equally well in the screen. This emphasises that for successful screening, the library composition and complexity are key considerations.

Increasing the odds with a haploid approach

Engineered from KBM7 fibroblasts, eHAP cells are a fully haploid cell line and are particularly suited to CRISPR-Cas9 screens because there is only one copy of any given gene to edit. We used the eHAP cells to examine the mechanisms of resistance to 6-thioguanine (6-TG), a purine antimetabolite that is used in the treatment of leukaemia. The biology of the DNA mismatch repair (MMR) system and factors that mediate resistance to 6-TG have been extensively studied and as such provide an excellent paradigm to test the power of CRISPR-Cas9 resistance screens in haploid cells. eHAP cells were infected with the whole genome GeCKOv2 library and were exposed to either 500nM 6-TG or vehicle control and maintained in culture to allow sgRNA enrichment and depletion to occur. As expected, NGS analysis revealed that sgRNAs targeting MLH1, MSH2 and MSH6, three genes that encode MMR proteins, were enriched in the screen (Branch et al., 1993; de Wind et al., 1995, Abuin et al., 2000, Buermeyer et al., 1999). To validate these targets as 6-TG resistance factors, MLH1 and MSH6 knockout HAP1 cells were generated using a CRISPR-Cas9 approach. These cells were able to proliferate in the presence of 6-TG unlike the parental cell line. Thus, CRISPR-Cas9 approaches were used to both identify the mechanisms of resistance and to validate them.

The future looks bright

CRISPR-Cas9 is a fast moving technology that has been embraced across the board. As discussed above, it has been used to successfully identify mechanisms of drug resistance in whole genome screens, but its applications extend further into animal model generation and genetic therapies. Its use, particularly in humans, is the subject of much debate that looks set to continue for years to come. However, in terms of drug discovery, CRISPR-Cas9 technologies look poised to replace RNAi as a new avenue for more effective drug discovery. Thanks to NGS, we are starting to catalogue the plethora of mutational changes that occur in the genome of any cancer cell. With this knowledge comes potential - novel mutated genes and the proteins that they encode, are candidates for prognostic markers and/or new drug targets. With the ability to rapidly engineer cells (and animal models) using CRISPR-Cas9 technologies, the generation of robust models needed for improved target identification and validation promise to be attainable.