WHITE PAPER

How CRISPR-based functional genomics is transforming drug discovery.

Introduction

One of the biggest challenges in disease research is the ability to assign functional characteristics from genomic sequences. Functional genomics provides valuable insights into the relationships between genotypes and phenotypes using large-scale data from genome-wide omics-based experiments. This approach enables researchers to explore how genes function together in complex pathways, how these pathways are altered in disease states, and how we can modulate these pathways via drug interventions. The past 20 years have seen an emergence of novel technologies that can be used to investigate the links between gene and protein function in a high-throughput manner. These include RNA interference (RNAi) and large-scale mutagenesis screens, as well as expression profiling and protein interaction analyses. More recently, CRISPR-based functional genomics tools have emerged as key players in pre-clinical drug discovery.

Since the initial reports of CRISPR-Cas as a programmable tool for genome modification, the field has experienced an explosion of activity. CRISPR-based tools are now being used in whole genome-scale knock-out screens and technologies have been developed that enable transcriptional regulation, epigenetic modification, and DNA and RNA editing on a whole-genome scale[1,2]. Here we discuss how these CRISPR-based technologies are being applied to the field of drug discovery across multiple stages of the pipeline, from target identification to patient stratification and mechanism of action studies.

Key advantages of CRISPR-based functional genomics tools

- Generate robust screening datasets
- Systematically explore factors that influence a drug response
- Identify patient stratification strategies
- Deeper investigations of gene expression with CRISPRa/i
- CRISPR base editing allows for the assessment of clinically relevant point mutations

Moving from RNAi to CRISPR screens

Historically, RNAi screens have been extensively utilized to identify novel genetic factors that moderate the cellular response to drug treatment. However, RNAi technologies can be prone to off-target effects due to partial complementarity as well as variability in phenotype due to the level of knockdown achieved. The addition of CRISPR-Cas9 to the functional genomic toolbox has enabled the development of screening platforms based on gene knockout rather than RNAi-mediated depletion. Notably, CRISPR-Cas9 screens have been shown to produce more robust data than RNAi because of their greater penetrance. They have also successfully identified moderators of drug response that were undetected by RNAi technologies.

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The first genome-scale CRISPR-Cas9 screens published by the Zhang lab[3] assessed the impact of gene knockout on resistance mechanisms to a therapeutic RAF inhibitor, Vemurafenib. Their experiments identified known and novel genetic factors contributing to the drug treatment outcome. Since then, numerous CRISPR-Cas9 screens have been performed to identify moderators of the cellular response to current therapeutic modalities[4–6].

Improving target identification

Many drug discovery pipelines have failed as a result of poorly validated target identification[7]. CRISPR-mediated functional genomics experiments are now being performed at an industrial scale to generate more robust observations which can guide drug discovery programs. One such study was published by the Garnett lab[8], where they performed CRISPR KO screens targeting 18,000 genes in 324 cell lines derived from 30 different cancer types. By combining the resultant data with the known genetic features of each cell line, they identified over 600 unique targets for potential pharmaceutical intervention.

RNAi-mediated depletion is widely used to identify novel targets and modifiers of drug response in situations where reduced gene expression rather than total knockout is required to generate the desired phenotype. This approach has been enhanced by combining CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) screens to determine how altered gene expression impacts drug sensitivity[2,9,10]. In addition, the advent of CRISPR Quantitative Trait Locus (QTL) mapping of regulatory elements allows researchers to test for correlations between gene expression and drug response[11].

More recently, the development of CRISPR base editors has shown utility for target identification. CRISPR base editors introduce point mutations more efficiently than Cas-mediated homology-directed repair (HDR)[12]. Studies where missense mutations have been introduced in parallel with point mutations designed to induce protein knockout have identified regulators of the cellular response to DNA damage, PARP inhibition, and interferon treatment[13–15].

While identifying the right target is important in the early drug discovery process, the success of clinical trials can be greatly enhanced if you know which patient cohort is most likely to respond favorably to treatment. This patient stratification strategy can be informed by functional genomic approaches. Classically, a drug is screened against a large panel of cell line models harboring a range of genetic perturbations. The drug response is then correlated with the genotypes[16]. Alternatively, the initial target identification can be performed in cell models expressing the disease-associated genetic alterations. A functional genomic approach using CRISPR or RNAi aims to systematically alter the expression of each gene to identify processes or pathways that impact the cell's, and thus potentially a patient's, response to the drug. An early RNAi-based functional genomic approach identified a synthetic lethal interaction between PARP inhibition and BRCA deficiency[17]. This finding has led to the treatment of BRCA1/2-deficient tumors with PARP inhibitors, such as Olaparib[18]. The introduction of CRISPR-based targeting as a screening tool has resulted in an expansion of the range of genetic mutations that can be assessed.

Understanding drug response mechanisms

Prolonged drug treatment often results in the development of acquired resistance. Functional genomic approaches can be used to determine which gene perturbations influence the drug sensitivity. These observations, combined with patient genotyping, can help predict which treatment combinations will lead to better patient prognosis. Base editors can be used to systematically screen single nucleotide variants found in patient cohorts to determine their prognostic value. In a recent study, Hanna et. al. used CRISPR base editing to uncover missense mutations in the MCL1 and PARP genes that confer resistance to BH3 mimetics and sensitivity to PARP inhibitors[13]. They also used a pooled screening approach to generate clinically reported variants and assessed the impact of the missense mutations on the cisplatin and hygromycin response. The results demonstrated that the introduction of missense mutations into many of the genes involved in the DNA damage repair pathway conferred sensitivity to the compounds. These approaches therefore provide clinicians and researchers with biomarkers and an improved understanding of the mechanisms of the drug response.

Determining the mode of action of any drug greatly enhances its successful clinical development. Loss of function screens can be combined with drug response data to investigate the mechanism of action, sensitivity, specificity, and isoform selectivity of a drug[19,20]. Saturated genome editing with CRISPR base editors will significantly increase the level of understanding of drug–target interactions.

Moving from *in vitro* to *in vivo*

All the screening strategies described above utilize *in vitro* cell lines, which do not recapitulate all aspects of *in vivo* tumour biology. Thus, it is necessary to produce more accurate model systems that better represent patient biology. Patient-derived primary cells are an attractive opportunity for a more nuanced research model. Due to limited material availability, this approach is mostly restricted to cells of the immune system that can be extracted from patient blood. The Marson lab recently established a high-throughput CRISPR screening platform in ex vivo human hematopoietic cells for a 77,000 guide library[21].

CRISPR-mediated *ex vivo* editing of primary immune cells represents an attractive opportunity to generate the next generation of cell-based therapeutics. Differentiated induced pluripotent stem cells (iPSCs) have the potential to generate any cell type found in the body with the benefit of being scalable, as they can be stably propagated in culture[22]. iPSCs can be edited with CRISPR, clonally expanded, and then banked for future use. However, both primary cells and iPSCs are sensitive to the double-strand DNA breaks created by CRISPR-Cas9, resulting in reduced cell viability. This provides a barrier to multiplex editing in these model systems. However, CRISPR base editors, which are not dependent upon double strand breaks, present an opportunity to create more complex cell-based therapeutics in these cell types.

Organoids are three-dimensional structures derived from patient or embryonic stem cells that retain many features of their respective organ[23]. Over the past few years, there have been a number of studies reporting genome-wide CRISPR screens in organoids[24–26] or the use of organoids to validate previous CRISPR screen results[27]. Although it is too early to say whether dependencies identified in organoids are significantly better drug targets, the observations that organoids can model clinically relevant drug responses is suggestive of their potential as a preclinical model.

Finally, *in vivo* CRISPR screens can be performed in animal models, which better represent the 3D architecture of tumor biology and the impact of the immune system on tumour growth[28,29]. A number of *in vivo*, genome-wide CRISPR screens have now been performed to identify new immune-regulatory genes that are potential drug targets in cancer[30].

Summary

The addition of CRISPR-based technologies to the functional genomics toolbox has identified many novel opportunities for the drug discovery pipeline. With recent developments such as CRISPR-mediated epigenetic modifications and CRISPR base editors, researchers can now generate more nuanced data. The potential for these perturbations to be introduced into more clinically relevant, preclinical models could help identify novel targets and improve the success rates of subsequent drug discovery programmes.

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Revvity
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
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