A New Era in Functional Genomics Using CRISPR/Cas9 Knockout Screening

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Abstract

In this commentary, we discussed the new exciting progress in CRISPR based screening technology field and highlight recent developments in the area of CRISPR-based functional genomics. High-throughput functional genomics using CRISPR-Cas9 revolutionized our ability to decipher cellular function in health and disease. Despite its limitations, the simplicity and effectiveness of CRISPR/Cas9 based screening, makes an enormous impact on genomic screening and thus scientific discovery.

Keywords: Genome-editing; CRISPR based screening; Genetic screening

Introduction

Genetic screening has been a powerful tool to identify gene function, in particular through studying cellular phenotypes arising from genome-wide perturbations. The main method for genome-wide loss-of-function screening is using short hairpin (sh) RNA or siRNA libraries in order to knock down mRNA transcript levels. More recently developed techniques utilizing Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) genome editing have significantly improved gain- or loss-of-function studies. It is now possible to make much more precise changes to endogenous genes and completely knock out their expression in vitro and in vivo [1-3]. As a powerful genetic tool, CRISPR/Cas9 has been used to study and potentially treat single gene disorders (e.g. sickle cell anemia and β-thalassemia), cardiovascular diseases (e.g. coronary heart disease due to higher LDL cholesterol levels) and HIV infection (e.g. inactivating HIV co-receptors CCR5 and CXCR4) [4,5].

Discussion

In 2014, two seminal publications in Science first demonstrated that CRISPR/Cas9 system can be used as a screening tool for genetic studies [6,7]. They developed genome-scale lentiviral pooled libraries targeting approximately 17,000 and 18,000 human genes (with 5'-6 gRNAs/gene), respectively. Both positive and negative selection screening was successfully carried out with CRISPR pooled library in mammalian cells. Importantly, the CRISPR based screening was demonstrated superior to shRNA screening because of its ability to knock out the genes efficiently. We have recently taken advantage of the genome-scale CRISPR-Cas9 knockout (GeCKO) library developed by the Broad Institute to study the mechanisms underlying FLT3 inhibitor resistance in acute myeloid leukemia (AML) [8]. In our screen, we identified SPRY3, an intracellular inhibitor of FGF signaling, and GSK3, a canonical Wnt signaling antagonist, and demonstrated that re-activation of downstream FGF/Ras/ERK and Wnt signaling as major mechanisms of resistance to the FLT3 inhibitor. In the last four years, numerous CRISPR based pooled genetic screens were performed to study various biological or pathological processes, uncovered mediators of drug resistance, pathogen toxicity, tumor growth/metastasis as well as defined cell-essential genes of the human genome and new roadblocks in reprogramming mouse embryonic fibroblasts etc. A genome-wide CRISPR screen in a mouse model of tumor growth and metastasis was conducted by transducing a CRISPR library into a non-small-cell lung cancer cell line and transplanted cells subcutaneously into immunocompromised mice [9]. Enriched single guide RNAs (sgRNAs) in lung metastases and late stage primary tumors were identified to target a small set of genes, suggesting specific loss-of-function mutations drive tumor growth and metastasis. A similar approach was used to identify tumor suppressor mechanisms of hepatocellular carcinoma as well as new immunotherapy targets [10,11]. More recently, Chow et al. delivered an adenovirus-mediated CRISPR library directly into the mouse brain that conditionally expressed Cas9 through stereotaxic injection to identify functional suppressors in glioblastoma [12].

Conventional pooled CRISPR screenings are limited to analyses of cell-population behavior during the screening process. This limitation was recently overcome through the combination of CRISPR screen with single-cell RNA-seq. The studies described CROP-seq [13], Perturb-seq [14,15], and CRISPR-seq [16]. CRISPR-UMI [17] use the CRISPR-Cas9 system to create up to thousands of genetic perturbations in parallel within a single sample, as with conventional pooled screens. But by using single-cell RNA-seq as readout, the approaches enable the gene knockout and phenotype of each cell to be examined simultaneously. These new methods have already been proved to be a powerful tool to study cellular signaling including the T-cell receptor signaling pathway in Jurkat cells, and mammalian unfolded protein response, the transcriptional program in the bone marrow-derived dendritic cells (BMDC) response to lipopolysaccharide (LPS), mouse embryonic fibroblasts reprogramming as well as regulatory circuits of innate immunity.

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| Representative study reference | Cas9 Protein | gRNA library | Cells | Methods | Scientific Implications |
|-------------------------------|--------------|--------------|-------|---------|-------------------------|
| Wang, et al. 2014, Science    | Dox-inducible WT cas9 | 7,114 genes, 73,151 gRNAs | Human leukemia cell lines (KBM7 and HL60) | Positive and negative selection | Established CRISPR/Cas9 screens as a powerful tool for systematic genetic analysis in mammalian cells |
| Shalem, et al. 2014, Science  | Constitutive WT cas9 | Human GeCKO | Human melanoma cell line (A375) | Positive and negative selection | Demonstrated feasibility and advantages of CRISPR/Cas9 system for pooled genome-scale functional screening |
| Hou, et al. 2017, Cancer Res  | Constitutive WT cas9 | Human GeCKO | Human leukemia cell line (MV4-11) | Positive selection | Identification of genes whose loss confer resistance to drug in AML |
| Zhou, et al. 2014, Science    | WT cas9       | 291 genes, 869 gRNAs | Human cervical carcinoma cell line (Hela) | Positive selection | Identification of genes essential for cell intoxication |
| Park, et al. 2016, Nature Genetics | WT cas9 | 18,543 genes, 187,536 gRNAs | Human CD4+ T cell line (CCR-F-CEM) | Positive selection | Identification of host genes important in facilitating virus infection |
| Hart, et al. 2015, Cell       | WT cas9       | 90K, TKO library | Human colorectal carcinoma cell line (HCT116), colorectal carcinoma cell line (LDL1), glioblastoma cell line (GBM), immortalized retinal epithelial cell line (RPE1), melanoma cell line (A375) | Negative selection | Expansion of the catalog of human cell line fitness genes and identification of genetic vulnerabilities and therapeutic targets |
| Tzalepis, et al. 2016, Cell Reports | WT cas9 | 18,010 genes, 90,709 gRNAs | Human AML cell lines (MOLM-13, MOLV-11, HL-60, OCI-AML2, OCI-AML3) | Negative selection | Identification of genetic vulnerabilities and therapeutic targets |
| Arroyo, et al. 2016, Cell Metabolism | WT cas9 | 18,335 genes, 74,687 gRNAs | Human CML cell line (K562) | Death screening | Genetic analysis using dead cells |
| Chen, et al. 2015, Cell       | WT cas9       | Mouse GeCKO | Mouse lung cancer cell line (KPD) | Mutated cells were subcutaneously injected into immunocompromised NuNu mice | Providing a road map for in vivo screening |
| Song, et al. 2017, Gastroenterology | WT cas9 | Mouse GeCKO | Mouse embryonic liver progenitor cell | Mutated cells were subcutaneously injected into immunocompromised NuNu mice | In vivo CRISPR-based genetic screening in tumor models |
| Manguso, et al. 2017, Nature  | WT cas9       | Mouse TSG  | Mouse melanoma cell line (B16) | Mutated cells were subcutaneously injected into mice treated with immunotherapy | In vivo CRISPR-based genetic screening in tumor models |
| Chow, et al. 2017, Nat Neurosci | Conditional expression | 56 genes, 288 gRNAs | Mouse primary astrocyte | Mutated cells were stereotaxically injected into the mouse brain | In vivo CRISPR-based genetic screening in tumor models |
| Dixit, et al. 2016, Cell      | WT cas9       | 24 transcription factors (67 gRNAs) | Mouse bone marrow derived dendritic cells | Cells were stimulated with LPS in 7 days after infection | Dissecting the transcriptional program in the BMDC response to LPS |
| Adamson, et al. 2016, Cell    | WT cas9       | 10 transcription factors (46 gRNAs) | Human CML cell line (K562) stably expressing Cas9 | Cells were stimulated with LPS in 7 days after infection | Global transcriptional modules predict individual TF functions |
| Jaitin, et al. 2016, Cell     | dCas9         | 9 three-guide vectors, 91 sgRNAs | Human CML cell line (K562) stably expressing dCas9-KRAB | Treatment of 4 mg/mL tunicamycin for 6 hrs | Revealing bifurcated UPR within a population and allows unbiased discovery of UPR-controlled genes |
| Datlinger, et al. 2017, Nat Methods | WT cas9-GFP | 57 gRNAs targeting 22 genes | CD11c+ myeloid cells sorted from Cas9-GFP transgenic mice | Cells were treated with lipopolysaccharide (LPS) | Rewiring of regulatory circuits in myeloid cells |
| Michils, et al. 2017, Nat Methods | WT cas9-GFP | 26,514 guides targeting 6,560 genes | Mouse embryonic fibroblasts (MEFs) | Positive selection screen | Identifying new roadblocks of cellular reprogramming |

Table 1: Representative CRISPR based screenings in vitro and in vivo and combined with single cell RNA-seq.

Note: Dox: Doxycycline; GeCKO: Genome-scale CRISPR-Cas9 knockOut library; sgRNA: Single guide RNA; LPS: Lipopolysaccharide.
Conclusion

Although CRISPR based screening has been reported to perform better with low noise, minimal off-target effects and experimental consistency, compared to knock down approaches using CRISPRi and shRNA [18], the application of the approach has its own limitations. The Cas9/gRNA does not always lead to knockout as the indels could be in-frame mutations, thereby keeping the gene function intact. Additionally, several studies have shown that the correlation between cellular lethality and the number of DNA double strand breaks (DSBs) in a cell, independent of the gene being targeted. Thus, CRISPR knockout based screens can identify false-positive hits for highly amplified genomic regions, including non-expressed genes [19,20].

Representations of the in vitro and in vivo screenings up to date are summarized in Table 1. Taken together, high-throughput functional genomics using CRISPR-Cas9 revolutionized our ability to decipher cellular function in health and disease. Despite its limitations, the simplicity and effectiveness of CRISPR/Cas9 based screening, promise many exciting new applications in the coming years.

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