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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 an 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 adeno-associated virus (AAV)-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
In vitro	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	Constituitive WT cas9	Human GeCKO	Human melanoma cell line (A375) andstem cell line (HUES62)	Positive and negative selection	Demonstrated feasisbility and advantages of CRISPR/Cas9 system for pooled genome-scale functional screening
	Hou, et al. 2017, Cancer Res	Constituitive 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 (CCRF-CEM)	Positive selection	Identification of host genes important in facililtating virus infection
	Hart, et al. 2015, Cell	WT cas9	90K, TKO library	Human colorectal carcinoma cell line (HCT116), colorectal carcinoma cell line (DLD1), 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
	Tzelepis, et al. 2016, Cell Reports	WT cas9	18,010 genes, 90,709 gRNAs	Human AML cell lines (MOLM-13, MV4-11, 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
In vivo	Chen, et al. 2015, Cell	WT cas9	Mouse GeCKO	Mouse lung cancer cell line (KPD)	Mutated cells were subcutaneously injected into immunocompromised Nu/Nu 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 Nu/Nu 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 WT cas9 expression	56 genes, 288 gRNAs	Mouse primary astrocyte	Mutated cells were sterotaxically injected into the mice brain	*In vivo* CRISPR-based genetic screening in tumor models
Single-Cell CRISPR Screening	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
		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
	Adamson, 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
	Jaitin, et al. 2016, Cell	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
			A pool of Cebpb, Irf8, Rela, Stat1, Stat2 and two control gRNAs	Mouse CD11c+ myeloid cells sorted from Cas9-GFP transgenic mice	Cells were treated with LPS after 7 days following transplantation	Uncovering the complexity of myeloid regulatory circuits in immune niches *in vivo*
	Datlinger, et al. 2017, Nat Methods	WT cas9	TCR-related 87 gRNAs (29 genes)	Human T-ALL cell line (jurkat) stably expressing Cas9	T-cell -receptor induction	Facilitating large screens by providing a vector that makes the guide RNA itself readable
			Non-targeting 20 gRNAs			
			Essential 9 gRNAs			
	Michils, et al. 2017, Nat Methods	Dox-inducible Cas9	26,514 guides targeting 6,560 genes	Mouse embryonic fibroblasts (MEFs)	Positive selection screen	Identifying new roadblocks of cellular reprogramming

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

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

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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