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Current Limitations in CRISPR/Cas9 Cancer Screening

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Abstract

CRISPR/Cas9 screening techniques are a relatively new application being used in the field of genomic cancer research. This new type of screening allows for the genomic identification of oncogenes in presenting cancers, and has proven to be quite effective in doing so. Two primary ways of CRISPR/Cas9 cancer screening are nuclease function–based and transcription regulation-based, each of these is unique in respect to their effectiveness and limitations. It was the goal of this review to assess these limitations to determine where/if the methods could be improved.

Keywords

CRISPR/Cas9, oncogene, cancer, nuclease, transcription

Introduction

Through a variety of new and developing methods, the field of functional genomics is providing substantial insights into the human genome. One of the more promising new methods being used, specifically to identify the function of unique genes in cancers, is CRISPR/Cas9 knockout screening. CRISPR/Cas9 is a molecular technique that allows for the editing of DNA (removal or addition), via the Cas9 enzyme, which cuts the DNA strand where targeted. Such targeting can be done with the incorporation of a small guiding RNA (sgRNA) sequence complementary to the DNA that is to be manipulated. With this method, scientists can knockout particular genes, and by assessing the function of the targeted components after knockouts are implemented can determine the function of the genes in question. The generation of gene knockouts is a cornerstone of functional genomics research. In very recent years, over a very short amount of time, the application of CRISPR technology has led to the rapid development of pooled-library CRISPR knockout screens in mammalian cells for functional genomics and identification of cancer vulnerabilities (4). By combining these insights along with growing knockout databases, it is possible that these screenings will provide new information into the components of particular cancers, providing researchers with the details necessary to develop more targeted and effective therapies. There is much promise in this screening method, however, these techniques are limited by variable efficiency and cumbersome assembly, and many sgRNAs have wide ranging modes of

activity (2). This review will seek to address some of the limitations in sgRNA efficiency and possible solutions to these issues.

CRISPR/Cas9 Nuclease Function–Based Loss-of-Function Screening

One common method for cancer screening with CRISPR is function-based loss-of-function screening. The goal of this implementation of the method is as it sounds, to produce a phenotypic loss-of-function in the model, and to then assess the resulting loss-of-function in said model to determine the function of the targeted genes. This, as mentioned in the introduction, is done with complementary sgRNAs, they are selected to remove a specific gene or introduce a loss-of-function mutation into the gene in order to knockout its expression in the system. Once this is completed, the resulting changes in the phenotype of the cells or model can be assessed to determine the function that the targeted gene previously had. This method is particularly useful in the identification of cellular factors in oncogenesis or the prevention thereof, in which the knockout of a certain gene can result in tumor growth or metastasis in a healthy model (3,5) or can result in an increased sensitivity to an inhibitor in a cancer model (1). A promising example of this screening was done in a study conducted by Kiessling, Michael K et al (2016). In their study they took mutant cell lines possessing two known oncogenes, with the use of an sgRNA library they then targeted drivers of oncogenesis for knockout. Upon this knockout they found that each of the cell lines were more susceptible to treatment with specific kinase inhibitors, demonstrating that the knockout took effect in the targeted oncogenes (1).

CRISPR/Cas9 Transcription Regulation–Based Screening

Transcription regulation-based screening is an alternative method to the aforementioned nuclease function based screening. This application of CRISPR/Cas9 is targeted at specific transcription regulating domains, as its name suggest, to induce changes in a system. Focusing on transcription targeted approach, the methods of screening are much like the nuclease function based screening; observing a loss or gain of function change in the system following the introduction of the sgRNA/Cas9. However, transcription promoter Cas9 possesses a significant advantage over nuclease Cas9, in that it does not require the permanent destruction of the gene being screened. Transcription promoter Cas9 allows for the suppression or promotion of gene expression, and at least a partial reversal of the induced effect, without 'cutting' the gene itself (11). This is advantageous for a couple reasons, first, the ability to reverse the induced changes in expression allows for more certain deduction of the function of the gene in question, and it also allows for researchers to work within the same system for multiple different screenings without killing or destroying their models. Beyond these key differences the transcription regulation application proceeds as the nuclease one does, once a gene's expression is changed in the model, the model can be reassessed to identify what has changed in its phenotype, and it can thus be deduced that the phenotypic changes observed are a result of the genetic changes caused by the introduction of the Cas9, demonstrating the function and/pathway of the gene in question.

Limitations in CRISPR/Cas9 Nuclease Function–Based Loss-of-Function Screening

Though both of these screening methods have proven to be effective in certain conditions, there still exist many difficulties in increasing their efficiency and accuracy. Beyond the general challenges in long-term cultures of primary human cells, this screening method is also difficult in regards to introducing the exogenous DNA into multiple cell types in a living system. However, this has been overcome in some transgenic mice that have Cas9 engineered into their genome, of course this is not an option in humans, so overcoming the culturing challenges in human cells is likely the only current course of action for improvement on this front (13). Additionally, false positives are also common if the sgRNA match at too many points in the genome, though this can likely be overcame with more stringent sgRNA design, thorough screening scrutiny, and better sgRNA libraries (9). With these limitations in mind, CRISPR/Cas9 screening techniques can be improved and very likely prove to be a significant factor in our genomic understanding of cancer.

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