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LETTER TO THE EDITOR

Utilizing CRISPR as a Novel Tool for the Induction of Cell Reprogramming

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Researchers can now target specific DNA sequences and easily modify them thanks to recent developments in CRISPR technology, enabling genome manipulation with unmatched precision. Furthermore, cell reprogramming is one of the most fascinating fields in which CRISPR-based techniques are being used. Nowadays, without using embryonic stem cells, scientists can change one type of cell into another by inserting particular genetic alterations. This has significant implications for regenerative medicine since it enables the creation of transplantable cell lines that are patient specific.

In 2014, Chakraborty et al. developed a CRISPR/Cas9-based system for reprogramming cell lineage specification. They used a synthetic transcription factor (sTF) approach where they engineered transcription factors (TFs), by using the CRISPR/Cas9 system to activate endogenous genes that are specific to the desired lineage. The sTFs were designed to recognize a specific DNA sequence and recruit the transcriptional machinery to activate gene expression. They demonstrated the feasibility of this approach by reprogramming mouse embryonic fibroblasts (MEFs) into induced motor neurons (iMNs) and induced hepatocytes (iHeps). The reprogramming efficiency was improved by adding small molecules and growth factors that enhance cell survival and proliferation. The authors also demonstrated that this approach can be used for lineage conversion of human cells by reprogramming human fibroblasts into iMNs [1].

Jiang et al. induced the expression of four transcription factors known to be crucial in cardiovascular development: Gata4, Mef2c, Tbx5, and Nkx2.5 using a CRISPR activation (CRISPRa) system termed SAM (Synergistic Activation Mediator). SAM is a modified CRISPR-Cas9 system that triggers the expression of genes by fusing the dCas9 protein with a transcriptional activation domain. They used a lentiviral vector to introduce the SAM system

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into mouse fibroblasts, and after that they looked for cells that had successfully turned on the four targeted genes. To confirm that the reprogrammed cells were indeed cardiovascular progenitor cells, a range of molecular and functional experiments were used to further characterization of the cells. The transplanted reprogrammed cells were then seen to considerably enhance heart function and lessen scar size in myocardial infarction mice models. Finally, the scientists discovered that the reprogrammed cells could develop into several types of cardiovascular cells in vivo [2].

In a different work, Jalil et al. used a lentiviral vector to deliver the Cas9 enzyme and a sgRNA (single guide RNA) targeting the SOX2 gene to the patient cells. In order to apply base editing and reprogramming in patient cells simultaneously, they used CRISPR-Cas9 and piggyBac transposon technology. Using a lentiviral vector, the researchers introduced a Cas9 enzyme and a sgRNA targeting the SOX2 gene to patient cells. Due to this, the SOX2 gene was disrupted, which is required for sustaining the fibroblast cell fate. The Yamanaka factors (OCT4, SOX2, KLF4, and c-MYC) were then introduced to the cells via a piggyBac transposon vector. The researchers also applied a novel base editing technique known as "transient HITI" (homology-independent targeted integration), which enables base editing and the simultaneous integration of a new gene into the genome of the host cell. In this instance, a GFP reporter gene was inserted into the iPSCs' AAVS1 safe harbor locus via transient HITI. The research demonstrated that this method effectively converted patient fibroblasts into iPSCs that expressed GFP, with a conversion efficiency of up to 10% [3]. In this study, iPSCs were created from human fibroblasts using CRISPRa. The researchers created CRISPRa constructs to activate the endogenous expression of OSKM genes in order to produce iPSCs with a lentiviral delivery method. Moreover, only after 12 days of culture, the researchers discovered that the CRISPRa-mediated reprogramming procedure was effective. It was discovered that the generated iPSCs displayed typical pluripotent characteristics, such as the capacity to develop into distinct cell types and the expression of pluripotency markers [4].

Shakirova et al. reviewed the use of CRISPR/Cas9-based transcriptional regulation systems in cell reprogramming. Some examples of this transcriptional regulation systems in cell reprogramming are: CRISPRa, CRISPR interference (CRISPRi), Epigenetic modifiers to introduce epigenetic modifications like DNA methylation or histone acetylation to reprogram cells, Genome editing to introduce precise genetic modifications to reprogram cells, such as deleting or inserting specific genes or mutations, and Synthetic gene circuits for create synthetic gene circuits that allow for precise control of gene expression and cell fate [5].

The developments in CRISPR gene editing tools have been especially beneficial for the field of cell reprogramming, where scientists are able to change one kind of cell into another without the use of embryonic stem cells by employing CRISPR-based technologies. Recent studies have shown that fibroblasts can be successfully reprogrammed into cardiovascular progenitor cells and induced pluripotent stem cells, and that tumor cells can be successfully engineered in situ using CRISPR-based techniques for cancer immunotherapy. The potential advantages of cell reprogramming, such as the capacity to create cells with desired properties or activities for medical applications, are probably the driving force behind these investigations.

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