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Leveraging hiPSCs and CRISPR/Cas9 for Advancements in Medical Research and Drug Discovery

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A B S T R A C T

The constant search for better resource in medical research assists in acting, constructing, and enhancing the approaches in the identification of therapeutic goals, diagnostic signs, and treatments. The purpose of this paper is to review comprehensive literature on the application of gene editing such as CRISPR/Cas9 in hiPSCs. hiPSCs are derived from skin hair, urine, blood and so on and therefore when compared to ES cells, the former are easier to derive since they are derived from embryonic stem cells that are still regarded as unethical. Thus, due to the ability of hiPSCs to be virtually identical copies of the human pathology, they can contribute to diseases studies, drug creation, and individualized treatment plans. HiPSCs are cultured to show the diseases' processes and the effect of drugs when it is brought into a three-dimensional structure that resembles the tissues. Regarding the principles important for the current subject, this review targets hiPSCs' utility for the reparative process of tissues, stimulating regenerative repair, and immunological rejection problems. CRISPR/Cas9 is one of the most efficient and versatile technique to make required alteration in genes of a organism due to its precision. HiPSC combined with CRISPR/Cas9 make it possible to model genetic diseases and therapeutic points as well as correct mutations. These integrations of nanorobots have made drug screening possible today, find new ways in treating diseases and enhancing the cellular therapy. However, the following are some of the shortcomings; the efficiency of reprogramming hiPSCs; inducing aging for diseases, which are normally developed in adulthood and possibly, some cones with gene editing. According to these studies, it is anticipated that the above technologies will be brought to new levels of development, the reprogramming techniques will be improved, and new approaches for using CRISPR/Cas9 in treating the aforementioned diseases will be identified. This review also emphasizes the significance of hiPSCs and CRISPR/Cas9 system in pharmaceutical and medical research institutions and notes that research using these are rapidly ongoing and providing new medicines and therapies.

Keywords: CRISPR/Cas9, Drug discovery, Gene editing, Personalized medicine, Zinc finger nucleases (ZFNs), CRISPR RNA (crRNA).

1. Introduction

The constant hunt for resources that can enhance and expedite the completion of tests has long been a hallmark of advances in medical research. intended to identify the factors influencing an illness, ultimately identifying therapeutic targets and medications, as well as prognostic and diagnostic factors. These goals are frequently achieved by combining important discoveries, as is the case with the combination of the most recent gene editing methods with the technology able to reprogram somatic cells to become pluripotent. Induced pluripotent stem cells, oriPSCs, have shown a lot of promise since their discovery [1]. One such possibility is the ability to allay worries about safety and ethics surrounding the use of embryonic stem cells. Currently, it is more practical to obtain human iPSCs (hiPSCs) thanks to the opportunity to access human cell lines by minimally invasive techniques such skin punch biopsy, hair, urine, or blood samples, as well as the collection of chorionic villus and amniotic fluid samples [2–5].

As a result, these cells are now employed in several research using both healthy and sick cell lines obtained from donors. As a matter of fact, hiPSCs have the potential to display characteristics that are almost identical to human pathology. As such, they may serve as a more predictive model for disease than the animal- or tumor cell-derived cells that are currently on the market [6]. When cultivated in circumstances that replicate tissue architecture in multicellular spheroids or organoids, hiPSCs can mimic human organs and their microenvironment, including physiology, pathology, and pharmacological response [7].When combined with the new information regarding the paracrine effects of hiPSCs [8], these characteristics make hiPSCs the perfect option for replacing damaged tissues following cellular transplantation or for promoting endogenous regenerative repair. They also help to preserve the patient's genetic background and reduce immunological rejections, all of which contribute to the goal of more individualized disease treatment [8, 9].Recently, the usage of hiPSCs has been closely linked to the application of gene editing, which targets the disease-causing gene in order to perform drug screening, further explore the pathophysiology, and enhance the possibility for cell therapy [10].Clustered, regularly interspaced, short palindromic repeats (CRISPR) and CRISPR-associated protein (CRISPR/Cas9) really constitute the most potent gene editing tool. This is arguably one of the most

potent and adaptable pieces of technology available for epigenetic modification as well as transcriptional control and gene editing. Scientific research will be significantly impacted by its assembly using hiPSCs [11].

This review's objective is to provide an overview of the critical roles that hiPSCs have played in pharmaceutical and medical research alongside the use of the CRISPR/Cas9 system, with a particular emphasis on how quickly these cells can be used to investigate novel medications and treatment options (Fig. 1).

2. Using hiPSCs as a tool for drug discovery

Utilizing hiPSCs to create a disease model has been utilized to evaluate multiple potential medications for various pathologies shortly after the reprogramming technology became widely available. This quickens the procedure that leads to increasing the identification of potential targets and medications, as well as selecting and stratifying trial participants in an optimal manner, with regard to the therapeutic application of a specific molecule, particularly if it has been previously approved for the treatment of other diseases [11]. While hiPSCs remain a viable technique for the successful reproduction of monogenic diseases, their applicability in sporadic disorders, where environmental factors may play a role in the de novo mutation's onset, may be limited [9, 12]. The reproduction of late-onset disorders presents another difficulty since differentiated cells derived from hiPSCs have fetal-like characteristics, necessitating the induction of cellular aging [13, 14]. Additionally, during the reprogramming process, factors related to the culture conditions may have an impact on the epigenetic status of specific clones of hiPSCs [12].

In addition to overcoming genetic background differences between patients and control hiPSCs, the opportunity to obtain an isogenic cell line of hiPSCs guarantees the establishment of a genetically defined condition. Other variables related to age or sex are also eliminated. This allows for the confirmation of drug screen results using a wide variety of controls, including wild type (WT)-hiPSCs, patient-hiPSCs, and corrected-patient-hiPSCs .

In order to model familial dysautonomia (FD, OMIM #223900), a peripheral neuropathy accompanied by the degeneration of autonomic and sensory neurons brought on by a point mutation in IkB kinase complex associated protein (IKBKAP8) that results in a tissue-specific splicing defect, the first large-scale drug screening using hiPSCs was carried out in 2009. Three crucial FD factors were discovered by Lee and colleagues [15] using a hiPSC illness model.

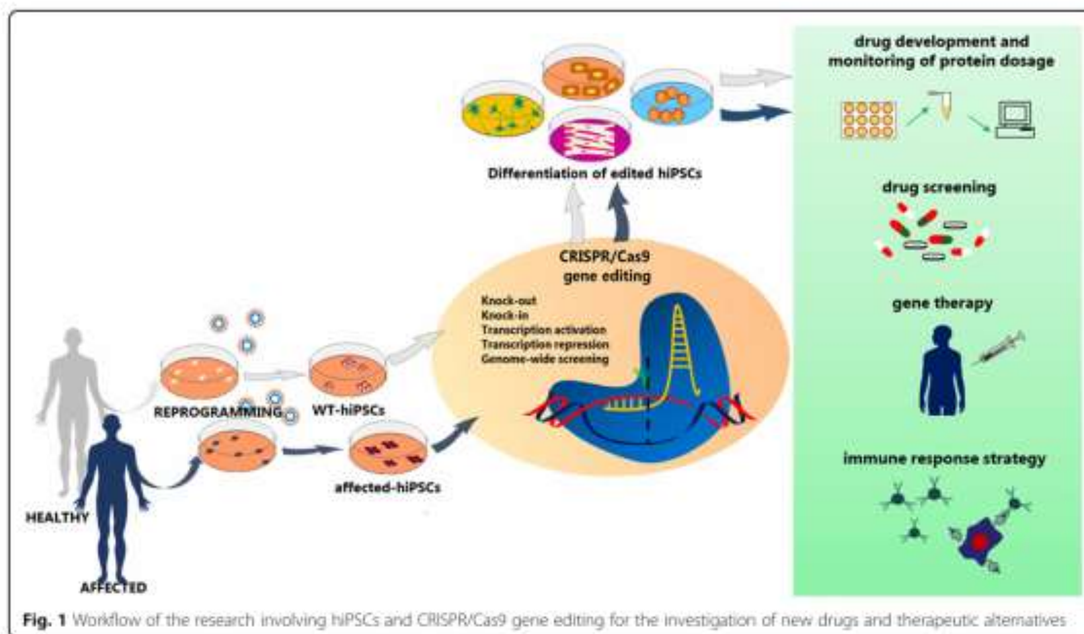

Fig. 1 Workflow of the research involving hiPSCs and CRISPR/Cas9 gene editing for the investigation of new drugs and therapeutic alternatives

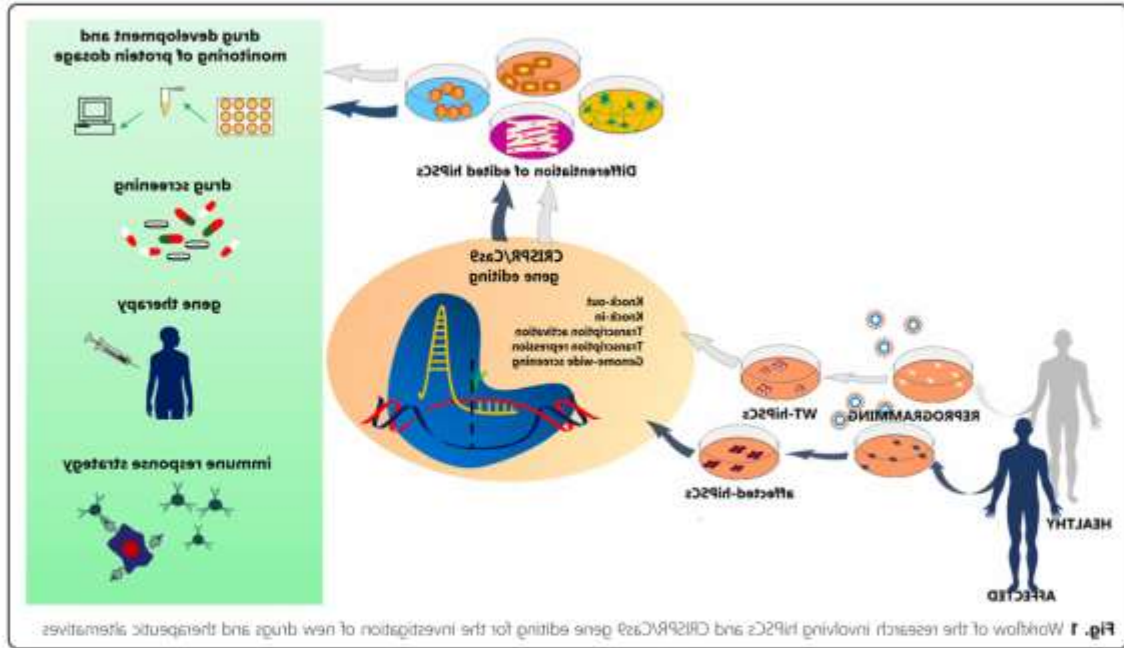

Fig.1.Workflow of the research involving hiPSCs and CRISPR/Cas9 gene editing for the investigation of new drugs and therapeutic alternatives.

After testing a few potential medications that might have affected the characteristics observed, they found that the plant hormone kinetin could lessen the pathological phenotype . Since the examination of several compounds for serious disorders was made possible by this cellular technique, numerous drug screening studies have been carried out onhiPSCs, for evaluating the toxicity and effectiveness of various substances. summarizes some of the most recent drug testing conducted on hiPSCs.

Recently, a clinical trial was initiated to evaluate the potential therapeutic benefit of bosutinib, an inhibitor of Src/c-Abl [16], as a molecular target treatment for amyotrophic lateral sclerosis (ALS, OMIM #105400). Cancer therapy is thought to target the tyrosine kinases Src and Abl, which are linked to angiogenesis, apoptosis, and proliferation [17]. Certain brain-penetrant tyrosine kinase inhibitors have been shown in many investigations to mitigate the phenotype of neurodegenerative illnesses linked to protein aggregates [18,19]. Using motor neurons produced from ALS patient-derived hiPSCs, a pharmacological phenotypic screening of available Src/c-Abl inhibitors was carried out prior to initiating the clinical study [20]. Since bosutinib had already received FDA approval for the treatment of chronic myelogenous leukemia (CML, OMIM #608232), this cutting-edge research led to the repurposing of a medication using hiPSCs [21]. The aforementioned experiment will assess the safety, tolerability, and efficacy of bosutinib in relation to the physical conditions of patients with ALS to date, particularly given that this medication may have certain side effects [22]. In reality, a lot of work is being done to reduce the expenses associated with medication development and expedite this process. This includes testing new drugs to ensure repeatability and reliability in the results of drug therapy, as well as looking for innovative methods that enable the prediction of drug side effects . All of these characteristics should be present in the ideal device, without ignoring genetic and epigenetic variations, environmental influences, or other circumstances that might encourage a rise in the complexity of pharmaceutical testing, such as potential drug-drug interactions [23].

Nowadays, the Organ-On-a-Chip (OOC) technology is the center of attention in order to address these needs. This is a three- or two-dimensional designed biomaterials microfluidic system with an extracellular matrix that allows for the replication and tracking of human cell behavior, from migration and adhesion to replication and differentiation . The OOC is made up of a variety of microfluidic channels that periodically and controllably perfuse biological fluids containing nutrition, biological components, and medications . Moreover, it might be paired with an automated microdevice-monitoring element, allowing for repeated assessments of physiological parameters to assess the toxicity and effects of medications. HiPSCs might be used to engineer the

OOC, giving rise to the possibility of creating a customized drug-testing platform that effectively absorbs human pathology and is tailored to the individual patient [24]. Nevertheless, there are a few limitations to the OOC technology, such as the difficulties in fabricating the device technically and the high expense and insufficiency of obtained human resources. Achieving appropriate human tissue development and differentiation is crucial since the device should also enable functional organ reproduction. This might be dangerous since using specific differentiation reagents and growth factors that are needed for a certain cell type could harm other cells [25].

The fact that hiPSCs can be used for both phenotypic and target-based screening is one of their advantages. Over the past few years, hiPSCs have played a major role in the reevaluation of phenotypic screens, primarily because they make it simple to access a variety of disease-related cell types, particularly those that are difficult to get. For instance, the inability to attract sufficient neuronal cell types has historically impeded phenotypic screening for pain studies [26]. It is now possible to generate sensory neurons from hiPSCs and use them to create a functional neuronal excitability assay that will be used to assess the phenotypic effects of a small fraction of targeted validation compounds [27]. Lately, high-throughput screening (HTS) has been used to screen cortical organoids using a 3D human stem cell platform, thanks to the creation of hiPSCs . Serum-Free Embryoid Bodies (SFEBs) were produced by assembling the reprogramming technology using high content imaging, which allowed for the analysis of neurite outgrowth and cellular composition. In this manner, multi-electrode array analysis and neurite morphology detection were performed . This strategy proved to be a good one for overcoming the experimental variability that is typical in 3D cultures; this work establishes the combination of SFEBs and HTS as the standard for phenotypic drug screening [28].

3.CRISPR/Cas9 system: The newest gene-editing technique

Gene editing serves as a technique for genome modification, enabling the insertion, deletion, or correction of genetic material into a particular DNA sequence. These days, the tools accessible to Unprecedented simplicity and accuracy in editing DNA are offered. This translates to the potential to discover the mutations responsible for the illness phenotype by either introducing the suspected cause lesions into cells obtained from healthy persons or repairing them in patient-derived cells . When genomic alterations were made in yeast and mice during the 1970s and 1980s, a number of techniques were investigated to modify a particular DNA region utilizing a gene target strategy based on the homologous recombination (HR) process [29]. The effectiveness of gene editing has grown recently, suggesting that the ability to cause a targeted DNA double strand break (DSB) in the desired sequence has been acquired. Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9 are, in fact, the three primary methods that can cause double-strand breaks (DSBs). High target specificity, usability, and efficiency characterize each of these technologies . Their potential has been shown in naturally occurring biological processes, and a nuclease is in charge of their action .Zinc finger DNA binding domains, which were first discovered in sequence-specific eukaryotic transcription factors, are essentially what make up zinc finger DNA binding domains (ZFNs) . These domains are coupled with DNA-cleavage domains from bacterial proteins. Their purpose is to identify triplets of three to six nucleotides, frequently involving the interaction of two or more zinc finger proteins [30]. The three primary components of ZFNs-derived genomic modification are the cleavage, the bond to the target sequence, and the genomic change brought about by the induction of endogenous DNA repair [31]. The restriction enzymes known as TALENs, which are released by plant pathogenic bacteria in order to facilitate infection, target these identical pathways. TALENs technologies are characterized by a DNA-binding domain and a DNA-cleavage domain that can introduce DSBs into the DNA target sequence. The basic one-to-one code of recognition between base pairs in the DNA target and protein modules serves as the foundation for the action's mechanism .Although TALENs and ZFNs are widely employed for both therapeutic and disease modeling applications, they are not as accurate or specific as CRISPR/Cas9. Furthermore, there are still issues with protein production, design, and validation that prevent their normal application . By the way, HR can proceed with the repair if homologous donor DNA is available. If not, non-homologous end joining (NHEJ) can be used to repair the break, albeit it carries the risk of introducing sporadic mistakes like tiny insertions and deletions (indels) [32].

The original purpose of the CRISPR/Cas9 system was to provide bacteria and archaea with an adaptable immune system against viruses and plasmids. Three kinds of CRISPR/Cas9 are known to exist . Because type II requires only one protein for RNA-guided DNA recognition and cleavage, it is the most well-known of them and is used in genome engineering. Type II is a two-component RNA-programmable system that is based on the endonuclease Cas9, which forms base pairs with DNA target sequences by utilizing RNA guide sequences [33]. Cas9 can insert a site-specific double-strand break in the DNA thanks to this identification. An RNA duplex consisting of crRNA (CRISPR RNA) and tracrRNA (transactivating crRNA) makes up the RNA-guide sequence. They have been designed as a single guide RNA (sgRNA) with two essential characteristics: a duplex RNA structure at the 3′ side that binds to Cas9, and a sequence at the 5′ side that uses base pairing to identify the DNA target location [34]. This device turned out to be straightforward because Cas9 may be made to target any desired DNA sequence by altering the sgRNA's guide sequence. The protospacer sequence, which must be complementary to the 5′ -end 20-nt sequence of crRNA, and the existence of a crucial short sequence called the protospacer adjacent motif (PAM), bound by Cas9, are the two important components of the targeted sequence recognition . Once the two requirements are met—the binding between Cas9 and the PAM sequence and the protospacer pairing to the 5′ -end 20-nt sequence—the CRISPR/Cas9 system will be able to cut and introduce a DSB . PAM is essential for the first DNA binding because, as some investigations have shown, without PAM, Cas9 is unable to identify target sequences that are completely complementary to the guide RNA [35].

Following systemic activation, DNA repair machinery operates to correct the DSB that was produced by NHEJ or HDR . Conformational reorganizations play a role in the CRISPR/Cas9-mediated genome targeting process. These modifications occur twice: first during binding to the guide RNA and again upon engagement with a target double-stranded DNA. In fact, Cas9 structures undergo guide RNA-induced reorientation to produce a central channel where DNA substrates are attached, as well as to delineate two lobes housing two nucleic acid clefts [36]. Although the majority of our understanding of CRISPR/Cas9 stems from microbiological research, it became evident in 2013 that this system could be effectively used to modify human cell genomes

. Recently, a variety of human cells, including embryonic stem cells, have been subjected to RNA-programmable S. Pyogenes Cas9-mediated gene editing . The objectives of these applications have been diverse and include the accurate replication of tumor-associated translocations, the examination of gene functions through loss-of-function genetic screening, and most importantly, the correction of genetic mutations causing inherited disorders .

Given its mode of action, CRISPR/Cas9 appears to be the most user-friendly and economical technique available, as altering the target site merely necessitates a modification in the guide RNA sequence [37]. Ultimately, in order to optimize the performance of this gene editing system, the correct sgRNA must be designed using the best sgRNA design tools, and potential off-target sites must be identified in order to improve sgRNA specificity. The off-target typically happens at locations that are partially complementary to the guide RNA sequence and have a PAM . Nonetheless, several efforts over time have been directed at improving the system to lower off-targets . The goal of these research has been to create an engineered Cas9 nuclease variation with increased specificity (eSpCas9) or a nickase form of Cas9 (D10A mutant) controlled by paired guide RNAs. Additional developments have focused on creating a CRISPR/Cas9 that can alter the genome without requiring DSBs.

Prime editing, a flexible and accurate genome editing technique that combines a catalytically inhibited Cas9 joined to an engineered reverse transcriptase, programmed with a Prime Editing Guide RNA (pegRNA), is the source of the most recent successful attempt to enhance this system . The unique characteristic is a pegRNA that, through its extension, allows the system to directly replicate the genetic material while also identifying the target spot and encoding the desired modification [38].

Anzalone et al. documented the possibility of using this novel instrument for gene editing without donor DNA or double-strand breaks (DSBs), accomplishing over 175 modifications in human cells. Its main advantage is that it exhibits significantly less off-target activity than Cas9. The mutations causing Tay Sachs illness (OMIM #272800) and sickle cell disease (OMIM #603903) were investigated using prime editing. Additionally, it has been tested for the insertion of a transversion in the DNMT1 gene in mouse primary cortical neurons, the introduction of a protective variant in the PRNP gene in HEK cells, and a comparison of prime editing and HDR in four human cell lines (HEK293T, K562, U2OS, and HeLa). With a lower degree of off-target and fewer byproducts than HDR, the results demonstrate the good efficiency of prime editing and its capacity to expand the goals of genome editing, correcting a significant percentage of known genetic variations related with human illnesses [39].

4. Overview of hiPSC gene editing using CRISPR/Cas9

Recently, two technologies that have the potential to drastically alter biological and medical research are genome editing and somatic cell reprogramming.

CRISPR/Cas9 may target a large number of genomic sequences through gene knockout or knockin, gene interference or activation, and other chromosome-related applications if the proper sgRNA is designed, all while preserving the majority of the genetic background [40].

Beginning with the fundamental biological research on hiPSCs, the CRISPR/Cas9 system has been applied in many ways depending on the goal of the study, as follows:

Since gene knockout is the most popular tool for establishing a link between an upstream molecular process and a biological event, it is mostly utilized to examine gene function . In stem cell research, the identification of particular markers is usually achieved using gene knock-in, which involves the introduction of an exogenous nucleotide sequence. Transcriptional activation or repression: certain Cas9 variations (such as dead Cas9, or dCas9) retain their endonucleolytic activity while still being able to form the gRNA/Cas9 complex. To modify the transcription of endogenous genes, these variations could be coupled with transcriptional activators or suppressors [41]. Genome-wide screening: gRNA libraries offer a vast number of genes for results analysis via the gathering of sequencing data. CRISPR/Cas9 can target transcription inhibitors or gene knock-outs, whereas RNA interference (RNAi) libraries reduce gene expression at the mRNA level. These mutations could be assessed for fundamental biological research on stem cells as well as for medication screening and disease modeling in medicine . One characteristic of the initial attempts to genetically edit hiPSCs was the requirement for the insertion of a floxed resistance cassette. Although homologous recombination with the antibiotic gene normally introduces the LoxP cassette, its removal may leave leftover LoxP sequences that could result in uncontrolled phenotypes . Prior to the development of a method enabling the isolation of single-base genome-edited hiPSCs without the need for antibiotic selection [42], isogenic cell lines for cell therapy and disease modeling were produced using CRISPR/Cas9. The goal of one of the earliest studies in this field was to create a particular gRNA to fix a point mutation in the HBB locus, which causes sickle cell disease (OMIM #603903). The gRNA, the Cas9 linked to a donor DNA template carrying the WT HBB DNA, and the later removal of the selection cassette were used in the construction of the molecule. The generation of HBB protein from the corrected allele in the erythrocytes formed from gene-edited hiPSCs was demonstrated by the authors, who differentiated both the corrected and parental hiPSCs into erythrocytes [43]. The likelihood of determining a gene's function and, consequently, the effects of a potential mutation are directly linked to the availability of an isogenic cell line, as stated in a recent study . This study reports the use of the CRISPR/Cas9 method to repair ATM mutations in patient-derived hiPSCs for Ataxia-Telangiectasia (OMIM #208900). The healing of DNA damage and oxidative stress response was demonstrated by gene corrected hiPSCs, demonstrating the advancements made possible by gene editing . In addition to creating isogenic cell lines, WT hiPSCs could also be treated with CRISPR/Cas9 technology to produce the precise mutation that causes the pathology under investigation. Recently, a model for autosomic dominant polycystic kidney disease (ADPKD, OMIM #613095) was reported.The PKD2 gene was knocked out using CRISPR/Cas9 using hiPSCs obtained from healthy individuals as the starting point for the investigation . The insertion of a patient-specific point mutation in the MEN1 gene into the WT cell line was accomplished using a same methodology, which involved the use of a donor oligonucleotide containing the mutation. Here, the molecular variations in the hypoglycemia phenotype displayed in two patients carrying the same mutation were explained by the correlation between gene editing and hiPSCs [44].

5. CRISPR/Cas9 application to hiPSCs for the development of novel therapeutic approaches

Considering the variety of ways that CRISPR/Cas9 can be applied to hiPSCs, their combination can be seen as a chance to create innovative therapeutic approaches.The primary methods for bringing CRISPR/Cas9 and hiPSCs closer to the field of pharmaceutical research are discussed and summarized here.

5.1 Drug development

It is feasible to incorporate specific genetic modifications into hiPSCs during the early stages of the drug discovery process that could be helpful for the production of a particular molecule or to Select the ideal medication candidate. Pretty recently, Kawai. [45] have made their research on the use of CRISPR/Cas9 to differentiate hiPSCs into intestinal epithelial-like cells (IECs) by inducing peptide transporter 1 (PEPT1) knock-out. This study's objective was to assess PEPT1-mediated intestinal absorption in order to provide the groundwork for the potential creation of peptide and peptide-mimetic medicines as PEPT1 substrates. Thus, pharmacokinetic testing found a role for the usage of hiPSCs. They proved that hiPSCs-IECs had PEPT1 activity and that the levels of PEPT1 expression were comparable to those in the adult human small intestine. For the first time, exon 21's specific glutamate—which is necessary for PEPT1 transport ability—was depleted using CRISPR/Cas9.As a result, a PEPT1-KO-hiPSCs IECs line was established, which may be used in a highly selective transporter test to assess PEPT1 substrates without the need for inhibitors [46].

Using hiPSCs with CRISPR/Cas9 gene editing to identify particular therapeutic targets is a further stage in the drug development process. Their combination was used in the investigation of a specific neurodegenerative illness known as multiple-system atrophy (MSA, OMIM #146500) . Autonomic failure is the hallmark of MSA, and the condition is classified according to the degree of parkinsonism, cerebellar ataxia, and pyramidal dysfunction. Nakamoto et al. [47] investigated a type of MSA linked to a functionally compromised COQ2 gene variation that is linked to a decrease in Q10 biosyntesis using neurons produced from hiPSC patients. It was feasible to rectify the ensuing functional deficiencies in the mitochondrial respiration and antioxidant system as well as the increased apoptosis by employing the CRISPR/Cas9 gene correction technique. As a result, our study links neuron dysfunctions to a reduction in the coenzyme Q10, suggesting that Q10 supplementation may be a beneficial alternative for MSA treatment [48].

5.2 Protein dosage monitoring

One of the primary goals in the study of illnesses marked by the accumulation of certain products is to track the quantity of specific proteins.After all, aberrant protein dosage can be caused by genetic changes that cause half-loss, functional impairment, or de novo increase of function. One illustration is the peculiar way that important regulators are stored in developmental diseases like FOXG1 syndrome (OMIM #613454). Early in the development of the brain, there is variable expression of forkhead transcription factor 1 (FOXG1). Human deletions or missense mutations on one FOXG1 allele have previously been shown to cause FOXG1 syndrome, a severe neurodevelopmental disorder characterized by a wide range of phenotypic manifestations, including severe intellectual disability, microcephaly, autism spectrum disorder, and epilepsy. For the first time, Zhu et al. [49] attempted to regulate the dosage of FOXG1 by integrating three technologies: hiPSCs, small molecule-assisted shut-off (SMASh), and CRISPR/Cas9.This innovative combination makes it possible to more effectively handle the dose control issues that are typically encountered when conventional knock-out or knock-down techniques are applied. Because self-removing degrons are present, SMASh technology can precisely and reversibly change the post-translational quantity of proteins upon the injection of tiny compounds (protease inhibitors) .The targeted gene was tagged with a SMASh in hPSC-derived interneurons using CRISPR/Cas9. They have been able to track how the dosage of FOXG1 influences the production of GABAergic interneurons using this design, which may help to understand the different clinical presentations of FOXG1 syndrome. Because it is simple to examine protein function when protein dosage is controlled by drug-induced breakdown or stabilization, the scientists adjusted this method to replicate a disease model. Nonetheless, this may have intriguing uses in the pharmaceutical industry for screening medications intended to return the amount of protein to normal in order to lessen or eliminate the severe clinical symptoms of syndromes caused by changes in protein content.

5.3 Gene therapy

Gene editing for cell therapy is one of the primary uses of hiPSCs combined with CRISPR/Cas9. Actually, hiPSCs make it possible to take patient cells where genome editing is viable and use those cells for them for transplanting autologous cells. The HBB gene repair of beta-thalassemia (OMIM #613985) patient-specific hiPSCs and their generated hematopoietic stem cell transplantation was demonstrated to give an optimal therapeutic strategy for treating the condition in 2015 [50].

Research on recessive dystrophic epidermolysis bullosa (RDEB, OMIM #226600), a severe inherited skin disorder brought on by COL7A1 gene alterations, has also advanced . Jackàw et al. employed CRISPR/Cas9 to repair hiPSCs produced from patients with RDBE. Gene-corrected hiPSCs were used to create three-dimensional skin equivalents (HSEs), which were subsequently implanted onto immunonodeficient mice after differentiating into keratinocytes and fibroblasts . Type VII collagen was expressed normally in animals. As a result, this set the stage for further clinical uses of cutting-edge autologous stem cell-based treatments for RDBE .The use of TALENs and CRISPR/Cas9 to repair the mutation that causes Duchenne muscular dystrophy (DMD) in patient-derived hiPSCs has been the subject of another investigation [51]. The dystrophin gene (DMD), which is found on chromosome X, has a loss of function mutation that leads to DMD (OMIM #310200), a severe muscular degenerative disease . Prior to their clonal proliferation, patient myoblasts were utilized to restore dystrophin protein, however this process was mediated by an oncogene like hTERT .

Alternatively, without requiring any additional steps, hiPSCs can be separated from patients while retaining their pluripotency and ability for self-renewal. The authors of this study [52] attempted to correct dystrophin using three different approaches: knocking in the missing exon 44 to restore the full protein coding-region, introducing small indels to modify the protein reading frame, and disrupting the splicing acceptor to skip exon. All of these strategies were helpful, according to the results, but only the knock-in strategy was able to restore the full-length dystrophin protein. Due in large part to the extended expression of CRISPR/Cas9, research on Duchenne illness has made significant progress in lowering the likelihood of immunogenicity and off-target mutagenesis. The extracellular nanovesicle-based ribonucleoprotein delivery system known as NanoMEDIC was created very recently by Gee et al. In order to recruit Cas9 protein and sgRNA into nanovesicles, this is based on chemical dimerization and viral RNA pack aging signal. They have used a variety of cell types to test this device, including skeletal muscle cells made from DMD-hiPSCs. The system's exon skip ping efficiency was over 90%. As evidenced by in vivo experiments, this novel approach appears to hold promise for DMD genomeediting therapy [53].

5.4 Immune response strategy

As mentioned earlier, CRISPR/Cas9 originated as an immune response in certain bacteria to eliminate viruses. HIV research has made use of this idea since the beginning Since currently known treatment approaches are unable to completely remove the virus from the body, numerous studies are currently attempting to combat this infection by genome editing. Regarding how hiPSCs might be used in this area of medical research, Liao and coworkers attempted to provide hiPSCs an antiviral defense mechanism akin to that of bacteria and archaea. In order to express a CRISPR/Cas9 system targeted at the reverse-transcribed products of the viral RNA genome, they generated hiPSCs. The outcome was the generation of HIV-targeted CRISPR/Cas9-expressing hiPSCs that were stable. These cells have the ability to develop into HIV reservoir cells, which will keep them permanently resistant to the virus. Thus, this may be characterized as an innovative treatment approach to combat viral infections [54]. Regarding the potential uses of CRISPR/Cas9 and hiPSCs in antiviral response, this tool can be useful in the increasingly urgent SARS Cov-2 research. The main aim is to create a testing platform that resembles a human lung, treat WT-hiPSCs with pseudoviruses that can imitate SARS-Cov-2 infection , and differentiate them into type II pneumocytes. Next, gene editing can be used to introduce known polymorphisms that may protect against or predispose to viral infection, as well as to repress or upregulate genes implicated in virus entrance and activity . Modified cells can also be used to evaluate various candidate drugs' ability to combat infections. Recently, a versatile and effective method for targeting virus RNA through CRISPR/Cas9 action has been put into practice; it can be utilized specifically to the RNA genome of SARS-CoV-2, restricting the virus's ability to reproduce [55]. This is a fantastic opportunity to combat viruses that could quickly evolve and become resistant . Oncology is another area of medical research that necessitates immune response activation.

Because the ability to recognize and correct such mutations as well as the identification of molecular targets are crucial goals of cancer treatment, CRISPR/Cas9 is already widely employed in cancer research. There aren't many cancer research, nevertheless, that take use of the pairing of CRISPR/Cas9 with hiPSCs. An intriguing study was reported in 2016 , in which researchers attempted to enhance the ability of NK cells to eradicate solid tumors by modifying the tumors' expression of a more stable version of CD16a. Through the process of antibody-dependent cell-mediated cytotoxicity, this protein plays a crucial role in the removal of cells that have been opsonized by antibodies (ADCC).

The production of ADAM17, a metalloprotease that facilitated the proteolytic cleavage of CD16a, may inhibit the activity of natural killer cells. Consequently, ADAM17 expression was suppressed in hiPSCs using CRISPR/Cas9 editing, and shortly after, the cells were modified to generate cleavage-resistant CD16a (S197P). These cells can now be distinguished as natural killer cells that target tumor cells, which is a strategy for allogenic cell-based immunotherapies [56].

5.5 Drug screening

Researchers now have a useful tool for drug screening thanks to the potential and accessibility of genome editing and reprogramming technology, particularly when patient samples are not easily accessible. Following gene editing, disease-targeting cells can be divided into distinct cell types, and various drugs can be screened to find those that can improve the disease phenotype. For instance, drug screening turned out to be the most effective method for identifying the perfect substance to combat the mtDNA depletion syndrome (MTDPS3, OMIM #251880), which is brought on by a deficiency in DGUOK, a mitochondrial kinase that phosphorylates purine deoxyribosides . Since the liver is the primary organ affected by this illness, the scientists generated hepatocytes from WT-hiPSCs by using CRISPR/Cas9 to induce DGUOK knockdown. These cells were utilized as a platform to find medications that might enhance mitochondrial activity and ATP generation since they replicated the mitochondrial abnormalities linked to the livers of MTDPS3 patients . The SPECTRUM collection library, which has over 1300 medications licensed for use in humans in the USA, Europe, and Japan, was utilized by the writers. The identification of substances that boost cellular energy generation was done using ATP levels. NAD was the medication that consistently increased ATP levels and the expression of every gene involved in the mitochondrial electron transport chain that was assessed in this screening . Therefore, a potential NAD treatment could be a good therapeutic option. This is an illustration of how combining gene editing and reprogramming might yield a high-throughput testing method for evaluating possible therapeutic formulations with various activity spectra[57].

6. Future challenges

Medical research has been made possible by reprogramming technology and the resultant ability to employ pluripotent stem cells produced from patients. This innovation has experienced an additional development with the identification of the CRISPR/Cas9 genome editing mechanism. These two instruments have a wide range of roles in translational and fundamental biological research, particularly in the areas of pharmacology and drug development. But there are a lot of obstacles to overcome. To prevent partial reprogramming and the start of de novo mutations, it is very important to

establish uniform protocols and increase the effectiveness of the reprogramming mechanism.It is challenging to imagine a system more basic than CRISPR/Cas9, given the revolution in genome editing sparked by the development of such a sophisticated tool based on a single protein. In any case, improving it is necessary to lessen the effects of genome editing that are not intended for human use and to develop innovative methods for delivering CRISPR/Cas9 into cells without the need for virus carriers. Facilitating the selection and growth of hiPSCs-CRISPR/Cas9-corrected clones, which are frequently arduous and time-consuming, would also be ideal. Furthermore, as human trials for the use of hiPSCs and CRISPR/Cas9 are anticipated, the safety must be assessed. An alternative viewpoint involves utilizing pluripotent stem cells to increase target accuracy. This approach can be effortlessly applied to preclinical and clinical research, ultimately increasing the success rate of clinical trials. Addressing these issues could have a significant impact on the drug development process, cutting down on costs and time[58].

Conclusion

In conclusion, the relentless pursuit of more efficient and ethical resources in medical research has propelled advancements in gene editing and cellular reprogramming technologies. The integration of hiPSCs and CRISPR/Cas9 represents a groundbreaking approach to disease modeling, drug discovery, and therapeutic development. hiPSCs provide an ethically sound and versatile platform for generating human cell lines that closely mimic human pathology, which, when combined with the precision of CRISPR/Cas9, enables unprecedented accuracy in gene editing. This combination facilitates not only the identification of disease mechanisms and potential therapeutic targets but also the development of personalized treatments with reduced risks of immunological rejection.The use of hiPSCs in drug discovery has accelerated the identification of therapeutic targets and drugs by providing a more predictive model of human diseases compared to traditional animal or tumor cell models. Despite challenges such as the limited applicability in sporadic disorders and the need for inducing cellular aging, hiPSCs remain a powerful tool for the replication of monogenic diseases and drug screening. The CRISPR/Cas9 system further enhances this by allowing precise genetic modifications, which are essential for understanding disease mechanisms and developing targeted therapies.As medical research continues to evolve, the combination of hiPSCs and CRISPR/Cas9 is poised to play a crucial role in the development of innovative therapeutic strategies. This includes gene therapy for conditions like sickle cell disease and Duchenne muscular dystrophy, as well as antiviral defenses against diseases like HIV and potentially SARS-CoV-2. The ability to model diseases accurately and screen drugs effectively using these technologies holds immense promise for advancing personalized medicine and improving patient outcomes.However, to fully realize the potential of these technologies, significant challenges must be addressed. Standardizing protocols, improving reprogramming efficiency, and minimizing unintended genomic effects are critical steps forward. As researchers continue to refine these tools and explore their applications, the future of medical research looks increasingly promising, with the potential for transformative impacts on disease treatment and prevention.

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