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Review Article

Stem Cells Applications in Therapeutics and Site-Specific Genome Editing Through CRISPR Cas9 System

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Abstract

Stem cells are immature cells that have ability to differentiate into all specific and mature cells in body. The two main characteristics of stem cells are self-renewable and ability to differentiate into all mature, functional and adult cells types. There are the two major classes a) pluripotent stem cells which have potential to differentiate in all adult cell and b) multipotent stem cells which have capacity to differentiate into many adult cells but not in all cell types. Due to the self-renewable ability stem cells are used in therapeutics, tissue regeneration, disease modeling, regenerative medicines and to treat cardiovascular diseases, neural disorders such as Parkinson's disease and most importantly to treat carcinomas. The human induced pluripotent stem cells provide a great platform to study and treatment of human diseases because these are able to differentiate into many functional and specialized adult cells of body. The genome editing tools such as CRISPR Cas9 system and TALENs are used to generate multiple DNA variants in hPSCs by inducing site specific mutations, frame shift mutation and deletion. In present days CRISPR Cas9 is more efficient and frequent method for genome editing which is derived from bacterial cell.

Keywords: Clustered regularly interspaced short palindromic sequences Cas9; TALENs; *sRAGE*; *AGE-Albumi*; Parkinson's disease; leukemia stem cells

Introduction

The cells that are able to differentiate into all kinds of cells of body are stem cells. These cells are unspecialized and have the unlimited self-renewal rate as compare to other body cells and the new progeny is phenotypically similar to parents. Stem cells are categorized into embryonic, adult stem cells that's are present in human body [1]. Stem cells are referred as ancestral progenitors for highly functional and specialized cells. Embryonic stem cells are isolate from inner mass of blastocysts and grow into whole organism and adult stem cells are arise from any adult organ. The adult stem cells are differentiating to only same organ from which they are obtained e.g., Mesenchymal Stem Cells (MSCs) which are isolate from bone marrow, cartilages and adipose tissues. Cancer cells and stem cell have common feature such as self-renewable and differentiation potential [2]. The stem cells have high potential role indifferent therapies such as in treatment of cardiovascular diseases, Parkinson's disease and a lot of other disorders. In CVDs they regenerate the myocardium. Bone marrow derived mesenchymal stem cells, mononuclear stem cells and skeletal myoblasts etc. are used in treatment of cardiovascular diseases [3]. Recent studies revealed that miRNAs enabling stem cells to overcome the G1/S check point in cell division. Stem cells provide high potential strategies to treat retinal disorders. The embryonic stem cells and induced pluripotent stem cells are pluripotent as they are differentiated into any kind of cell in body. Oligopotent stem cells are able to differentiate into several cells and myeloid stem cells are differentiate into white blood cells not in red blood cells. Fibroblasts are the first source of iPSCs. Now a days the stem cells therapies are possible for the treatment of kidney disorders, spinal cord injury, lungs injury, retinal disorders and many other genetic disorders are treated with use of adult and embryonic stem cells.

Stem Cells Potential Role in Therapeutics

As the stem cells are characterized by their ability to differentiate into all kind of cells, their self-regenerative ability and their unspecialized status. Multipotent stem cells differentiate into limited number of cells pluripotent into all kind of cells in body. It has been noted that the hMSCs are differentiated and proliferate into adipocytes, osteoblasts and chondrocytes when cultured in a specific medium which contain reprogramming factors such as OCT4, SOX2, Nanog, KLF4 and MYC these are also called Yamanaka factors. The reprogramming factors are responsible for inducing pluripotency into any somatic cell and proliferate it into desired cells or tissue. When DNA polymerase inhibitors aphidicoline and mitomycine C are added, the hMSCs are adipocytes at high density and osteoblast at low density but when hMSCs are cultured without soluble differentiation factors the low density inhibit the adipogenic commitment while high density prevent osteogenesis. The actin cytoskeleton is involved in cell shape commitment. When cytochalasin D which is actin disrupting agent decrease osteogenesis and increase adipogenesis but effect of cytochalasin D is unclear whether it effect the cell shape or disrupting the actin cytoskeleton. It also examined that low level of active *RhoA* related to adipogenic condition and high level with osteogenic conditions [4]. For the retinal disorder treatment multipotent stem cells are derived from fetal stem cells from neural lineage, adult stem cells from neural lineage. Furthermore, fetal retinal progenitor cells, fetal cortical progenitor cells, ciliary epithelium derived stem cell, muller glial cells, umbilical cord derived cells, bone marrow derived stem cells are important for retinal disorders. Embryonic stem cells are pluripotent cells and isolate from inner mass of blastocyst and differentiate into all three germ layers.

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As the chances of immune are present in use of these cells then to avoid the immune rejection the induces pluripotent stem cells are generated from somatic cells reprogramming [5]. Mesenchymal stem cells are adult stem cells which are differentiate into mesodermal lineages and other embryonic lineages such as adipocytes and cartilages and bone. The mesenchymal stem cells are isolate from bone marrow. With respect to other stem cells mesenchymal stem cells have low ethical concern and low immune rejection chances. Recent studies suggested that human umbilical cord blood is an alternative source of MSCs. Umbilical cord derived cells have high rate of differentiation, expression and proliferation as compare to bone marrow derived cells [6]. MSCs are vehicle for gene therapy in lipopolysaccharides induced lung injury as they are migrated into lung and adopt the shape of lung cells and injured part recovered. MSCs based Ang1 gene therapy using lentivirus vector is developed for the lung injury. This therapy reduces the lung inflammatory injury and pulmonary vascular permeability [7]. Embryonic stem cells are used to replace the abnormal cells in Parkinson's disease patients. In this disease dopamine generating cells are damaged and transplantation of ES cells result in replacement of these damaging cells and new healthy cells are produced. It has been noted that CRIPTO is important regulator of ES cells. The suppression of cripto stimulate cells to differentiate to neural fate and cellular poo for dopaminergic differentiation is increase [8]. The mutations in *LRRK2* are linked with Parkinson disease. Two strains of induced pluripotent stem are generated with *LRRK2* gene knockouts by using CRISPR cas9 system. The guided RNA can delete the target gene at exon 9 and generate knockouts. These strains have the same morphology, expression rate and ability to differentiate into three germ layers endoderm, mesoderm and ectoderm. These genomic edited induced pluripotent strains are useful to examine Parkinson disease [9]. Recently it has been noted that the Advanced Glycation End product albumin (*AGE-Albumin*) is also responsible for the PD. Accumulation of *AGE-Albumin* stop the transmission of signals and dopamine generating cells are destroyed. The soluble receptors of *AGE-Albumin* are inhibiting their accumulation so the continuous supply of *sRAGE* can inhibit the cell death in substantia nigra and carpus striatum. The umbilical card derived mesenchymal stem cells are edited by using CRISPR cas9 system. The *sRAGE* coding sequence is inserted in stem cell by using PzDonor vectors to then *sRAGE*-secreting UCB-MSCs are produced which are able to secrete *sRAGE* continuously and inhibit cell death as well as increase number of normal cells in brain [10]. The endogenous cardiac stem cells are differentiated into smooth muscle cells, cardiomyocytes and endothelial cells to regenerate the heart. Cardiovascular disease such as coronary heart disease can stop blood supply to heart cells and damaged these cells. It has been reported that stem cell therapy can replace the damaged heart cells and avoid the heart diseases [11]. End stage kidney failure is the complete loos of function of kidney. It is believed that kidney cells have ability to regenerate to some extent. As the adult stem cells are differentiate into that organ from which they are derived. But it is not easy to isolate adult stem cells from kidney due to lack of definitive markers of kidney stem cells. The label retaining cells are isolate from the proximal tubules of mouse. These label retaining cells are able to regenerate the damaged cells of kidney but this approach is not completely successful to derived adult stem cells from kidney. The side population cells in kidney are isolated and these cells are show multipotency and improve renal function through humoral role [12]. Cancer is the chronic disease that dangerously effect the human life. Many strategies are developed such as radiotherapy, chemotherapy and surgery to treat the cancer. These strategies effective against some tumors because of metastasis and resistance to radio and chemotherapy restrict the treatment. The cancer stem cells provide platform for understanding and treatment of cancer. These cells are firstly identified in leukemia and isolate through $CD34^+$ and $CD38^-$ surface marker expression. The activities of CSCs are regulated by intracellular and extracellular factors and these factors are used as drug target for cancer treatment. CSCs have self-renewable ability and expand to form tumor. The major transcription activator of CSCs is *OCT4*, *SOX2*, *Nanog*, *KLF4* and *MYC*. In SCID mice the cells grow and form tumor which use as platform to study and cure tumor [13]. Myeloid leukemia is caused by mutations in BCRABL1. This gene encodes chimeric protein of 210KD with tyrosine kinase activity. The with BCRABL1 that cause chronic leukemia are termed as CML leukemia stem cells. It has been noted that some progeny of these cells has stem cell features with biological ability to cause CML. Some researchers argue that presence of Ph^1 chromosomes on B cells lineages prove that CML must begin in stem cell. Tyrosine kinase inhibitors block the biochemical activity of BCRABL1 and cure patients with chronic CML phase [14]. It has been investigated that dipeptidyl peptidase-4 (CD26) inhibit Stromal Cell Derived Factors (SDF1). CD26 cause cleavage of SDF1-CXR4 axis and implicated in release of CML leukemia stem cells from bone marrow into blood and act as marker of chronic phase CML-LSCs. $CD26^+$ LSCs are decreased by TKI treatment but TKI resistance numbers of CD26 expressing cells are increase in bone marrow and blood [15]. Acute myeloid leukemia is clonal disease of hematopoietic stem cells. It has been shown that mRNAm^6A reader YTHDF1 is overexpressed in huma AML. YTHDF1 can decrease the half-life of m6A transcript which contribute with integrity of leukemia stem cells functions along with tumor necrosis factors receptors whose upregulation in YTHDF1 deficient leukemia stem is responsible for apoptosis. In general, YTHDF1 not essential for normal hematopoietic stem cells but its deficiency enhances HSC activity. Hence YTHDF1 is a unique therapeutic agent which selectively target leukemia stem cells and promoting HSC activity [16]. Some genes are involved in regulation LSCs In CML such as p53, pten, Alox5, Alox15, PML1, Bim-1, Hedhehoh and BCL6. However limited numbers of studies are performed for specific targeting of LSCs by target genes. For example, blockage of Hedgehog signaling by SMO inhibitors, inactivation of BCLR by retroinverse BCL peptide inhibitor RI-BPI have been performed to inhibit CML development by inhibit LSCs. HIF1α pathway inhibition by echinomycine is effective in suppressing LSCs as well as deficiency of Alox15 and inhibition of Alox15 function leads to suppressing of LSCs [17]. Previously it has been deen studied that oxidative phosphorylation have important role for survival of leukemia stem cells. Generally, LSCs are depend on amino acid metabolism for driven of oxidative phosphorylation and in overall 20 amino acids cysteine is important for this purpose. We already know that cysteine is metabolized into glutathione. So, depletion of cysteine debilitated glutathione synthesis which results reduction in glutathionylation succinate dehydrogenase. A which is major component of electron transport chain II. Reduction in succinate dehydrogenase A glutathionylation damage activity of electron

transport chain and inhibit oxidative phosphorylation and ATP production diminishes which leading death of leukemia stem cells. Due to this reason cysteine depletion is recognized as a therapeutic agent to suppress LSCs [18].

Genome Editing of Stem Cells by CRISPR Cas9 System

As we know the stem cells are using now a days to cure the genetic disorders. For this purpose, the genome of stem cells edited with normal gene to replace the mutated gene in damaged tissue or organ. So, the most important and successful tool for the genome editing is CRISPR Cas9 system. CRISPR that is the abbreviation of Clusters of regularly interspaced palindromic repeats is new tool for sequence specific genome of mammalian cells. This system is present in bacteria to protect them from viruses by targeting the virus genome. CRISPR is isolate from streptococcus pyogenes which use cas9 nuclease protein and sgRNA of about 20 nucleotides and introduce site specific double stranded break [19]. It has been studied that the targeting of cas9 and sgRNA complex to DNA is done by base pairing between small guided RNA and target DNA in the presence of NGG PAM which is protospacer adjacent motif sequence. The double stranded breaks are induced at 3bp upstream of PAM site and the genome editing occur *via* non homologous end joining which result in frameshift mutation or deletion which cause loss of gene function or sometime by homology directed repair for insertion of point mutation at the target locus [20]. It has been studied that small molecules can block or activate the DNA repair pathways. A fluorescent reporter system was established to characterize the CRISPR mediated HDR efficiency. For this purpose, embryonic stem cells are used because they show better HDR efficiency then somatic cells. By following the series of methods, it is investigated that small molecules such as azidothymidine and trifluridine decrease the HDR pathway about 3-fold these both are anti-viral drugs while the Brefeldin A enhance the HDR mechanism. The editing of SNPs through single stranded oligodeoxynucleotides (sgRNA) template is important application of genome editing in gene therapy and disease modeling. The 200 nucleotides *ssODN* template was synthesized to induce an A4V mutation in human SOD1 locus in such a way that introduction of mutation also effect the NGG PAM sequence which prevent further targeting by *ssODN* to A4V allele. The vectors with CRISPRCas9 and sgRNA is transfected into human induced pluripotent stem cells. The results show that the small molecules L75507 enhance frequency of A4V mutation about 9-fold. In short, the identified small molecules can modulate the genome editing frequency and show minimum cell toxicity and work in all cell types used to enhance large template mediated gene insertion and *ssODN* mediated SNP editing [21]. Parkinson's disease is neurodegenerative disorder and human induced pluripotent stem cells are good resources for modelling of PD due their easy isolation and have the immune mimicry with patient body so the chances of rejection are very low. It has been reported that by using CRISPR genome editing tool two novel knock in cells lines which carry green fluorescent protein reporter for TH. Human induced pluripotent stem cells are derived from healthy donor. The guided RNA synthesized which guide the cas9 protein to cut the *LRRK2* gene at exon 9. The guided RNA, gene of interest and vector is insert into hPSCs by electroporation [22]. Due to unlimited self-renewal capacity and ability to differentiate into all kinds of adult cells the human pluripotent stem cells including human induced pluripotent and human embryonic stem cells provide a great platform for the disease and biological studies. The programmable site-specific nuclease has been significantly facilitating the target genome editing in culture cells types. The nuclease induces double stranded DNA breaks at site specific loci and trigger endogenous DNA repair *via* two pathways error prone nonhomologous end joining which lead to insertion/deletion mutation or by homology directed repair which introduce precise nucleotide alteration using a homology DNA template. For the further improvement of genome editing efficiency in hPSCs a new platform iCRISPR is develop with the help of TALEN mediated gene targeting. After doxycycline treatment hPSCs cell lines are transfected along with sgRNA to generate biallelic knockout hPSCs lines for multiple genes. The target integration and inducible expression of Cas9 from safe harbor locus provide reliable access to express invariable components of CRISPR system. The Cas 9 expressing hPSCs are easily transfected due to small size. iCas9 hPSCs are generated by TALENs mediated gene targeting into *AAVS1* locus because this site shows rapid transgene expression. TALENs pair designed to generate double stranded breaks in first intron of *AAVS1* locus and two donor template plasmid vectors are electroporated with *AAVS1*-TALENs construct. Cas9 donor plasmid have doxycycline inducible gene expression system along puromycine and at the end HDR of double stranded breaks cause the insertion of puroCas9 in *AAVS1* locus. So Cas9 expression induce through doxycycline treatment in established iCas9 lines in colonial form [23]. TALENs and CRISPR are emerged as powerful site-specific nuclease for genome editing. TALENs are designed as pair to bind the genomic sequences flanking the target site. Every TALEN arm has a sequence specific TALE DBA binding domain which link with non-specific DNA cleavage domain which isolate from bacterial restriction endonuclease *fokI* [24]. The iCISPR can also facilitate other types of gene editing in hPSCs by replacing the Cas9 with varients such as Dcas9, dCas-KRAB or dCas9-VP16. It is also use to create deletion in non-coding RNA [25].

Future Prospective

The genetic mutations are unable resolve by pharmaceutical agents, to resolve these mutations or increase expression of target gene specific tools are need. These tools are TALENs, ZFN and CRISPR. The CRISPR is most efficient and reliable platform for genome editing. It used nucleases that's are able to cut at specific site. It is also informed that viral base genome editing sometimes cause irrelevant gene editing or transgene delivery not occur [26]. To resolve this problem CRISPR based genome editing is preferred and in future recombination DNA technology and development of regenerative medicines is totally depend on CRISPR Cas9 system [27]. It has been studied that induced pluripotent stem cells are more useful in tissue engineering and personalized regenerative medicines development because iPSCs have huge potential to differentiate into all kinds of cells or tissues. To achieve rapid proliferation and differentiation of iPSCs, the expression of specific gene should be enhanced which is regulate by CRISPR Cas9 system. In future stem cells genome editing *via* CRISPR Cas9 system has most important role in clinical therapeutics, to treat genetic disorders and to produce desired traits in plants [28].

Conclusion

It is concluded that stem have the great potential to develop into all kinds of cells of body that performed a specific function in body. Pluripotent stem cells are differentiating into all cell types. Embryonic stem cells are pluripotent stem cells and multipotent stem cells are not differentiating into all kind but just in some kinds mesenchymal stem cells are multipotent stem cells. Due to their self-renewable activity, stem cells are used to in therapies. Mostly used stem cells in therapies are human induced pluripotent stem cells because these cells have low immune rejection chances as compere to other kinds of cells. By genome editing with the help of CRISPR Cas9 system hPSCs are great platform for diagnosis and treatment of disorders. In future the stem cells with genome editing by CRISPR are useful tools for treatment of cancer, Parkinson's disease and other genetic diseases.

Main Points

- The hMSCs are differentiated and proliferate into adipocytes, osteoblasts and chondrocytes when cultured in a specific medium which contain reprogramming factors such as *OCT4*, *SOX2*, *Nanog*, *KLF4* and *MYC* these are also called Yamanaka factors.
- Advanced glycation end product albumin (*AGE-Albumin*) is also responsible for the PD. Accumulation of *AGE-Albumin* stop the transmission of signals and dopamine generating cells are destroyed.
- A new platform iCRISPR is develop with the help of TALEN mediated gene targeting.
- The targeting of cas9 and sgRNA complex to DNA is done by base pairing between small guided RNA and target DNA in the presence of NGG PAM which is protospacer adjacent motif sequence.

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