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Small molecules and small molecule drugs in regenerative medicine

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Regenerative medicine is an emerging, multidisciplinary science that aims to replace or regenerate human cells, tissues or organs, to restore or establish normal function. Research on small molecules and small molecule drugs in regenerative medicine is currently increasing. In this review, we discuss the potential applications of small molecules and small molecule drugs in regenerative medicine. These include enabling novel cell therapy approaches and augmentation of endogenous cells for tissue regeneration, facilitating the generation of target cells for cell therapy, improving the interactions between cells and biomatrices for tissue engineering, and enhancing endogenous stem cell function for tissue regeneration. We also discuss the potential challenges for small molecule drugs in regenerative medicine.

Regenerative medicine replaces or regenerates human cells, tissues or organs, to restore or establish normal function [1]. The major distinction between regenerative medicine and traditional molecular medicine is that regenerative medicine restores normal function through the replacement or regeneration of human cells. It involves interdisciplinary research including, but not limited to, tissue engineering, cell therapy, gene therapy and protein pharmaceutics. Small molecule drugs have long been a focus of traditional molecular medicine. Owing to their pharmacological control and ease of use, there have been many recent efforts to search for small molecule drugs for regenerative medicine. In this review, we introduce the application of small molecules and small molecule drugs in regenerative medicine.

An introduction to regenerative medicine

Regenerative medicine studies three important components to restore normal function: cells, biomatrices and signaling cues. Whether the goal is to replace nonfunctional or missing cell types, or to regenerate failed organs, human cells are the core component of regenerative medicine. Currently, human pluripotent stem cells {hPSCs [embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs)]}, adult stem cells, and terminally differentiated cells are all being studied in regenerative medicine.

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Human cells in regenerative medicine

hESCs

The establishment of hPSCs in 1998 [2] provided a potential limitless cell source for regenerative medicine. With different differentiation protocols, these cells have been differentiated into many cell types. Nonetheless, the use of hESC-differentiated cells in clinical trials has been lagging. Currently, hESC-derived retinal pigmented epithelial cells are being tested to treat Stargardt's macular dystrophy and dry age-related macular degeneration (AMD) in clinical trials, with the first report published in 2012 [3]. The challenges responsible for this lag are: (i) immune rejection by the host; (ii) the inefficient differentiation and risks of tumorigenicity; and (iii) the lack of efficient cell transplantation methods.

hiPSCs

To overcome the challenges of immune rejection associated with hESC, hiPSCs were generated by forced expression of transcription factors [4], following Yamanaka's pioneering work of generating mouse iPSC cells with the transcription factors octamer-binding transcription factor 4/SRY (sex determining region Y)-box 2/Kruppel-like factor 4/C-MYC (OCT4/SOX2/KLF4/C-MYC) [5]. Although there are some differences, hiPSC resemble hESC in that both are pluripotent and have an unlimited capacity for proliferation. Similar to hESC, hiPSC can be induced to differentiate into many cell types, such as neural precursors [6], hepatocytes [7],

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hematopoietic progenitors [8], β cells [9], retinal pigmented epithelial cells [10], skeletal myoblasts [11], motoneurons [12] and cardiomyocytes [13]. Owing to the safety concern of introducing oncogenes (e.g. c-myc) during reprogramming by integrating viral vectors, generating hiPSC using nonintegrating methods (e.g. by using proteins, mRNAs, nonintegrating episomal plasmids and viruses) are available with moderate efficiency. Another strategy is to generate hiPSC with small molecules, which is discussed further below. Recently, the first clinical trial using integration-free hiPSC-derived cells to treat AMD was approved in Japan.

Adult stem cells

Adult stem cells, also called somatic stem cells, can self-renew and generate the cell types comprising the organs where the stem cells reside. Adult stem cells have been described in the hematopoietic system (hematopoietic stem cells), central nervous system (neural stem cells), liver (liver stem cells), skin (follicle and epidermal stem cells), cornea (the corneal stromal stem cells), gut (intestinal crypt stem cell), skeletal muscle (satellite cells), and adipose tissue and bone marrow (mesenchymal stem cells). Some stem cell types (e.g. amniotic fluid stem cells) are neither hESC/iPSC nor adult stem cells. These cells might also find applications in regenerative medicine owing to their proliferation capacity and differentiation potential.

Compared with hESC and hiPSC, adult stem cells have limited proliferation and differentiation capacity *in vitro*, but they confer a low risk of tumor formation after being transplanted to patients, as shown by the intravenous infusion of human adipose tissue-derived mesenchymal stem cells in humans [14]. There are many ongoing clinical trials using adult stem cells to restore normal cell function in various diseases, including neurodegeneration, spinal cord injury, graft-*versus*-host disease and diabetes. The National Institutes for Health (NIH) database currently registers over 1700 open clinical trials using hematopoietic stem cells, over 100 open clinical trials using mesenchymal stem cells (bone marrow-, cord blood- and adipose-derived) and five open clinical trials using neural stem cells. The US Food and Drug Administration (FDA) has approved several cord blood progenitor cell products for cell therapy; for example, Hemacord from the New York Blood Center, HPC from Clinimmune Labs, and Ducord from Duke University School of Medicine.

Terminally differentiated cells

Terminally differentiated cells also have uses in regenerative medicine. For example, fibroblast cells have been used for skin regeneration to treat burns and other ulcerations, and the FDA has approved several products for skin regeneration that contain live fibroblasts [15]. Chondrocytes are being studied to treat osteoarthritis and cartilage defects [16]. Currently, there are more than ten open clinical trials using chondrocytes.

Biomatrices in regenerative medicine

Cells actively interact with the biomatrices they contact. In neo-organ engineering, synthetic or natural biomatrices are necessary in addition to cells. Biomatrices serve several important functions: (i) they provide the structural integrity and organizational backbone for cells to organize and assemble; (ii) they mimic the functions of the native extracellular matrix (ECM) to provide support and anchorage for the cells; and (iii) they can be engineered to incorporate bioactive factors to enhance the survival and guide the differentiation of the cells. For example, neo-bladders engineered and transplanted in patients were generated with polyglycolic acid (PGA) and collagen [17], where PGA provided the rigidity to form the shape of the bladder, and collagen (used to create an ECM) enhanced cellular attachment and growth.

There are three types of biomatrix: (i) naturally derived materials, such as collagen, hyaluronic acid (HA), alginate, chitin/chitosan, keratin and silk; (ii) synthetic polymers, such as PGA, polylactic acid (PLA) and poly(-lactic-co-glycolic) acid (PLGA); and (iii) decellularized organ scaffolds. Recently, interest in decellularized organ scaffolds has increased, because these structures preserve the host organ architecture, especially the vascular structure. Acellular matrices from complex organs, such as heart [18], liver [19], lung [20] and kidney [21], have been prepared, and can support cell proliferation and differentiation upon recellularization. Bioengineered airway tissues using decellularized matrix have been transplanted to patients [22,23].

Following this brief introduction to the cells and the biomatrices, the most important components for regenerative medicine, we now discuss the potential applications of small molecules and small molecule drugs in regenerative medicine. We believe that small molecules can be used: (i) *in vitro* to facilitate the production of target cells and to improve cell survival in cell therapy and tissue engineering; and (ii) *in vivo* to enhance the proliferation and differentiation of endogenous stem cells to promote tissue regeneration.

Enabling novel cell therapy approaches using small molecules

Currently, cell therapy with or without biomaterials is still the major strategy in regenerative medicine. Although the use of small molecule drugs in regenerative medicine is still in an early stage, interest in their use is increasing owing to a better understanding of signaling pathways that control cell fate (especially stem cell fate) and the development of technologies for high-throughput screening (HTS). For a comprehensive review on small molecules regulating the cell fate of various stem cells, readers are referred to a recent review by Schultz *et al.* [24]. Here, we discuss the applications of small molecules in cell therapy and tissue engineering.

Facilitating the manipulation and maintenance of hESC and/or hiPSC with small molecules

Expanding pluripotent hESC and/or hiPSC is crucial for harnessing their therapeutic potential. Spontaneous differentiation is a challenge in hESC and/or hiPSC expansion. Rho-associated protein kinase (ROCK) inhibitor Y-27632 was found to diminish dissociation-induced apoptosis, increase cloning efficiency and facilitate subcloning [25]. The glycogen synthase kinase (GSK)-3β inhibitor 6-bromoindirubin-3′-oxime (BIO) helped to maintain hESC in an undifferentiated state [26]. Many small molecules facilitate the maintenance and proliferation of hESC and/or hiPSC (Table 1) and their use has made these cells more accessible for clinical application. For example, rapamycin and Y-27632 facilitated the large-scale suspension expansion of human ESC [27].

Guiding the differentiation of PSCs with small molecules

hESC and hiPSC are limitless sources for cell therapy, but pluripotency carries the potential to form teratomas. Although PSCs