Please cite this article as: Liu et al., Chemical Modulation of Cell Fate in Stem Cell Therapeutics and Regenerative Medicine, Cell Chemical Biology (2016), http://dx.doi.org/10.1016/j.chembiol.2016.07.007

Cell Chemical Biology
Review

Chemical Modulation of Cell Fate in Stem Cell Therapeutics and Regenerative Medicine

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http://dx.doi.org/10.1016/j.chembiol.2016.07.007

Regenerative medicine aims to repair and regenerate injured tissues and restore their impaired functions. Recent developments in stem cell biology have attracted significant interest in their applications in regenerative medicine. Chemical approaches using small molecules have yielded exciting results in induction and differentiation of pluripotent stem cells, lineage conversion of somatic cells, and ex vivo as well as in vivo modulation of adult stem cells. In this review, we discuss recent progress, new insights, and future challenges of the chemical approaches in stem cell biology and regenerative medicine.

Introduction

The use of stem cells in regenerative medicine holds tremendous promise for the treatment of various human diseases (Amabile and Meissner, 2009). Regenerative medicine aims to restore normal tissue function by replacing injured tissues with healthy ones via cell-based therapy or induction of endogenous reparative/regenerative processes (Xu et al., 2008). The approach of cell-based therapy involves isolating somatic cells (or adult stem cells) from a healthy donor or patient, inducing (and/or expanding) stem cells, correcting their genetic defects or arming the cells with enhanced/new functions, differentiating stem cells into tissue-specific cell types, and finally transplanting the desired cell population. The approach of inducing endogenous repair and regeneration involves in vivo modulation of tissue-specific cell types by therapeutic agents, stimulation of endogenous tissue repair and regeneration through resident (progenitor) cell activation, proliferation, differentiation, or reprogramming (Dimmeler et al., 2014). Both approaches require an in-depth understanding of basic stem cell biology, including the underlying mechanisms of stem cell induction, self-renewal, differentiation, trafficking, and integration into target tissue.

With their unique advantages, small molecules have served as essential tools to modulate cellular processes and probe fundamental cell biology (Li et al., 2013b). Rapid and dose-dependent in general, pharmacological modulations by small molecules can fine-tune the timing and magnitude of their cellular effects. Unlimited structural variations in small molecules can be explored to achieve desired pharmacological and pharmacokinetic properties. Properly targeted delivery and controlled release of small molecules can spatially and temporally regulate their effects in vivo. Already widely applied in stem cell biology, small molecules hold tremendous potential in regenerative medicine. In the following sections, we will review the applications of small molecules in the key areas of stem cell biology, including pluripotency induction, directed differentiation of pluripotent stem cells (PSCs), lineage conversion of somatic cells, and the modulation of adult stem cells (Figure 1).

Small-Molecule Modulation of Pluripotency Induction

PSCs can self-renew indefinitely and give rise to all cell types of the three germ layers. Human PSCs (hPSCs) can provide an unlimited source of desired cell types for regenerative medicine (Wu and Hochedlinger, 2011). Early and recent studies of pluripotent, human embryonic stem cells (hESCs) have enriched our knowledge of hPSCs. Therapeutic applications of hESCs, however, are controversial because of ethical issues. Researchers have sought to generate hPSCs without manipulating human embryos. Before 2006, somatic cell nuclear transfer and cell fusion were the only established routes to obtain PSCs from somatic cells (Hochedlinger and Jaenisch, 2006). However, these approaches have been proved challenging for clinical applications because of ethical liability and technical difficulties (Jaenisch, 2004).

The groundbreaking discovery of induced PSC (iPSC) technology by Takahashi and Yamanaka in 2006 directly addressed these issues (Takahashi and Yamanaka, 2006). They used the forced expression of four transcription factors (TFs) (i.e., Oct4, Sox2, Klf4 and c-Myc, OSKM) to reprogram mouse fibroblasts into iPSCs. In the following year, the reprogramming of human somatic cells to human iPSCs (hiPSCs) further paved the way for the entry of iPSCs into regenerative medicine (Takahashi et al., 2007).

The original method of iPSC generation involved viral transduction of the four Yamanaka TFs, which leads to permanent genome modification. Alternative methods that avoid the use of integrating viruses quickly emerged, including non-integrating viral vectors, excisable vectors, transfection of mRNAs, and recombinant proteins (Gonzalez et al., 2011). In addition, alternative combinations of TFs have been developed to exclude Klf4 and c-Myc to reduce the oncogenic risks for potential clinical application. Despite their benefits and advantages, these alternative methods may still present certain challenges, such as low efficiencies, slow kinetics, and/or genome alteration during iPSC reprogramming.

In the last decade, substantial progress has been made to improve the efficiency and safety of iPSC reprogramming

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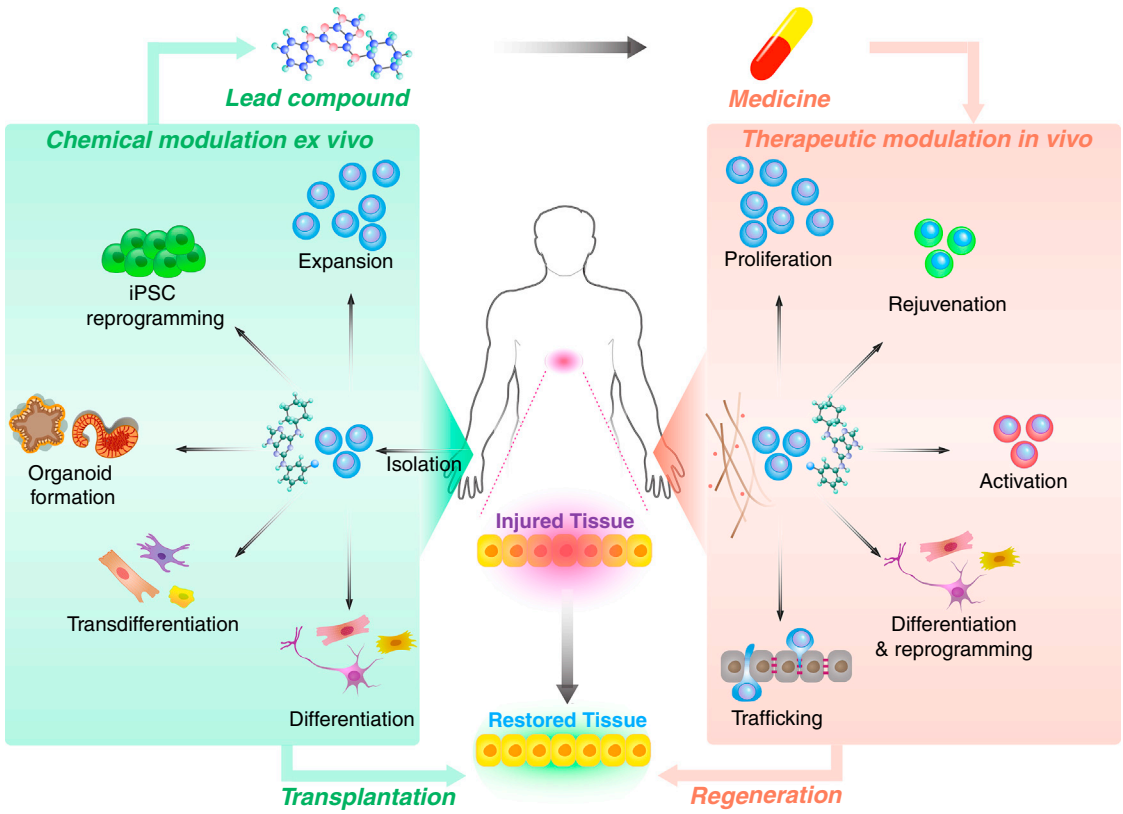

Figure 1. Chemical Modulation of Cell Fate in Stem Cell Therapeutics and Regenerative Medicine
Ex vivo chemical modulation (left) is applied to isolated somatic cells (or adult stem cells) from a healthy donor or patient to facilitate the induction and/or expansion of stem cells, to enhance the generation of functional tissue-specific cell types through differentiation or transdifferentiation, and to enable the formation of organoids as cell sources for cell-based transplantation therapy. In addition, the chemicals that can regulate appropriate cell fate or function ex vivo could be further developed as small-molecule therapeutics for modulating endogenous cells in vivo underlying disease or injury conditions. Utilizing such small-molecule therapeutics, therapeutic modulation in vivo (right) can be applied to induce endogenous reparative/regenerative processes involving resident (progenitor) cell activation, rejuvenation, proliferation, trafficking, and differentiation. Under disease or injury conditions, normal tissue function can be restored by transplantation of healthy tissues derived via ex vivo approaches or by endogenous tissue regeneration via in vivo approaches.

through supplementation with small molecules (Li et al., 2013b). Several mechanistically distinct classes of small molecules have been identified to functionally complement and even replace exogenous TFs for iPSC reprogramming (Yu et al., 2014). These small molecules not only serve as chemical tools to explore the reprogramming mechanisms (Figure 2) but also pave the way to generate iPSCs without exogenous TFs.

Epigenetic Modulation for Reprogramming

iPSCs and somatic cells share essentially the same genome but differ drastically in gene expression. Reprogramming of somatic cells into iPSCs requires activation of pluripotency-related genes and repression of somatic genes (Buganim et al., 2013). Since epigenetic modifications are important mechanisms that regulate gene expression, proper modulation of these mechanisms is indispensable for the establishment of pluripotency. A large number of small molecules were found to modulate DNA methylation, histone acetylation, and histone methylation. These small-molecule, epigenetic modulators exert significant effects on cell reprogramming.

DNA methylation is an important epigenetic mechanism to repress gene expression (Miranda and Jones, 2007; Suzuki and Bird, 2008). In differentiated somatic cells, the CpG-rich regulatory regions of pluripotency-related genes were found to contain high levels of DNA methylation (Meissner et al., 2008). During cellular reprogramming, loss of DNA methylation from these regions is required to activate the silenced pluripotency-related genes. DNA methyltransferases (DNMTs) are known to contribute to the DNA methylation dynamics in mammalian cells (Jurkowska et al., 2011). Inhibiting DNMTs can presumably facilitate alteration of the DNA methylation patterns required for cell reprogramming. 5-Azacytidine (5-aza; Figure 3) and RG108 (Figure 3) are the two most widely used DNMT inhibitors effective in enhancing OSKM-mediated cell reprogramming (Li et al., 2009b). Treatment with 5-aza was also found to complete the establishment of pluripotency in partially reprogrammed cells (Mikkelsen et al., 2008). As an analog of DNA nucleoside cytidine, 5-aza inhibits DNMTs at low doses but also causes toxicity by incorporation into both DNA and RNA strands at high doses (Christman, 2002). In contrast, 5-aza-2′-deoxycitidine (5-aza-dc) is exclusively incorporated into DNA and thus causes a DNMT depletion effect at a lower concentration. In a recent study by Deng's group, 5-aza-dc was used to promote the conversion of the extraembryonic endoderm (XEN)-like state to the pluripotent state during chemical reprogramming (Zhao et al., 2015).