MINI-REVIEW

Small Molecules

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Introduction

Stem cell biology research offers extraordinary potential to the future of regenerative medicine. Stem cells have the potential to form the basis of cellular therapies for diseases affecting organ systems with limited regenerative capacity, to provide enhanced systems for drug screening and toxicity testing as well as to gain insight into early human development obviating the need for human embryos. All of these applications, will require efficient, reproducible, and cost-effective methods for generation, maintenance, and differentiation of stem cells. Small molecules can be used as important reagents to help us better understand the underlying biology in stem cells, and efficiently regulate them.

Our understanding of biological processes often develops from discovering or designing ways to perturb a given process and observing the subsequent effects. Although genetic and cytokine/protein-based approaches have been widely used for this purpose, small molecules offer some distinct advantages:

- Can be cell permeable and have the ability to affect signaling pathways within the cell
- Effects can be finely tuned by varying concentration
- High purity and low lot-to-lot variability in activity
- Defined, more stable, and cost-effective than growth factors

Target-based screening

Small molecules with known mechanisms of action and/or molecular targets can be used in the place of their respective pathway ligands. For example, the GSK3 inhibitor, CHIR99021 can replace WNT in a number of applications. Similarly, the importance of a particular pathway or protein in a biological process can be demonstrated using small molecules that up- or down-regulate the pathway. For example, molecules such as CHIR99021 and PD0325901 have been used to stimulate self-renewal of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), demonstrating the roles of GSK3/WNT and MEK/ERK signaling in the maintenance of pluripotency.[1-3] In another example, activation of the WNT pathway with CHIR99021 followed by inhibition of the WNT pathway with compounds such as IWP-2 was shown to induce cardiomyocyte differentiation in pluripotent stem cells, thereby demonstrating the importance of biphasic WNT signaling in cardiomyocyte differentiation.[4] These studies illustrate how small molecules can be as effective at regulating stem cell fate decisions as more expensive growth factors, and give further insight into the molecular pathways involved.

Target-based approaches have also been used to probe and improve the reprogramming process for making iPSCs. Histone deacetylase (HDAC) inhibitors (e.g. Sodium Butyrate, Trichostatin A, Valproic Acid), histone methylation inhibitors (e.g. 3-Deazaneplanocin A, BIX01294) and DNA methyltransferase inhibitors (e.g. 5-Azacytidine, RG108) all have enhancing effects on reprogramming efficiency, demonstrating the importance of an open chromatin state during reprogramming. The use of these chromatin modifiers, along with small molecules promoting stem cell self-renewal and survival, has vastly improved reprogramming efficiency. For example, small molecules such as the combination of PD0325901, Thiazovivin and SB431542, have been used to increase the efficiency of reprogramming human fibroblasts to iPS cells.[5] More recently, small molecules (CHIR99021, Forskolin, Tranylcypromine, Valproic Acid, and 3-Deazaneplanocin A) have been utilized to reprogram mouse fibroblasts without the use of genetic factors,[6] providing encouraging news for the future of cell-based therapies.

High-throughput Screening

Small molecules are routinely used in high-throughput screening in the pharmaceutical industry as a means for unbiased biological discovery. Recent technological advances, such as access to small molecule libraries and advances in robotics, are making these approaches accessible to academic laboratories, especially

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as many research institutes develop their own screening facilities. These advances have increased the speed at which small molecules are being discovered and the identification of new targets and pathways that regulate stem cell fate and biology.

Several challenges in pluripotent cell culture have been addressed through the high-throughput screening approach. Human pluripotent stem cells have low single cell viability, which makes genome-editing and cloning techniques difficult. Compounds such as Y-27632[7] and Thiazovivin[8] were identified in high-throughput screens and have been shown to increase survival of single cells through inhibition of RHO/ROCK signaling. Screening for molecules that promote reprogramming in the absence of one or more canonical reprogramming factors led to RepSox,9 a TGF-β inhibitor. Regulators of lineage-specific differentiation are critical for the realization of regenerative therapies, and high throughput screening methods have identified small molecules such as Purmorphamine, a Hedgehog pathway activator that promotes differentiation of mesenchymal stem cells,[10,11] IDE1 and IDE2 which induce definitive endodermal differentiation of mouse and human pluripotent stem cells,[12] and Cardiogenol C, found to induce cardiomyocyte differentiation of mouse ES cells.[13]

Conclusion

It is an exciting time for the field of stem cell research as new biological discoveries are being made with tremendous potential for regenerative medicine. Small molecules are becoming an indispensable tool for the field, both in the discovery process and in the development of efficient, defined, and cost-effective protocols.

References

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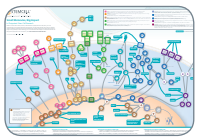

WALLCHART

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