Review

Cell PRESS

The use of small molecules in somatic-cell reprogramming

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Pioneering work over the past years has highlighted the remarkable ability of manipulating cell states through exogenous, mostly transcription factor-induced reprogramming. The use of small molecules and reprogramming by transcription factors share a common history starting with the early AZA and MyoD experiments in fibroblast cells. Recent work shows that a combination of small molecules can replace all of the reprogramming factors and many previous studies have demonstrated their use in enhancing efficiencies or replacing individual factors. Here we provide a brief introduction to reprogramming followed by a detailed review of the major classes of small molecules that have been used to date and what future opportunities can be expected from these.

Embryonic stem cells (ESCs) and induced pluripotency

ESCs show great potential for regenerative biology and provide a unique platform for studying basic cell biology. Their capacity for unlimited self-renewal allows long-term maintenance of ESC lines in laboratory culture conditions, making them one of the few non-transformed cell types with this ability. As a result, ESCs have proved a useful model for studying early human embryology and, more generally, decisions of cell fate throughout differentiation and specification [1–3]. Furthermore, the system allows for disease modeling in human cells, which facilitates the study of underlying disease mechanisms in a relevant setting [4–6]. Beyond basic research, the prospective clinical uses for personalized cellular therapy using pluripotent cells cannot be overstated. The first clinical trial assessing the use of ESCs for acute spinal cord injury began in 2009 and another trial investigating their use in age-related macular degeneration began in 2010 with promising results [7]. Although cellular therapy with donor ESCs is indeed exciting, the ultimate goal lies in treating patients with genetically matched stem cells derived from their own somatic tissue.

In the past, several approaches have been used to achieve somatic-cell reprogramming including somatic cell nuclear transfer (SCNT) and cell fusion [8,9]. Clinical translation of both techniques has proven difficult, because SCNT requires scarce donor oocytes and is accompanied by ethical trepidation, and the fused tetraploid cells are unsuitable for use in human patients.

The seminal work of Takahashi and Yamanaka eloquently addressed the shortcomings of these reprogramming approaches by transforming fibroblasts into pluripotent cells via forced expression of four transcription factors: Oct4, Sox2, Klf4, and c-Myc (OSKM) [10,11]. The cells produced with this method, termed induced pluripotent stem cells (iPSCs), show morphological, transcriptional, epigenetic, and phenotypic similarity to ESCs. Likewise, they can differentiate into all embryonic germ layers and incorporate into a developing mouse embryo to give rise to chimeric adults [8]. Many widely available cell types provide the starting point for mouse and human iPSC reprogramming [12,13]. The original protocol relies on viral transduction of the four factors but subsequent efforts quickly focused on methodologies to avoid the use of integrating viruses. Although variable, the kinetics of the process remains slow (2–4 weeks) and inefficient (0.01–0.1% of cells reprogram) [13]. Moreover, the introduction of the oncogenic proteins Klf4 and c-Myc is best avoided when considering clinical translation [14,15].

Improvements to the Yamanaka protocol address these concerns by utilizing alternative methods of transduction, substituting genetic factors with macromolecules, or treating with small molecules that abrogate the need for certain genetic factors [13]. Non-integrating viral vectors, excisable vectors, and direct transfection of plasmid DNA with OSKM can all reprogram fibroblast cells, but the efficiency and kinetics of these processes remain prohibitively low [5,16–18]. Transduced, modified mRNA can substitute for DNA introduction during reprogramming and its transient stability allows for iPSC generation while avoiding integration and permanent introduction of oncogenes [19]. Recombinant OSKM proteins tagged with a cell-penetrant polyarginine sequence have also been used and eliminate

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Keywords: induced pluripotent stem cells; small molecules; reprogramming; chemical biology; epigenetics.

0962-8924/$ – see front matter
 http://dx.doi.org/10.1016/j.tcb.2013.09.011

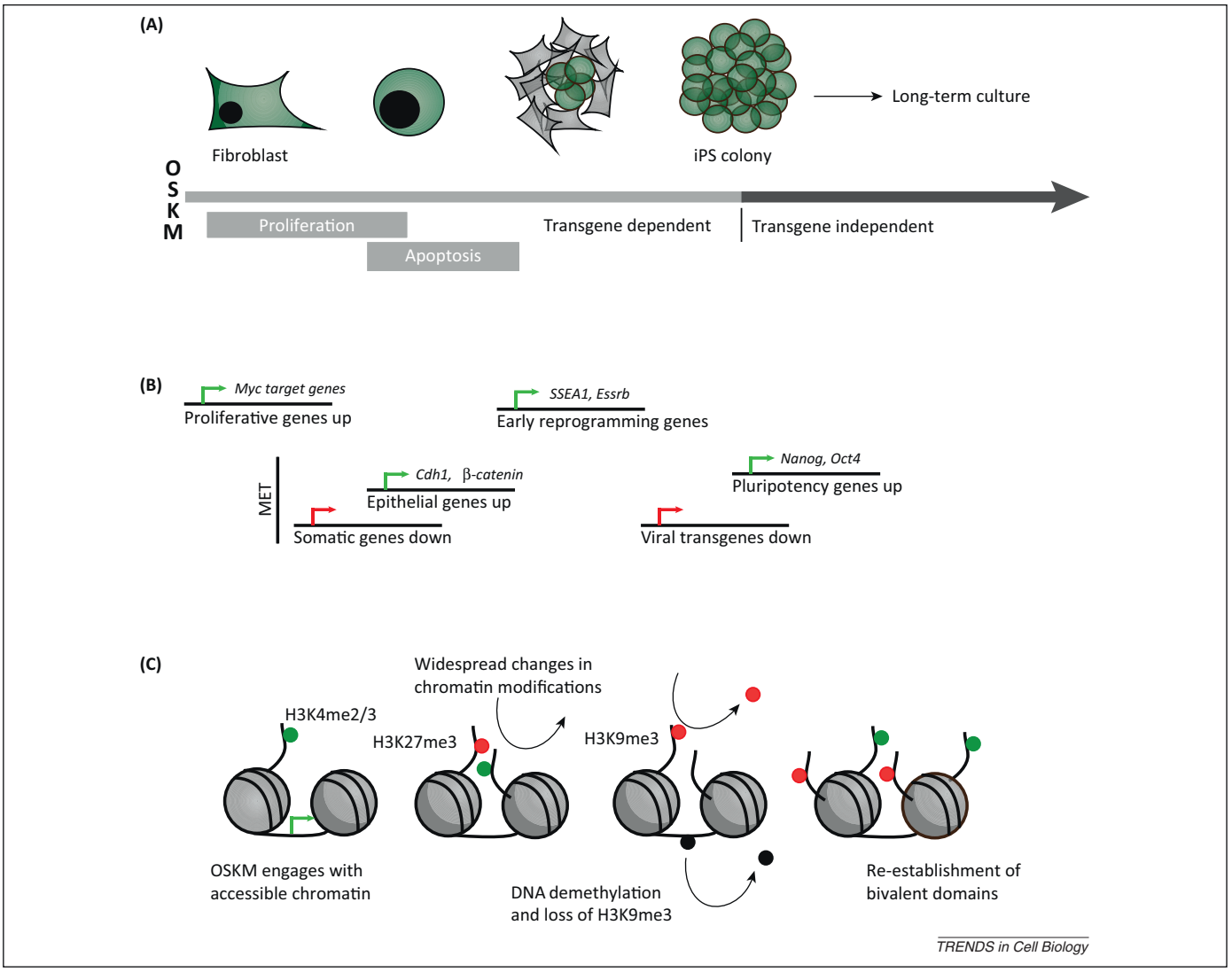

Figure 1. Dynamics of selected molecular events during reprogramming. The reprogramming of somatic cells occurs in two general phases, the first being transgene dependent wherein removal of exogenous expression of OSK(M) results in the reversion to a differentiated state. During the second phase, removal of exogenous transgene expression no longer prevents the final transition to pluripotency. **(A)** A set of morphological changes are notable during the transgene-dependent phase, as early reprogramming cells divide rapidly, becoming round and beginning to form clusters. Widespread apoptosis is seen following this expansion, and eventually the cells form compact colonies of fully reprogrammed iPSCs [79]. **(B)** Many of the early changes in transcription result from a c-Myc driven effect. This is followed by the mesenchymal to epithelial transition seen at an intermediate phase in reprogramming, ultimately leading to the silencing of transgenes and the permanent re-activation of the core pluripotency network. **(C)** The initial binding locations of the OSKM factors are defined by the chromatin landscape of the somatic cell. Early in reprogramming, widespread changes in histone modifications are seen, followed by a general loss of repressive histone and DNA modifications. In fully reprogrammed cells, bivalent domains are re-established at loci important for development as seen in ESCs. Chromatin remodeling continues during the transgene-independent phase with X-chromosome reactivation and telomere elongation. Abbreviations: ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; OSKM, Oct4, Sox2, Klf4, and c-Myc.

the need for the transfer of genetic material [20]. However, this process is inefficient, requires large amounts of recombinant protein and has not been widely used. In addition to the inefficiencies with these second-generation reprogramming methods, the various techniques remain technically challenging, forcing most laboratories to continue to generate iPSCs through classic viral transduction approaches. However, iPSC generation can be facilitated and improved by supplementation with small-molecule compounds. Modulation by small molecules is more technically tractable than alternative genetic approaches and their activity can be restricted temporally and spatially, ensuring an additional level of safety in clinical applications.

The reprogramming process requires coordinated, correctly timed transitions in gene expression, which are mediated by OSKM [21–25] (Figure 1). To date, a small collection of compounds has already been shown to collaborate with OSKM to facilitate iPSC reprogramming (Table 1), all of which exert their effects through gene-regulatory pathways, in particular through inhibiting chromatin regulators or kinase signaling pathways. Furthermore, the first report of somatic-cell reprogramming using only small-molecule compounds was published earlier this year [26]. Following this rapidly advancing field, we review published compounds, their effect on somatic-cell reprogramming, their mechanism of action, and the pathways they act on. A better understanding of how these molecules interact with each other as well as their interaction with the cellular environment will allow for more efficient reprogramming and eventually small molecule-based control of cell states.