Molecular Aspects of Medicine 34 (2013) 841–848

Contents lists available at SciVerse ScienceDirect

Molecular Aspects of Medicine

journal homepage: www.elsevier.com/locate/mam

Review

Cellular reprogramming to reset epigenetic signatures

Kyle J. Hewitt [a], Jonathan A. Garlick [b,*]

[a] Department of Cell and Regenerative Biology, University of Wisconsin-Madison, 1111 Highland Ave., Madison, WI 53705, USA
[b] Department of Oral and Maxillofacial Pathology, Tufts University, 55 Kneeland Ave., Boston, MA 02111, USA

ARTICLE INFO

Article history:
Available online 5 September 2012

Keywords:
Induced pluripotent stem cells
DNA methylation
Epigenetics
Profiling
Reprogramming

ABSTRACT

The controlled differentiation of induced pluripotent stem cells (iPSC) towards clinically-relevant cell types has benefitted from epigenetic profiling of lineage-specific markers to confirm the phenotype of iPSC-derived cells. Mapping epigenetic marks throughout the genome has identified unique changes which occur in the DNA methylation profile of cells as they differentiate to specific cell types. Beyond characterizing the development of cells derived from pluripotent stem cells, the process of reprogramming cells to iPSC resets lineage-specific DNA methylation marks established during differentiation to specific somatic cell types. This property of reprogramming has potential utility in reverting aberrant epigenetic alterations in nuclear organization that are linked to disease progression. Since DNA methylation marks are reset following reprogramming, and contribute to restarting developmental programs, it is possible that DNA methylation marks associated with the disease state may also be erased in these cells. The subsequent differentiation of such cells could result in cell progeny that will function effectively as therapeutically-competent cell types for use in regenerative medicine. This suggests that through reprogramming it may be possible to directly modify the epigenetic memory of diseased cells and help to normalize their cellular phenotype, while also broadening our understanding of disease pathogenesis.

Contents

* Corresponding author. Tel.: +1 617 636 2478.
E-mail address: Jonathan.Garlick@tufts.edu (J.A. Garlick).

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http://dx.doi.org/10.1016/j.mam.2012.08.002

1. Introduction

Development of technologies for the generation of induced pluripotent stem cells (iPSC) is an exciting and rapidly-evolving field with great potential for clinical translation. Adult somatic cells can be reprogrammed by a variety of methods to a cellular state that is phenotypically similar to human embryonic stem cell lines (ESC) isolated from the inner cell mass of human embryos (Takahashi et al., 2007; Yu et al., 2007). The unlimited expansion potential and ability to generate therapeutically-relevant and patient-specific cell types makes these pluripotent cells valuable progenitors for the regeneration of diseased cells and tissues (Robinton and Daley, 2012; Yamanaka, 2009). Before iPSC can be implemented for therapeutic applications, rigorous phenotypic analysis and thorough testing of function and biological potential of cells differentiated from them is needed (Yamanaka, 2009). Despite the immense opportunities that iPSC technologies have provided to study development and disease-progression, there is still considerable debate over the extent of epigenetic changes associated with reprogramming (Lister et al., 2011) and implications of this for human therapies remain largely unknown.

In this review, we will discuss the contribution of epigenetic profiling, particularly the use of DNA methylation profiling, for characterization of iPSC- and ESC-derived cell populations and cells differentiated from them to specific lineage fates, and assessing the therapeutic safety and efficacy of iPSC-derived cells. In addition, there is growing evidence that reprogramming may serve to reset the cellular "biological clock" through epigenetic rearrangements (Marion and Blasco, 2010; Suhr et al., 2010). This intriguing concept could lead to vastly-improved therapies to replace cells or repair tissues that have been damaged due to degenerative diseases and/or aging. Furthermore, since several diseases are now linked to an altered epigenetic profile (Leung et al., 2012), there may be opportunities to correct these disease phenotypes through reprogramming approaches.

2. Epigenetics of pluripotency

A critical step in further developing pluripotent cell sources for future cellular therapies and for disease modeling is elucidating the underlying epigenetic mechanisms that establish and maintain the pluripotent state. Sophisticated tools have been developed to screen the epigenetic profile from a wide range of cellular phenotypes (Spivakov and Fisher, 2007) allowing assessment of how differences in epigenetic status distinguish between cell lineage fates as well as direct and maintain their cellular phenotype (Sant et al., 2012).

2.1. Pluripotency

Within the nucleus of pluripotent stem cells, the epigenetic state of DNA is in an open configuration, in which lineage-specific genes are not expressed, yet have not been repressed, by epigenetic rearrangement (Bernstein et al., 2006; Spivakov and Fisher, 2007). This orientation allows for rapid differentiation and lineage specification, facilitated by global changes in nuclear organization, DNA methylation, and histone modifications. Genome-wide mapping of this epigenetic state has identified a signature of pluripotent cells that distinguishes them from differentiated cell types (Bibikova et al., 2006; Doi et al., 2009). The chromatin structure of pluripotent cells contains a bivalent chromatin structure marking key developmental genes, such as Sox1, Pax3 Msx1, Irx3 that are not expressed in pluripotent cells (Bernstein et al., 2006). The chromatin pattern of these lineage-associated genes have been epigenetically modified with a combination of activating and repressive histone modifications that prime them for expression or repression upon induction of cell lineage specification (Gan et al., 2007).

2.2. DNA methylation

One extensively studied epigenetic mechanism that maintains differentiation potential in pluripotent cell types is DNA methylation. DNA methylation primarily occurs at cytosine residues throughout the genome, and the majority of DNA methylation events occur at the 5 position of cytosine residues at a CG dinucleotide (or CpG site) (Adams, 1990). Since spontaneous deamination of 5-methylCytosine (5mC) to thymidine is known to happen frequently throughout the mammalian genome, these dinucleotides are relatively rare and are thought to be evolutionarily-conserved gene regulatory regions (Wiench et al., 2011). Methylation events at these dinucleotide pairs can be localized into regions known as CpG islands, or are dispersed throughout the genome. Often, the CpG sites are localized near gene promoters and high levels of methylation at these promoters have been typically associated with repression of gene expression (Trowbridge and Orkin, 2010). The mechanism of DNA methylation-mediated repression involves interference with binding of activating transcription factors, as well as recruitment of repressors and realigning chromatin structure (Khavari et al., 2010; Nagae et al., 2011). The catalysis of DNA methylation is carried out by DNA-methyltransferases (DNMTs), and the expression and localization of these enzymes are determinants for whether CpG methylation is lost, gained, or stably maintained on the newly-synthesized strand following DNA replication and cell division (Chen et al., 2003).

Several whole-genome methods for DNA methylation profiling (Bisulfite NextGen sequencing, MeDIP sequencing, RRBS, etc.) have increased the depth of profiling data and enabled the accessibility of these technologies into novel experimental systems. By mapping the genomic distribution of methylated sequences, or methylome, it is possible to generate a detailed