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Short communication

Epigenetic modulators promote mesenchymal stem cell phenotype switches

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ABSTRACT

Discoveries in recent years have suggested that some tissue specific adult stem cells in mammals might have the ability to differentiate into cell types from different germ layers. This phenomenon has been referred to as stem cell transdifferentiation or plasticity. Despite controversy, the current consensus holds that transdifferentiation does occur in mammals, but only within a limited range. Understanding the mechanisms that underlie the switches in phenotype and development of the methods that will promote such type of conversions can open up endless possibilities for regenerative medicine. Epigenetic control contributes to various processes that lead to cellular plasticity and DNA and histone covalent modifications play a key role in these processes. Recently, we have been able to convert human mesenchymal stem cells (hMSCs) into neural-like cells by exposing cells to epigenetic modifiers and neural inducing factors. The goal of this study was to investigate the stability and plasticity of these transdifferentiated cells. To this end, neurally induced MSCs (NI-hMSCs) were exposed to adipocyte inducing factors. Grown for 24–48 h in fat induction media NI-hMSCs reversed their morphology into fibroblast-like cells and regained their proliferative properties. After 3 weeks approximately 6% of hMSCs differentiated into multilocular or plurivacuolar adipocyte cells that demonstrated by Oil Red O staining. Re-exposure of these cultures or the purified adipocytes to neural induction medium induced the cells to re-differentiate into neuronal-like cells. These data suggest that cell plasticity can be manipulated by the combination of small molecule modulators of chromatin modifying enzymes and specific cell signaling pathways.

1. Introduction

Regeneration is arguably among the most awe inspiring biological phenomena known to exist. It can be defined as the natural ability of living organisms to replace worn out parts, repair or renew damaged or lost parts of the body, or to reconstitute the whole body from a small fragment during the post-embryonic life of an organism. Regeneration is thus the reactivation of development in post-embryonic life to restore missing tissues. All species, from bacteria to humans, have been shown to possess some capacity for this process. Although certain species are capable of subtotal regeneration, namely the hydra and planarian flatworms, others have a far more limited scope by which damaged tissues can be restored. The current known mechanisms associated with natural regeneration are: regeneration via pluripotent or tissue and organ specific stem cells and regeneration through dedifferentiation and transdifferentiation. Regeneration through dedifferentiation, transdifferentiation and pluripotent stem cells are mostly typical to more primitive animals such as Hydra and planarians, as well as to lower vertebrates such as zebrafish, newts, and salamanders (Echeverri and Tanaka, 2002; Henry and Tsonis, 2010; Siebert et al., 2008; Tanaka and Reddien, 2011; Zhang et al., 2013). While the regeneration through these mechanisms in lower vertebrates is not as striking as in Hydra and planarians, they are able to replace their lens, retina, intestine, cardiac ventricle, upper and lower jaws, limbs, and tail following injury (Tanaka and Reddien, 2011).

In the adult mammals, the regeneration was thought to exclusively occur through the action of organ or tissue-restricted stem cells (i.e. haematopoietic stem cells making blood; gut stem cells making gut, etc.). However, a large body of recent work has challenged the long-held belief that organ-specific stem cells are lineage-restricted (Eguizabal et al., 2013; Hombach-Klonisch et al., 2008; Jopling et al, 2011; Shen et al., 2004). In particular, haematopoietic, mesenchymal and neural stem cells appear to be the most versatile at cutting across lineage boundaries (Kopen et al., 1999; Lagasse et al., 2000; Orlic et al., 2001a,b; Petersen et al., 1999;

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Song et al., 2009; Vescovi et al., 2002a). As demonstrated in these studies bone marrow stromal cells can be turned into astrocytes, cardiomyocytes and hepatic oval cells, hematopoietic cell can be transdifferentiated into hepatocytes, and neural stem cells can be transdifferentiated into penile smooth muscle cells and hematopoietic cells.

Despite the large amount of literature demonstrating transdifferentiation, the ability of tissue specific adult stem cells to give rise to an unrelated cell type has been controversial. The current consensus holds that transdifferentiation does occur in mammals, but only within a limited range (Morshead et al., 2002; Shih et al., 2002; Vescovi et al., 2002b). Thus, although gene-restriction programs are established during embryonic development when cell lineages are formed, stem cells retain a degree of flexibility that is essential for tissue regeneration. Understanding the mechanisms that involved in the regulation of these processes will give us clues how to promote and/or enhance the plasticity of adult stem cells of mammals that have mostly lost during evolution.

It is now well established that epigenetic regulation plays a key role in governing stemness, lineage commitment, differentiation and maintenance of these states. The key components of these machinery include DNA methylation at CpG islands, noncoding RNA molecules, chromatin remodeling complexes, histone-modifying enzymes (histone "writers" and "erasers"), and proteins that specifically recognize and then translate the histone marks (histone "readers") (Lunyak and Rosenfeld, 2008; Pasque et al., 2011). Recent several studies suggest that plasticity and the fate of cells can be manipulated by the alteration of epigenetic marks on histones or DNA. (Chen et al., 2009; Harris et al., 2011; Lyssiotis et al., 2007; Schmittwolf et al., 2005). As demonstrated in the majority of these reports, the biological active substances that involved in the regulation of covalent modifications of chromatin play a key role in the modulation of cells plasticity, and in appropriate culture conditions (specific medium, extracellular matrix, growth factors and modulators of specific cell signaling pathways) promote cell lineage conversions.

In our previous studies, using a chemical genetics approach for cell reprogramming (specific combination of small cell-permeable biological active compounds that involved in the regulation of covalent modifying enzymes of chromatin, and specific cell signaling pathways important in neural differentiation), we have been able to generate neural-like cells from feline and human MSCs (hMSCs) (Alexanian, 2007, 2010; Zhang and Alexanian, 2012, 2014; Zhang et al., 2011). Neurally induced hMSCs (NI-hMSCs) exhibit several neural characteristics such as neural morphology, expression of specific neural markers, and secretion of neurotrophic factors.

Differentiation of these cells into neuronal progenitors and mature functional neuronal cells has been achieved by addition of SMAD inhibitors to our neural induction protocol (Alexanian et al., 2013). These chemically induced neuronal precursors (CiNPCs), grown for 2–3 weeks in this culture conditions, exhibited electrophysiological properties of maturing neurons and formed synapses with differentiated human neurons in co-cultures. Ninety percent of cell in these cultures exerted neuronal morphology and were positive to neuronal progenitor and mature neuronal markers demonstrated by immunocytochemistry, real time PCR and Western blot. Further differentiation of CiNPCs with specific morphogens, growth factors and cell signaling modulators produced specific neuronal phenotypes such as dopaminergic (DA), GABAergic and Cholinergic. In particular, we have been able to develop efficient method for generation of DA-like cells (Funk and Alexanian, 2013). These data demonstrate that CiNPCs exhibit neuronal progenitor-like properties and in appropriate culture condition may produce different neuronal subtypes similar to embryonic- or iPSCs-derived neuronal precursors.

The goal of this study is to investigate the effect of epigenetic modifiers on the stability and plasticity of these transdifferentiated cells.

2. Materials and methods

2.1. Expansion of hMSCs

Human bone marrow MSCs (frozen at passage 1) was provided by Texas A&M Health Science Center, Institute for Regenerative Medicine. According to the product specification sheet, human bone marrow aspirate was drawn and mononuclear cells were separated using density centrifugation. The cells were plated to obtain adherent human marrow stromal cells, which were harvested when cells reached 60–80% confluence. These specimens were considered passage zero (P0) cells. These P0 cells were expended, harvested and frozen at passage 1 (P1) for distribution. Prior to release, two trials of the frozen P1 cells were analyzed over three passages for Colony Forming Units, cell growth, and differentiation into fat, induction protocols. These characterized hMSCs from P1 were expanded and used for all experiments.

2.2. Neuronal differentiation

Neuronal differentiation was performed as described before (Alexanian et al., 2013; Funk and Alexanian, 2013). In short, hMSCs from early passages (P2–P4) were plated on poly-l-ornithine/laminin (P/L) coated six-well plastic with the density of 25,000/cm^2 as well as on P/L coated 12 mm glass coverslips in 24-well tissue culture plates with the density of 20,000/cm^2 and exposed to neural induction medium with the following composition: 200 nM Trichostatin A (TSA, Santa Cruz, Santa Cruz, CA, USA), 10 μM RG-108 (Calbiochem, Billerica, MA, USA), 300 μM 8-BrcAMP (Sigma, St. Louis, MO, USA), 1 μM Rolipram (Sigma, St. Louis, MO, USA), 2 μM Dorsomophin (Sigma, St. Louis, MO, USA), 2 μM SB431542 (Sigma, St. Louis, MO, USA) in the medium of NeuroCult Basal Medium (StemCell Technologies, Vancouver, BC, Canada) supplemented with N2 (10 μl/mL) (Invitrogen, Carlsbad, CA, USA) and 20 ng bFGF (R&D Systems, Minneapolis, MN, USA). After 2 weeks, cultures were used for immunocytochemistry or for adipocyte differentiation studies.

2.3. Immunocytochemistry

For immunocytochemistry, cells were fixed with 4% paraformaldehyde and stained for mature neural markers such as mouse monoclonal anti-β-III-tubulin (B3T) (1:750; Covance, Princeton, NJ, USA), and rabbit polyclonal anti-Synaptophysin (1:300, abcam, Cambridge, MA, USA). Immunoreactive cells were visualized with Texas Red (TxR)-conjugated goat anti-mouse IgG or fluorescent-conjugated (FITC) goat anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA, USA). DAPI used for nuclear staining. To reduce fluorescence quenching, glass coverslips were mounted in ProLong Antifade reagent (Invitrogen, Carlsbad, CA, USA) and dried on microscope slides. Representative images were captured by a Nikon microscope equipped with color digital camera (Spot II). NIS-Elements v4.0 (Nikon Laboratory imaging) was used for analyzing the images and counting the cells.

2.4. Adipocyte differentiation

For adipose differentiation, neurally transdifferentiated cells that had been grown in neural induction medium for 2 weeks were incubated in adipocyte inducing medium (α-MEM, 4.5 g/L glucose, 1 μM dexamethasone, 0.2 mM indomethacin, 1.7 μM insulin,

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