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An inner nuclear membrane protein induces rapid differentiation of human induced pluripotent stem cells

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ABSTRACT

The ability of iPSCs (induced pluripotent stem cells) to generate any cell type in the body makes them valuable tools for cell replacement therapies. However, differentiation of iPSCs can be demanding, slow and variable. During differentiation chromatin is re-organized and silent dense heterochromatin becomes tethered to the nuclear periphery by processes involving the nuclear lamina and proteins of the INM (inner nuclear membrane). The INM protein, Samp1 (Spindle Associated Membrane Protein 1) interacts with Lamin A/C and the INM protein Emerin, which has a chromatin binding LEM (Lap2-Emerin-Man1)-domain. In this paper we investigate if Samp1 can play a role in the differentiation of iPSCs. Samp1 levels increased as differentiating iPSCs started to express Lamin A/C. Interestingly, even under pluripotent culturing conditions, ectopic expression of Samp1 induced a rapid differentiation of iPSCs, of which some expressed the neuronal marker βIII-tubulin already after 6 days. This suggests that Samp1 is involved in early differentiation of iPSCs and could potentially be explored as a tool to promote progression of the differentiation process.

1. Introduction

Induced pluripotent stem cells (iPSCs) displays similar morphology and pluripotency markers as embryonic stem cells (Constantinescu et al., 2006; Takahashi and Yamanaka, 2006) but have a greater potential in generating patient-derived cells for cell replacement therapies and as model systems for human diseases and disorders (Tiscornia et al., 2011). The difficulty however, lays in finding a robust and quick differentiation procedure for generating the cell type of choice. Although many advances for neuronal differentiation have been made recently (Hu et al., 2010; Mattis and Svendsen, 2011; Pre et al., 2014), improvements are urgently needed especially concerning the temporal aspect.

The nuclear envelope (NE) is an incredibly complex structure between the nucleoplasm and the cytoplasm. Underlining the nuclear envelope is the nuclear lamina, composed of lamins and other nuclear envelope proteins for example nuclear envelope transmembrane proteins (NETs). Only a few of the hundreds of NETs have been characterized and only some of them have so far been linked to differentiation. For example, TMEM120A and B have been reported to be involved in adipocyte differentiation (Batrakou et al., 2015) and the entire nuclear lamina undergoes a dramatic reorganization during adipogenesis (Verstraeten et al., 2011). In muscle, NET9, NET25, NET32, NET37 and NET39 are up-regulated and predicted to have roles in myogenesis (Chen et al., 2006). NET37 glycosidase activity is proposed to be important for the IGF-II maturation during myoblast differentiation (Datta et al., 2009). On the other hand, NET39 functions as a negative regulator of myoblast differentiation by reducing IGF-II production (Liu et al., 2009). The LEM-domain proteins NET25 (LEMD2), Emerin and Man1 are also up-regulated and shown to be essential for myogenesis (Huber et al., 2009).

During myogenic and neuronal differentiation the composition of nuclear pore complex (NPC) changes (D'Angelo et al., 2012). The nuclear pore membrane protein gp210/Nup210 (Wozniak and Blobel, 1992) is absent in both myoblasts and embryonic stem cells (ESCs) but strongly expressed during myogenesis and in neuroprogenitor cells (D'Angelo et al., 2012). Gp210 is required for initiating transcription of genes essential in cell differentiation but also appears to play a role in preventing ER-stress during differentiation (Gomez-Cavazos and Hetzer, 2015).

The majority of the characterized NETs bind lamins and/or chromatin binding proteins (Harr et al., 2016; Kind et al., 2013; Wong et al., 2014). Chromatin proteins in pluripotent stem cells display hyperdynamic plasticity (Chen and Dent, 2014; Meshorer et al., 2006). During differentiation, when stem cells lose their pluripotency, chromatin becomes more static and organized (Ricci et al., 2015). Cells tether tightly packed heterochromatin to the nuclear periphery, in a Lamin A/C dependent (A-tether) and/or Lamin B receptor dependent (B-tether) manner (Solovei et al., 2013). Chromatin is first tethered by the B-tether and then during differentiation by the A-tether. The B-tether has a generally silencing effect and is made up by Lamin B and Lamin B receptor. Whereas the A-tether is important for differentiation, and involves Lamin A/C and tissue-specific inner nuclear membrane (INM) proteins, including

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chromatin binding LEM-domain proteins. Disruption of these tethering proteins affects gene expression and cellular differentiation, for example in muscle differentiation (myogenesis) (Solovei et al., 2013). The nuclear envelope is highly specialized in each specific cell type, with most NETs expressed in a tissue-specific manner, comparing three tissues (muscle, liver and leukocytes) only 16% of the NETs were shared (Worman and Schirmer, 2015). Many, not yet identified, proteins might be involved in this tethering process (Czapiewski et al., 2016) and could have their own unique function in the diversity of tissue-specific differentiation.

In this paper we used human iPSCs (hereon referred to as just iPSCs) as a model for stem cell differentiation, to study if the INM protein Samp1 (spindle associated membrane protein 1) could have a role in the differentiation process. Samp1 (Buch et al., 2009) (also called NET5 in rat liver (Schirmer et al., 2003) and Tmem201) binds directly to Emerin (Gudise et al., 2011; Jafferali et al., 2014) and is functionally associated to proteins of the A-type lamina network (Borrego-Pinto et al., 2012; Jafferali et al., 2014). Accordingly, we asked whether Samp1 could act as an A-tether component and promote cell differentiation. In this report we find that Samp1 levels increased in the nuclear periphery during differentiation in parallel with expression of the early differentiation marker Lamin A/C, and was able to induce a rapid differentiation of iPSCs despite pluripotent culturing conditions.

2. Methods

2.1. Cell culture

Human induced pluripotent stem cells (ATCC ACS-1011) were cultured in humidified atmosphere containing 5% CO2 at 37 °C, according to manufactures instructions (ATCC Human ES/iPS Cell Culture Guide: Protocols for Feeder-Free and Feeder Dependent Systems). Pluripotent stem cell SFM XF/FF (ATCC ACS-3002) medium supplemented with 0.5% penicillin-streptomycin (v/v) (Gibco™, 10378-016) was changed daily. For the undirected differentiation of the iPSCs, pluripotent stem cell medium was exchanged to DMEM:F-12 Medium (ATCC 30-2006) supplemented with 10% fetal bovine serum (FBS, v/v) (Gibco™ 10500-056) with 0.5% penicillin-streptomycin (v/v) leading to what we have termed SID (serum induced differentiation).

2.2. Subculturing

Cells were passaged every week, using a micromanipulator to scratch a section from the monolayer center of the colony to isolate undifferentiated cells without potential differentiated peripheral cells. The iPSC sections where placed on 35 mm glass bottom dishes (P35G-1.5-20-C) from MatTek Corporation, coated with CellMatrix basement membrane gel (ATCC ACS-3035), containing pluripotent stem cell media with ROCK inhibitor Y27632 (ATCC ACS-3030).

2.3. Transfection

iPSCs were transiently transfected with plasmids encoding YFP (pEYFP-N1, BD Biosciences), YFP-Emerin (Shimi et al., 2004),YFP-Samp1 or Samp1-YFP (Buch et al., 2009).

Transfection was performed for each well as follows. Plasmid solution: 1 μg of plasmid was diluted to a total volume of 50 μl with Opti-MEM® reduced serum medium (Gibco™ 11058-021). Lipofectamin solution: 3 μl Lipofectamin® 2000 (Life Technologies 11668-030) and 47 μl Opti-MEM® medium were mixed. The plasmid solution was added to the lipofectamin solution and incubated for 30 min before addition to each well containing cells and medium.

2.4. Immunofluorescence microscopy

The whole procedure was performed at room temperature and all solutions were based on PBS (1×) (Gibco 18912-014). Cells cultured on glass bottom dishes were washed 3 times for 2 min in PBS. Fixed for 30 min in 3.7% paraformaldehyde. Permeabilized in 0.5% Triton X-100 for 30 min. Followed by 3 washes and blocked in 2% Bovine serum albumin containing 0.01% NaN_3 (blocking solution) overnight. The samples were then incubated for 1 h with primary antibodies in blocking solution followed by four 2 min washes in blocking solution. The samples were then incubated with secondary antibodies for 1 h in blocking solution followed by four washes in PBS with 0.1% Tween for 2 min each before imaging directly in PBS.

Primary antibodies used for immunofluorescence were rabbit polyclonal anti-Samp1 (1:500) (Buch et al., 2009), mouse monoclonal anti-Emerin antibodies (1:500) from Santa Cruz Biotechnology (sc-25284), mouse monoclonal anti-Lamin A/C (1:1000) from Abcam (ab40567),

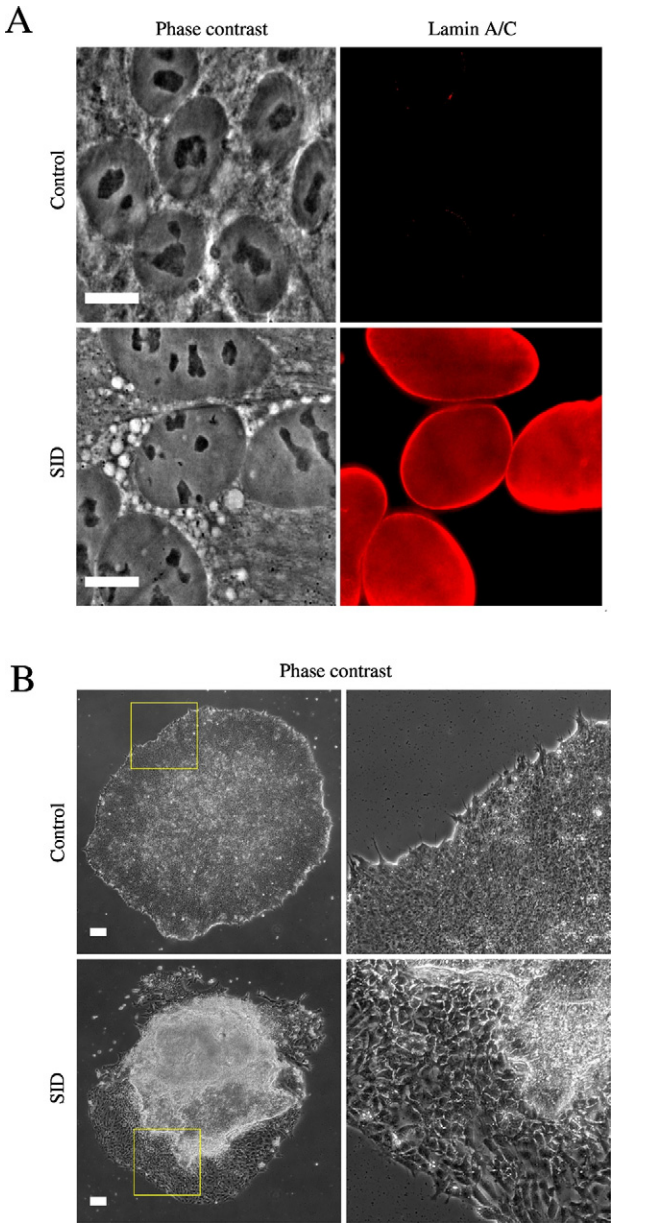

Fig. 1. Characterization of undifferentiated and differentiated iPSCs. (A) iPSCs were cultured for 6 days, in pluripotency medium (upper) or subjected to SID (lower). Immunofluorescence and phase contrast microcopy show that control (undifferentiated) iPSCs had no detectable Lamin A/C staining at equal intensity settings and typical iPSC morphology in contrast to differentiated iPSCs (that showed strong Lamin A/C staining and differentiated morphology). Scale bar, 10 μm. (B) Phase contrast images of a control (upper) and a SID (lower) colonies with 4× magnified regions (right). Undifferentiated (control) iPSCs grow in compact colonies with defined edges and uniform morphology, compared to differentiated (SID) iPSCs. Scale bar, 100 μm.