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miR-200c and GATA binding protein 4 regulate human embryonic stem cell renewal and differentiation☆

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Abstract Human embryonic stem cells (hESCs) are functionally unique for their self-renewal ability and pluripotency, but the molecular mechanisms giving rise to these properties are not fully understood. hESCs can differentiate into embryoid bodies (EBs) containing ectoderm, mesoderm, and endoderm. In the miR-200 family, miR-200c was especially enriched in undifferentiated hESCs and significantly downregulated in EBs. The knockdown of the miR-200c in hESCs downregulated Nanog expression, upregulated GATA binding protein 4 (GATA4) expression, and induced hESC apoptosis. The knockdown of GATA4 rescued hESC apoptosis induced by downregulation of miR-200c. miR-200c directly targeted the 3′-untranslated

Abbreviations: hESCs, human embryonic stem cells; EB, embryoid body; TGF-β, transforming growth factor-β; FGF, fibroblast growth factor; miRNAs, microRNAs; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

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region of GATA4. Interestingly, the downregulation of GATA4 significantly inhibited EB formation in hESCs. Overexpression of miR-200c inhibited EB formation and repressed the expression of ectoderm, endoderm, and mesoderm markers, which could partially be rescued by ectopic expression of GATA4. Fibroblast growth factor (FGF) and activin A/nodal can sustain hESC renewal in the absence of feeder layer. Inhibition of transforming growth factor-β (TGF-β)/activin A/nodal signaling by SB431542 treatment downregulated the expression of miR-200c. Overexpression of miR-200c partially rescued the expression of Nanog/phospho-Smad2 that was downregulated by SB431542 treatment. Our observations have uncovered novel functions of miR-200c and GATA4 in regulating hESC renewal and differentiation.

Introduction

Isolated from the inner cell mass of blastocysts, embryonic stem cells (ESCs) are characterized by their ability for unlimited self-renewal and pluripotency. ESCs are able to develop into almost all cell types in the body. Thus, ESCs are important for the study of both developmental biology and regenerative medicine. Human embryonic stem cells (hESCs) presumably preserve the molecular signatures of early development. Cultured in suspension, hESCs spontaneously form three dimensional spheroid aggregates called embryoid bodies (EBs) that differentiate into ectoderm, mesoderm, and endoderm, which mimic the developmental stages of the embryo from the blastocyst to gastrulation and the egg-cylinder formation (Desbaillets et al., 2000; Itskovitz-Eldor et al., 2000; Leahy et al., 1999). The observation of parallel tissue-specific gene expression patterns and signals during EB formation and embryo differentiation supports the hypothesis that ESC differentiation into EBs can serve as a tool to investigate the differentiation processes of different lineages (Keller et al., 1993; Risau et al., 1988; Sanchez et al., 1991; Wang et al., 1992). However, our knowledge about the molecular basis of the hESC–EB transition is still in its infancy. Only limited molecular details have been revealed. For example, the suppression of PI3-Kinase delays the formation of compact mouse EBs by 12 h (Gurney et al., 2011), and knockout of GATA6 induces apoptotic gene expression in mouse EBs without abrogating EB formation (Rong et al., 2012).

Transforming growth factor-β (TGF-β)/activin A/nodal signaling is essential for the self-renewal and pluripotency of hESCs (James et al., 2005). TGF-β, activin A, and nodal all belong to the TGF-β superfamily, and regulate Smad2/Smad3 signaling pathways (Heldin et al., 1997; Vallier et al., 2005). TGF-β/activin A signaling directly regulates the Nanog promoter through Smads (Xu et al., 2008). Inhibition of TGF-β/activin A/nodal signaling by SB431542, an inhibitor of TGF-β type I activin receptor-like kinase (ALK) receptors, downregulates phosphorylation of Smad2 and/or Smad3, and the expression of Oct4 and Nanog (Besser, 2004; Inman et al., 2002; James et al., 2005; Valdimarsdottir and Mummery, 2005). Activin A/nodal and the basic fibroblast growth factor (bFGF) pathways cooperate to maintain pluripotency of hESCs in the absence of feeder cells (Vallier et al., 2005). Furthermore, phosphorylation of Smad2/Smad3 induced by TGF-β signaling is decreased during early differentiation (James et al., 2005).

MicroRNAs (miRNAs) are another class of critical regulators in development. miRNAs are small (18–25 nucleotides in length), endogenous non-coding RNA molecules that regulate target genes either by degradation of mRNA transcripts or by inhibition of mRNA translation (Lee and Shin, 2012; Nelson et al., 2003). miRNAs have been proposed to play important roles in cell fate decisions and embryonic development (Gill et al., 2011; Wang et al., 2012b). Knockout of dicer, an enzyme required for miRNA biogenesis, leads to embryonic lethality in mice on day 7.5 (Bernstein et al., 2003). DGCR8 is an RNA-binding protein that functions together with the RNase III enzyme Drosha in processing of miRNAs. Mouse ESCs without DGCR8 or dicer display defects in differentiation and proliferation (Kanellopoulou et al., 2005; Murchison et al., 2005; Suh and Blelloch, 2011; Wang et al., 2007). However, the functions of the ESC miRNAs are not fully characterized (Wang et al., 2009).

The miR-200 family of miRNAs includes miR-200a, miR-200b, miR-200c, miR-141, and miR-429. Among them, miR-200a and miR-141, but not miR-200c, were observed to be regulated by c-Myc in mouse ESCs (Lin et al., 2009). The overexpression of miR-200a and miR-141 attenuated mouse ESC differentiation upon the removal of leukemia inhibitory factor (LIF) (Lin et al., 2009). In hESCs, the miR-302–367 cluster was shown to regulate cell growth, metabolism, and transcription (Barroso-del Jesus et al., 2009). The combination of miR-200c, miR-302s, and miR-369s reprogram both mouse and human somatic cells into a pluripotent ESC-like state (induced pluripotent stem cells, iPSCs) (Miyoshi et al., 2011; Samavarchi-Tehrani et al., 2010). Oct4 and Sox2 can regulate miR-200 family expression and mesenchymal–epithelial transition during iPSC generation (Wang et al., 2013). However, the functional roles of the miR-200 family in hESCs have not yet been determined.

In this paper, we have discovered a critical role for miR-200c in hESC renewal and the differentiation of all three developmental lineages that is partially mediated by directly targeting GATA4, and observed that miR-200c was reciprocally regulated by the TGF-β/activin A/nodal-Smad pathways.

Materials and methods

Materials

All cell culture reagents and qRT-PCR (quantitative real-time polymerase chain reaction) reagents were purchased from Invitrogen (Carlsbad, CA, USA), and all chemicals were obtained from Sigma (St. Louis, MO, USA), unless otherwise specified.

Cell lines and culture conditions

The hESC line H9 was purchased from WiCells (Madison, WI, USA) (Thomson et al., 1998), while HUES6 cells were kindly provided by Dr. Douglas A. Melton (Harvard University, Boston, MA, USA) (Cowan et al., 2004). hESC lines were maintained in