The regulation of confluence-based HGF and hypoxia signaling on Oct4 expression in mouse somatic cells

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ABSTRACT

The goal of this mini review is to explain the mechanism of Oct4 expression regulation by post-confluence HGF and hypoxia signaling effects. Stat3 can be activated ligand-independently by cell confluence, thus trigger cells aggregation. HGF signaling is preserved after confluence and functions in sustaining cells survival and proliferation, which might lead aggregated cells to form spheres. Hypoxia condition in spheres induces expression of Hif1α and Aid, the two regulators for Oct4 expression. Post-confluence HGF signaling stimulates β-catenin and Stat3 activity; they are critical for Oct4 expression. Nuclear receptors Esrrb, Lrh1/SF1 and RARs interact with each other to initiate Oct4 transcription through recruiting chromatin remodeling complex, as well as basal transcriptional machinery complexes and pol II. cAMP signaling produces SF1/Lrh1 ligand. Esrrb agonist or estrogen might function in activating Esrrb. RAR related Tlx could be induced by hypoxia. Taken together, it is proposed that to culture post-confluence cells with media containing HGF, Esrrb agonist or estrogen, and cAMP agonist, might lead to expression of Oct4.

Key words: Oct4 expression, confluence-dependent signaling, hepatocyte growth factor, hypoxia signaling, mouse.

INTRODUCTION

Induced pluripotent stem (iPS) cells yielded conventionally through ectopic expression of key pluripotency transcription factors, Oct4, Sox2, Klf4 and Myc in murine (Takahashi et al., 2006; Wernig et al., 2007; Okita et al., 2007; Maherali et al. 2007) and human (Takahashi et al., 2007; Park et al., 2008), or Oct4, Sox2, Nanog and Lin28 in human (Yu et al., 2007), or Oct4, Sox2, Klf4 in murine and human (Nakagawa et al., 2008). These protocols involve risk of insertional mutagenesis. The improvement methods such as non-integrating approaches (Stadtfeld et al., 2008; Sommer et al., 2009), free of vector approaches (Kaji et al., 2009; Soldner et al., 2009; Yu et al., 2009) therefore have been studied. Although it is showed that foreign genes are silenced or removed after reprogramming, those approaches are low reprogramming efficiency, and either leave residual vector sequences, or require tedious steps. Alternative non-integrating strategies based on administration of synthetic mRNAs (Warren et al., 2010) and recombinant proteins (Kim et al., 2009) are also reported, they are challenging to generate and purify in the quantities required.

It is reasonable to settle these issues by a system that reprograms somatic cells more safely and efficiently. A report showed that a set of chemical compounds is sufficient to reprogram mouse fibroblasts to iPS cells (Hou et al., 2013); this indicates the endeavor of the biological community in this direction. iPS cells can be obtained by ectopic expression of Oct4 alone from progenitor cells that already express other genes needed to make iPS cells (Kim et al., 2009), or from somatic cells treated with chemical compounds (Zhu et al., 2010). These studies showed that Oct4 is a central regulator in reprogramming of iPS cell. The molecular mechanisms of Oct4 reprogramming involve induction of MET (mesenchymal-to-epithelial transition) (Li et al., 2010) and overcoming epigenetic barriers (You et al., 2011). The present review discusses the mechanism of
regulating Oct4 expression by confluence-based HGF and hypoxia signaling. The points might be helpful for finding a novel strategy to reprogram somatic cells.

**CONFLUENCE-DEPENDENT STAT3 ACTIVATION TRIGGER CELLS AGGREGATION**

In both cancer and normal epithelial cell lines, it is revealed that Stat3 (signal transducers and activators of transcription) activity is partially suppressed by cdk2 in growing cells and derepressed upon cell confluence, that is, Stat3 can be activated ligand-independently by cell confluence (Vultur et al., 2004; Steinman et al., 2003). This envisioned a role of jak-dependent Stat3 signaling in modulating the survival-related physiological functions during cell confluence. Furthermore, ligand-independent Stat3 activation could trigger cell behavior changes after confluence. For instance, confluence-dependent Stat3 activation leads to its interaction with and recruitment Sp1/Sp3 to the NHE3 (sodium hydrogen exchanger-3) proximal promoter region, regulates NHE3 expression, thus triggering three-dimensional multicellular "domes" or hemicysts/aggregates formation above the plane of the monolayer (Su et al., 2007; Su et al., 2009) (Figure 1b). Notably, whatever Stat3 activity and its recruitment of Sp1/Sp3 to the promoter region of target gene are important for the Oct4 expression (Marin et al., 1997).

**HGF SIGNALING STIMULATES STAT3 AND β-CATENIN ACTIVITY IN POST-CONFLUENCE CELLS, AND RESULT IN PROLIFERATING OF AGGREGATES TO FORM SPHERES**

Sub-confluent versus confluent cells has differential responses to growth factor stimulation. The reason may be due to a number of factors, including gradients of the stimulatory factors around the site of cells, confluence-dependent regulation of receptor expression/activation, and confluence-dependent regulation of downstream intracellular signaling pathways. For instance, it is demonstrated that EGF (epidermal growth factor) receptor is altered in fully confluent cells, thus diminishing EGF-dependent receptor activation (Takahashi et al., 1996), and resulting in contact inhibition.

Hepatocyte growth factor (HGF), a mesenchymal-derived heparin binding growth factor also known as
scatter factor, is a pleiotropic cytokine. Unlike EGF receptor, HGF receptor c-Met is equally activated in confluent and non-confluent cells, thus sustaining cells survival and proliferation after confluence. HGF binds to receptor c-Met and activates multiple downstream signaling pathways, including the ERK (extracellular signal regulated kinase) and PI3K (phosphatidylinositol 3-kinase) pathways (Weidner et al., 1996; Fixman et al., 1997), triggers multiple cellular responses, including proliferation, migration and survival in several cell types (Weidner et al., 1993; Birchmeier et al., 2003). HGF signaling activate PI3K via two ways—mediated by adapter protein Gab1 (GRB2-associated-binding protein 1) and Fak (focal adhesion kinase) respectively. Following HGF stimulation, c-Met phosphorylates Gab1, Gab1 activates PI3K through creating docking sites for the p85 subunit of PI3K (Weidner et al., 1996; Laffargue et al., 1999). Meanwhile, c-Met mediates Fak activation, active Fak recruits p85 subunit to a Fakpaxillin protein complex at the cell membrane and subsequent PI3K activation. Activation of the PI3K stimulates Akt phosphorylation and Rac activation (Reiske et al., 1999) (Figure 1a).

In non-confluent cells treated with HGF, the high level of Akt activation results in inhibitory phosphorylation of GSK3β (glycogen synthase kinase-3 β) and increases β-catenin nucleus signaling; the increased Rac levels mediate the activation of actin cytoskeletal rearrangement that is necessary for morphogenic cell dedifferentiation and migration. In confluent cells, HGF signaling is selectively diminished. The fraction of c-Met/Fak/PI3K is diminished, while the fraction of c-Met/Gab1/PI3K is preserved; PI3K/Rac downregulated to prevent actin cytoskeletal rearrangement and cell migration; PI3K/Akt decreased but still remains to maintain low level of β-catenin (Figure 1b). Generally, nucleus β-catenin diminished after cells confluence because of growth factor (such as EGF) receptors inactivation (Ishibe et al., 2006), thus to prevent cell growth. But in case of HGF, although nucleus β-catenin signaling decreased followed c-Met/Fak downregulation, it still remains at low level to play a role in preventing the apoptotic response because of mediation by preserved c-Met/Gab1 (Ishibe et al. 2006). In conclusion, HGF signaling sustains cells survival and proliferation after confluence. These effects could result in proliferating of post-confluence aggregates to form spheres. Cell contact signaling in spheres induces Oct4 expression as mentioned below.

HGF also influences Stat3 signaling directly or indirectly. It is reported that HGF could stimulate Stat3 recruitment to c-Met at the plasma membrane, its phosphorylation on tyrosine 705, nuclear translocation, and binding to the specific promoter Sis-inducible element (Boccaccio et al., 1998). Other study reveals that HGF do not activate Stat3 directly, while HGF-Met signal cascade stimulates IL-6 production via PI3K pathway, leading to Stat3 phosphorylation as a secondary effect (Lee et al., 2009) (Figure 1b, c). Taken together, HGF signaling stimulates Stat3 and β-catenin activity in post-confluence cells. It is well known that the two factors are critical for Oct4 expression in mouse ESCs (embryonic stem cells) as effectors of pluripotency signaling (Li, 2010).

CELL CONTACT SIGNALING IN SPHERES INDUCES OCT4 EXPRESSION

It is reported that cell-cell contacts and hypoxic conditions in sphere induce Oct4 (Liu et al., 2013). When mouse fibroblasts were forced to form spheres in suspension culture, the sphere formation induce expression of Angiomotin (Amot), the Taz/Wwtr1 (transcriptional co-activator with PDZ-binding motif) -binding factor in tight junctions. Whereby retaining the Hippo cascade transcription factor Taz in the cytoplasm of cells in spheres. Translocation of the Taz to the nucleus induces expression of Zeb1 (zinc finger E-box binding homeobox 1), a transcription factor that causes EMT (epithelial-to-mesenchymal transition) (Lei et al., 2008). Therefore, cytoplasmic retention of Taz resulted in rapid loss of Zeb1. And, this loss of Zeb1 is associated with rapid MET. Subsequently, hypoxia in the interior of the spheres and loss of Zeb1 repression synergized to cause super-induction of Hif1α (hypoxia inducible factor 1 alpha). Hif1α bound to and activate the Aid (activation-induced cytidine deaminase) promoter, combine the loss of repression by Zeb1 to induce Aid. The expression of Aid coincides with demethylation of the Oct4 promoter/enhancer; Hif1α binds the Oct4 promoter and play a role in induction of Oct4 (Liu et al., 2013) (Figure 1c).

The Oct4 activators induced by hypoxia might also include RAR/RXR (retinoic acid receptors/retinoid X receptors) like nuclear receptors. Tlx is a retinoid X acid receptor-related nuclear receptor. Tlx has been identified as a mediator for proliferation and pluripotency of neural progenitors upon hypoxia. Following hypoxia, Tlx is recruited to the Oct4 proximal promoter, augmenting the gene transcription and promoting progenitor proliferation and pluripotency (Figure 1c). Tlx recruitment on the core promoter correlates with the active H3K9 acetylation and increased pol II recruitment (Chavali et al., 2008). The transcription activity of RAR/RXR on Oct4 expression depends on its interaction with other nuclear receptors, such as Esrrb (estrogen-related receptor beta) and Lrh1 (liver receptor homologue 1) as stated below.

NUCLEAR RECEPTORS COMPLEX FOR OCT4 EXPRESSION

Ligand dependent RAR/RXR activation globally counteracts core pluripotency factors action in pluripotent cells and cooperates with differentiation-associated transcription factors to repress pluripotency and induce differentiation (Chatagnon et al., 2015). On the other hand,
it is found that RARs worked as activators of Oct4 expression in a ligand-independent manner (Barnea et al., 2000; Ben-Shushan et al., 1995; Pikarsky et al., 1994). In undifferentiated cell, the activating role of RARs for Oct4 expression depends on it binding on HRE (hormone-responsive element) within the Oct4 proximal promoter and acting in synergy with Esrrb-Lrh1 (Barnea et al., 2000; Ben-Shushan et al., 1995; Pikarsky et al., 1994; Wang et al., 2011). They interact with Sp1, and recruit Pml-NB (Promyelocytic leukemia-nuclear body) and Brgl-dependent chromatin remodeling complex, as well as basal transcriptional machinery complexes and pol II. This complex creates/maintains a nucleosome-free region for Oct4 gene activity and to initiate transcription (Aoto et al., 2006; Chuang et al., 2011; Ho et al., 2009; Jin et al., 2011) (Figure 1c).

SF1 (steroidogenic factor 1) and Lrh1 supports the expression of Oct4 in pluripotent cells (Barnea et al., 2000; Chuang et al., 2011; Gu et al., 2005). SF1 is known to be related to β-catenin signaling. Lrh1 is closely related to SF1, particularly in the DNA binding domain, and has the same DNA response element as SF1. It is reported in human adrenocortical cells, CAMP-stimulated transcription of DGKθ (diacylglycerol kinase θ) which is a lipid kinase that phosphorylates diacylglycerol to form phosphatidic acid. Phosphatidic acid is a ligand for the nuclear receptor SF1 (Cai et al., 2013). So the application of cAMP agonist Forskolin will play function for producing SF1/Lrh1 ligand (Figure 1c). Forskolin is used as one of the compounds that chemically induced pluripotent stem cells lines (Hou et al., 2013), which revealed its role for somatic cell reprogramming.

Esrrb may play a generalized role in Oct4 transcriptional activation, because Esrrb interacts physically with components of the basal transcriptional machinery complexes thyroid hormone receptor-associated protein (Trap)/Mediator, TFIIID, and RNA pol II, as well as with the Trx/Mll (Trithorax/mixed-lineage leukaemia) chromatin-modifying complex and Ncoa3 (nuclear receptor coactivator 3) (van den Berg et al., 2010). Mediator, Trx/Mll, and Ncoa3 also bind to the ligand-binding domain of the estrogen receptor, which is related to Esrrb, and are essential cofactors for estrogen receptor-dependent transcriptional activation in mammary cells (Kang et al., 2002; Mo et al., 2006; Shang et al., 2000). These studies indicate that estrogen receptor may have the same function as Esrrb, or estrogen may function as Esrrb ligand. Alternatively, Esrrb can be activated by agonists. The soy isoflavones (genistein), and two highly similar synthetic small molecules GSK4716 and DY131, are known to function as agonists of Esrrb (Divekar et al., 2016) (Figure 1c).

PERSPECTIVES

Taken all considerations together, it is postulated that Oct4 expression might be induced when culturing confluent somatic cells with media containing LIF (leukemia inhibitory factor), HGF, Esrrb agonist or estrogen, Forskolin, and vitamin C. Under this culture condition, confluence-dependent Stat3 activation result in cell aggregation (Su et al., 2007). HGF signaling down-regulated but preserved in the fraction of c-Met/Gabl/P13K/Akt/β-catenin pathway (Ishibe et al., 2006), thus sustains β-catenin nucleus signaling and regulates Stat3 expression subsequently (Boccaccio et al., 1998; Lee et al., 2009). HGF stimulation and confluence effects establish co-activity of Stat3 and β-catenin in signaling in confluence cells. Also, HGF stimulated cell proliferation might result cell spheres formation; this provides cell-cell contacts and hypoxic conditions that maybe induce Oct4 expression (Liu et al., 2013). cAMP signaling produces SF1/Lrh1 ligand to activate Lrh1 (Cai et al., 2013). Esrrb agonist or estrogen might function in activating Esrrb. Lrh1 and Esrrb are essential for Oct4 expression (Li, 2010). Vitamin C is found to be of benefit for somatic cells reprogramming. It can promote MET and accelerates gene expression changes to a fully reprogrammed state (Esteban et al., 2010). LIF is a widely used factor to derive and maintain mouse ESCs. The maintenance of mouse ESCs depend on the balance between LIF signaling and BMP4 (bone morphogenetic protein 4) signaling (Li, 2010). Additionally, during long term culture without passage after cells confluence and aggregation, because of spatiotemporal change, some effects probably take place also. For example, the establishment of autocrine LIF niche which has been shown to be crucial in ESCs (Davey et al., 2006; Davey et al., 2007; Zaragosi, 2006), or inactivation of the integrin signaling that has been demonstrated crucial in promoting mouse ESCs self-renewal (Hayashi et al., 2007). All these synergistic effects might initiate Oct4 expression (Figure 1c).

Master stem cell genes such as Oct4 are silenced in somatic cells generally (Aoto et al., 2006; Marikawa et al., 2005). But studies also found that the epigenetic status of Oct4 among a population of somatic cells are heterogeneous (Marikawa et al., 2005), which indicates that at least in some cells Oct4 expression are quite possibly induced by present strategy. As a target gene of Oct4, Sox2 expressed subsequently (Catena et al., 2004); β-catenin interacts with Oct4-Sox2 complex then regulates the Nanog expression (Yukinari et al., 2007). When expression of Oct4, Sox2 and Nanog initiated, positive feedback regulation gradually makes them accumulate to a threshold that alter genome state to pluripotency.

REFERENCES


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