The role of ARF6 in human embryonic stem cell pluripotency and differentiation

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ABSTRAC

ARF6 is a low molecular weight GTPase localized to the plasma membrane and endosomal compartments. As ARF6 cycles through its active (GTP-bound) and inactive (GDP-bound) form, it regulates cell surface ligand internalisation, post internalization trafficking along the endocytic pathway(1), endosomal recycling (2) and fusion of recycling vesicles with the plasma membrane (3). Through its regulator proteins, ARF6 affects many cellular functions including receptor signaling, cell motility, adhesion (4),abscission (5) and lipid homeostasis (6). ARF6 is indispensable during embryonic development, as Arf6 knock-out leads to a lethal phenotype in mice (7).

We are interested in the membrane receptor trafficking and signaling output of the TGF-β superfamily members (TGF-β, Activin A and BMP4) in the pluripotency and differentiation of human Embryonic Stem Cells (hESCs). The ActivinA/TGF-β family ligands, maintain the pluripotent profile of hESCs (7,8), and signal through heteromeric complexes of type I and type II transmembrane serine/threonine kinase receptors which phosphorylate SMAD2/3 proteins (9). The phosphorylated SMAD2/3 proteins oligomerize with SMAD4, translocate to the nucleus and regulate transcription using a large network of interactions with transcription factors, co-activators and co-repressors (10). On the other hand, the BMP4 family ligand, which signals through SMAD1/5/8, promotes differentiation of hESCs through a similar mechanism (11). Previous results from our lab indicate that ARF6 is implicated in Activin A / TGFβ signalling. Using hESCs that over-express ARF6 or CRISPR-KO lines, we addressed the role of ARF6 in the phosphorylation of SMADs upon ligand induction. We found significant alterations in SMAD phosphorylation upon activation or inactivation of ARF6, suggesting that ARF6 is a key player in the responses of hESCs to Activin A / TGFβ family ligands.

Here we extend these studies and address the role of ARF6 in differentiation of the above genome edited hESCs to 3 germ layers, mesoderm, endoderm and ectoderm (12–14). Our results are consistent with an effect of ARF6 in the differentiation to all germ layers. KO ARF6 hESCs exhibit enhanced expression of key markers of mesoderm (MIXL1, WNT3) following induction by BMP4 (14). Markers of endodermal differentiation (SOX17, GATA4, FOXA2) induced by Activin A were also enhanced by

ARF6 KO. In addition, PAX6, a marker of neuroectoderm differentiation induced under chemically defined conditions (12) was also enhanced in the absence of ARF6. We present our findings and discuss their significance.

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