The Role of ARF6 in TGF-β family signaling in differentiated and human Embryonic Stem Cells

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ABSTRACT

ARF6 is a low molecular weight GTPase localized to the plasma membrane and endosomal compartments where it regulates many processes including endocytosis [1], exocytosis, recycling and actin remodeling [2]. In early embryonic development, ARF6 has been found to be indispensable, as Arf6 knock-out leads to a lethal phenotype in mice. 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 Activin A/TGF-β family ligands are key players in sustaining the pluripotent profile of hESCs, whereas BMP4 is involved in differentiation [3]. These members signal through heteromeric complexes of type I and type II transmembrane serine/threonine kinase receptors, which phosphorylate SMAD proteins. Phosphorylated SMADs oligomerize with SMAD4, translocate to the nucleus and regulate transcription using a large network of interactions with transcription factors, co-activators and co-repressors. Here we describe a novel role of Arf6 in regulating Activin A/TGF-β responses. Using differentiated cells and 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 and differentiated cells to TGF-β family ligands. We have addressed the significance of these results and present our findings.

REFERENCES

1. Schweitzer, J. K., Sedgwick, A. E., & D'Souza-Schorey, C. ARF6-mediated endocytic recycling impacts cell movement, cell division and lipid homeostasis. *Sem. Cell & Developmental biology*, *22*(1), 39-47, Sep. 2010.
2. C. D'Souza-Schorey and P. Chavrier, "ARF proteins: roles in membrane traffic and beyond.," *Nat. Rev. Mol. Cell Biol.*, vol. 7, no. 5, pp. 347–58, May 2006.
3. R.-H. Xu, X. Chen, D. S. Li, R. Li, G. C. Addicks, C. Glennon, T. P. Zwaka, and J. a Thomson, "BMP4 initiates human embryonic stem cell differentiation to trophoblast.," *Nat. Biotechnol.*, vol. 20, no. 12, pp. 1261–4, Dec. 2002.