

Defective insulin receptor signaling in hPSCs skews pluripotency and negatively perturbs neural differentiation

Friday, 30 Apr 2021



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Published in *J. Biol. Chem* on 3rd March 2021

Abstract

Human embryonic stem cells are a type of pluripotent stem cells (hPSCs) that are used to investigate their differentiation into diverse mature cell types for molecular studies. The mechanisms underlying insulin receptor (IR)-mediated signaling in the maintenance of human pluripotent stem cell (hPSC) identity and cell fate specification are not fully understood. Here, we used two independent shRNAs to stably knock down IRs in two hPSC lines that represent pluripotent stem cells and explored the consequences on expression of key proteins in pathways linked to proliferation and differentiation. We consistently observed lowered pAKT in contrast to increased pERK1/2 and a concordant elevation in pluripotency gene expression. ERK2 chromatin immunoprecipitation, luciferase assays, and ERK1/2 inhibitors established direct causality between ERK1/2 and OCT4 expression. Of importance, RNA sequencing analyses indicated a dysregulation of genes involved in cell differentiation and organismal development. Mass spectrometry-based proteomic analyses further confirmed a global downregulation of extracellular matrix proteins. Subsequent differentiation toward the neural lineage reflected alterations in SOX1⁺PAX6⁺ neuroectoderm and FOXG1⁺ cortical neuron marker expression and protein localization. Collectively, our data underscore the role of IR-mediated signaling in maintaining pluripotency, the extracellular matrix necessary for the stem cell niche, and regulating cell fate specification including the neural lineage.

Figure:

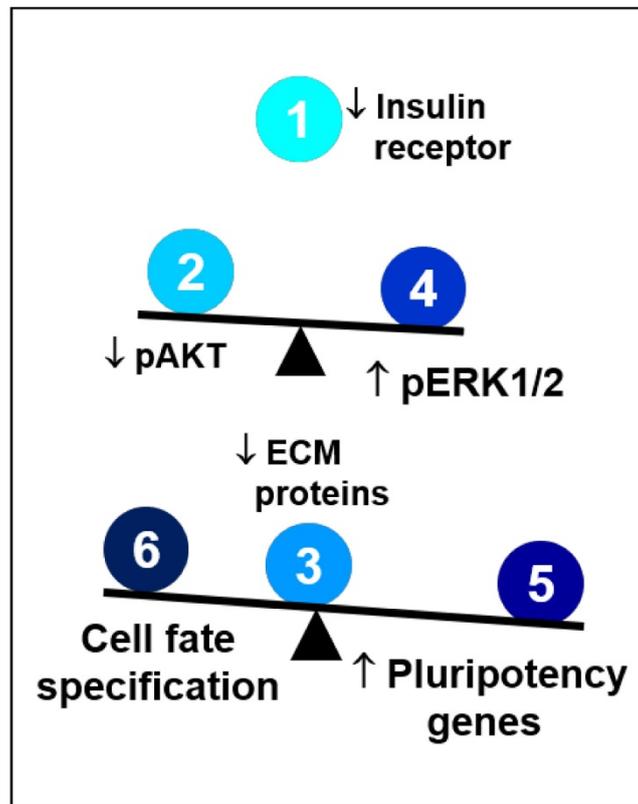


Figure legend: Knockdown of IR in hPSCs perturbs neuroectoderm differentiation and subsequent formation of cerebral organoids. Summary model depicting (1) shIR-hPSCs with decreased (2) pAKT and (3) ECM protein expression, (4) elevated pERK1/2, and (5) elevated pluripotency gene expression, resulting in (6) perturbations in cell fate commitment gene expression, including that of the neural lineage. hESC, human embryonic stem cell; hPSC, human pluripotent stem cell; IR, insulin receptor.