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Notch1 signaling promotes neuro-ectodermal differentiation of embryonic stem cells via the Notch target gene Sox9
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The Notch pathway plays a pivotal role in the control of cell fate decisions during differentiation of various tissues. In early embryogenesis, activated Notch signaling favours the formation of ectoderm at the expense of mesendoderm. In the development of the nervous system, Notch promotes the maintenance of neural stem cells, inhibits differentiation into neurons and commits neural progenitors to a glial fate. Recently we have shown that Notch signaling regulates expression of genes playing key roles in cell differentiation, cell cycle control and apoptosis in a highly context dependent manner. In embryonic stem cells under neuro-ectodermal differentiation conditions, Notch1 activation results in the upregulation of Pax6 and Sox9 RNA and protein. Sox9 and Pax6 expression is induced by activated Notch1 also in the absence of protein synthesis, suggesting that Pax6 and Sox9 may be direct Notch1 target genes. Pax6 promotes neural stem cell maintenance and is required for the specification of a neuronal fate. Sox9 specifies glial cells, promotes glial differentiation and inhibits neuronal differentiation. To understand the molecular mechanisms by which Notch mediates the neuro-ectodermal lineage choice, we combined a tamoxifen-inducible system to activate Notch signaling in embryonic stem cells differentiating in vitro towards the neural lineage with an siRNA-based strategy to simultaneously knock down Notch target genes. Using a neural monolayer differentiation protocol that mimics neural differentiation in vivo, we activated Notch1 signaling and simultaneously knocked down Sox9 or Pax6 and monitored differentiation along the neuro-ectodermal lineage by analyses of morphology, RNA expression and FACS analysis of neuronal and glial cell specific markers. Activation of Notch signaling in ESC inhibited neuronal differentiation and promoted gliogenesis. These effects were reversed by adjusting Sox9 expression levels back to uninduced levels during Notch1 activation. Knock down of Notch1-induced Pax6 expression in ESC did not alter the outcome of Notch signaling on neural differentiation. We thus conclude that Sox9 but not Pax6 is involved in mediating Notch1-induced neuro-ectodermal lineage determination.