Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700013

Notch1 regulates chondrogenic and neuroectodermal differentiation via the novel target gene Sox9

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Abstract

Objectives: Notch signaling is a crucial cell-cell communication pathway affecting cell-lineage decisions, proliferation, apoptosis, self-renewal and differentiation processes. In the canonical pathway, Notch is thought to mediate its function via the transcription factor RBP-J mainly by increasing the expression of the target proteins of the Hes/Hey family. Recently, we described novel cell context dependent Notch1 target genes that comprise a high percentage of transcription factors. Among these we found Sox9 to be regulated under various differentiation conditions.

Methods/Results: Here, we describe that during mesodermal differentiation as embryoid bodies this Notch1 induced upregulation of Sox9 had its maximum at day 4 and diminished at day 10. Furthermore, by inhibition of protein synthesis as well as luciferase experiments with RBP-J binding site mutants of Sox9 promoter reporter constructs, Sox9 was shown to be a direct target gene. To further investigate the role of Sox9 for Notch1 mediated effects, we employed a siRNA strategy to specifically quench the Sox9 peak induced by Notch1 induction without affecting induction of other Notch1 target genes. For chondrogenic differentiation we found that a temporary activation of Notch1 during the early stages of EB formation resulted in a strong increase in chondrogenic differentiation during later stages. This increase in cartilage development could be entirely reversed by the application of Sox9 siRNA, whereas the known blockage of cardiac differentiation by Notch1 was unaffected by the reduced Sox9 levels. In another set of experiments we used the same Sox9 siRNA strategy to investigate the role of Sox9 in Notch-mediated cell lineage decisions during neuroectodermal development. Notch is known to play a role in the decision between glial and neuronal cells as well as for the induction of neural crest differentiation, though the molecular basis if these effects are not understood. Our experiments revealed that in neuroectodermal differentiation conditions Sox9 is also a direct target gene of Notch1. Notch1 induction led to a strong increase in glial cell formation while inhibiting the generation of neurons. Quenching of the Sox9 peak induced by Notch1 signaling using Sox9 siRNA led to a significant decrease of glial cells, demonstrating the pivotal role of Sox9 in mediating Notch1 signals in this context.
Conclusion: In summary, our data indicate that the effects of Notch signaling are not only mediated through the well-described Hes/Hey family. The novel direct Notch1 target gene Sox9 described here plays an essential role, both, in chondrocytic development as well as in neuroectodermal differentiation, thus emphasizing the importance of alternative mechanisms in Notch signal transduction.

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Published online 10 Jun 2012