P55 Notch signaling as a key regulator of hESC self-renewal and differentiation potential

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Objectives:

1. Notch signaling is critical for the maintenance of undifferentiated state of hESCs?
2. To reveal how Notch signaling control the self-renewal/differentiation decision, and coordinate the proliferation/differentiation balance.

Materials and Methods:

1. Immunostaining for antibodies to Notch1, SSEA4, Tra-1-60 and Oct-4.
2. Undifferenetiated ES colonies are selected by AP staining after hESCs were cultured in ES media + GSIs for 4-5 days.
3. Quantitative RT-PCR analysis of Notch related genes expression during the spontaneous differentiation of hESCs into either EB or AEB (attached embryoid body).

Results:

1. Intact Notch1 signaling is highly active in hESCs. RT-PCR analysis of Notch1 and its downstream target genes including RBP-J, Deltex1, Hes-1, Hey-1 is shown in hESCs, EC cells and STO cells.
2. Notch1 signaling is downregulated as hESCs spontaneously differentiate. Two ligands of Notch are also downregulated as hESCs spontaneously differentiate.
3. Inhibition of Notch signaling disrupts an undifferentiated state of hESCs. Inhibition of Notch signaling leads to accelerated differentiation even in the presence of exogenous bFGF.
4. Blocking Notch signaling results in heterogeneous lineage differentiation of hESCs.

Conclusions:

Here, we found that endogenous Notch signaling was active in hESCs *in vivo* and downregulated as hESCs differentiated spontaneously and show that Notch signaling is required for self-renewal and inhibition of differentiation. Our findings suggest Notch signaling as a key regulator of hESC self-renewal and differentiation potential.

Key words: Notch, Self-renewal, Differentiation, RBP-J, hESCs