Cell Prolif. 2009 Feb;42(1):63-74.

Continuous hypoxic culturing maintains activation of Notch and allows long-term propagation of human embryonic stem cells without spontaneous differentiation.

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OBJECTIVE: The maintenance of pluripotency of human embryonic stem cells (hESCs) requires a high efficiency of self-renewal. During in vitro propagation, however, hESCs have a propensity to differentiate spontaneously. In this study, we assessed the nature of hESC responses to hypoxic conditions. MATERIALS AND METHODS: Human embryonic stem cells were grown in normoxic and hypoxic conditions, and the cells expressing Oct4 and stage-specific embryonic antigen-1 were identified by indirect immunofluorescence. The transcriptional expression of Nanog, Notch1, and Oct4 was determined by a real-time reverse transcription-polymerase chain reaction, and the inhibition of Notch-mediated signalling was achieved with a gamma-secretase inhibitor. RESULTS: In contrast to culture at 21% oxygen, where the colonies displayed a marked degree of differentiation, we found that during exposure to 5% oxygen, the hESC colonies displayed a homogenous and flat morphology that was consistent with the presence of Oct4-positive phenotype, indicating no spontaneous differentiation. When cultured at 5% oxygen for either 4 weeks or up to 18 months, high levels of Nanog and Notch1 transcriptional expression were detected, albeit the expression was significantly lower during longer exposure. The suppression of differentiation was rapidly reversed on transfer of the hypoxic cultures to normoxic conditions. Looking into the molecular mechanisms of the maintenance of self-renewal at low oxygen tensions, we found that inhibition of Notch signalling fully abrogated the hypoxic induction of undifferentiated phenotype. CONCLUSION: Our data, thus, indicate that hypoxic exposure has the capacity to sustain long-term self-renewal of hESCs and that this effect is mediated through activation of Notch.