

LOW OXYGEN TENSION MAINTAINS SELF-RENEWAL AND ENHANCES IN VITRO DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

Hsin-Fu Chen^{1,2}, Hung-Chih Kuo³, Chung-Liang Chien⁴, Ming-Shan Chiang¹, Hong-Nerng Ho¹

¹ Dept Of Ob/Gyn College of Medicine and The Hospital, National Taiwan University (Taiwan)

² Graduate Institute of Clinical Genetics, College Of Medicine And The Hospital, National Taiwan University (Taiwan)

³ Institute of Cellular and Organismic Biology & Genomics Research Center, Academia Sinica (Taiwan)

⁴ Department of Anatomy and Cell Biology, College Of Medicine NTU (Taiwan)

Purpose: Hypoxic (low oxygen tension, at the range of 2-5% O₂) culture is theoretically more physiological than normoxic culture (20-21% O₂) for embryos and human embryonic stem (hES) cells, due to much lower oxygen tension in uterine endometrial cavity than in atmosphere. It has been reported that hypoxic culture improves the growth of hES cells, although in practice the beneficial effects occur only in certain conditions (for example, after prolonged culture) as was reported by us recently. However it remains un-established whether hypoxic culture also exerts any (beneficial) effects on the differentiation of hES cells and whether it exerts its action through modulations of the major signaling transduction pathways, including FGF, TGF/GMP and Wnt pathways.

Methods And Materials: NTU1 hES cells which had been derived, published and continuously maintained in this lab for a long period were cultured in different O₂ tensions (2% and 21%) for self-renewal (undifferentiation state) and/or differentiation to embryoid bodies. The cellular development, imprinting status, gene expression and protein products of FGF, TGF/GMP and Wnt signaling pathways of hES cells, the development to embryoid bodies and their differentiated gene expression were analyzed by morphological criteria, bisulfite sequencing, real-time quantitative PCR, Western blot analysis, and flow cytometry.

Results: The morphology of hES cells was improved in hypoxia after prolonged culture without splitting (for 10-14 days), but the cells proliferated more slowly. Real-time PCR and Western blot analysis showed that most of the tested genes for undifferentiated hES cells were enriched and down-regulated upon differentiation (Oct4, Nanog, FGF2, FGF13, FGFR1, FGFR4, GDF3, LeftyA, LeftyB, Nodal, Cripto, FRAT2, Fzd5), though there were differential gene expressions between hypoxia and normoxia, including FGFR1, FGFR2 and FRAT2, but not genes in TGF/GMP pathway. Although HIF-1 transcript remained unchanged, HIF-1 protein level was increased in hypoxia, confirming the action of hypoxia. The bisulfite sequencing data showed that the imprinting status (H19, Kv, PEG3 DMR) were not different between the two oxygen tensions. The efficiency (morphology and number) of embryoid body formation was enhanced in hypoxia. In addition there was differential gene expression patterns in the embryoid bodies, including the increased expressions of Sox17 (endoderm) but decreased expressions of and CDX2 (placental formation), Desmin (mesoderm) and Pax6 (ectoderm) in hypoxic culture.

Conclusions: 2% O₂ culture was truly hypoxic to the hES cells. This hypoxic culture maintained significantly more favorably the undifferentiated state of hES cells than in normoxic culture and the imprinting status of hES cells is stable in hypoxia. In addition, hypoxic culture is especially useful for the in vitro differentiation of hES cells to embryoid bodies. Hypoxia likely exerted its action through modulation of the major signaling pathways (FGF, TGF/GMP and Wnt pathways), and some lineage-specific genes, which makes hypoxia a specific choice for maintaining or differentiating hES cells to specific lineages.