Genetic profile of human embryonic stem cells (hESC) grown under different O_2 concentrations

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CENTRO DE INVESTIGACION

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Introduction

The development of mammalian embryos during the early stages takes place in a hypoxic environment. After 11 weeks of pregnancy the mother's blood begins to flow freely in the placental space increasing the partial pressure of oxygen [1-3]. Therefore, we hypothesized that *in vitro* growth of hESC in normoxic conditions (21% O_2) may change the profile and differentiation potential of hESC.

In this study, we employed microarray analysis to examine gene expression in hESC, H9 line, grown under hypoxic (5% and 1% O_2) or normoxic (21% O_2) conditions.

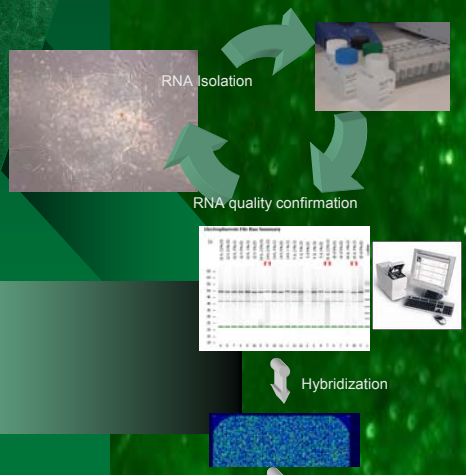
Materials and Methods

The cells grown under hypoxic (5% and 1% O_2) or normoxic (21% O_2) conditions in hESC medium [Knockout Dulbecco's modified Eagle's medium (DMEM, Invitrogen), 100µM beta-Mercaptoethanol (Sigma-Aldrich), 1% nonessential amino acids (Invitrogen), 1% glutamax (Invitrogen), 20% serum replacement (Invitrogen)], were collected at different time points: 0, 12, 24 hours and 5, 10 and 15 days. The experiments were repeated at least twice in a identical fashion.

RNA was extracted from the cells using the High pure RNA Isolation kit (Roche) according with the manufacturer's protocol.

RNA was quantified by spectrometry (NanoDrop ND1000, NanoDrop Technologies) and the quality was confirmed by RNA 6000 Nano Bioanalyzer (Agilent Technologies) assay. 800 ng of total RNA were used to produce Cyanine 3-CTP-labeled. Labeled cRNA was hybridized with Whole Human Genome Oligo Microarray Kit (Agilent p/n G4112F) containing 41,000+ unique human genes and transcripts.

Microarray data were normalised using the packages "Limma" and "Affy" (Bioconductor project) [4]. Differential gene expression was assessed using "Limma" and functional profiling analysis was performed using FatiScan tool (Babelomics project) [5].



Aim

To study the effects of normoxia and hypoxia on growth and genetic profile of hESC.

Results and Discussion

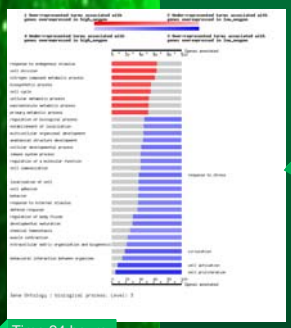
FatiScan allows to integrate the biological information in combination with the experimental results with the purpose of finding groups of genes with a significant statistically change in the behaviour (Babelomics project) [5].

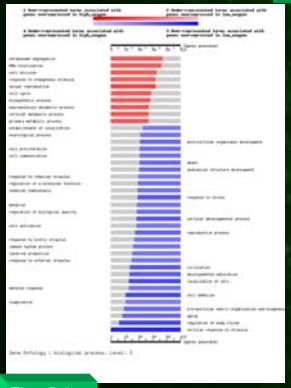
The next panels are representation of the significant terms associated with genes overrepresented in high and low oxygen concentration during the different time points (see materials and methods). The red labels are related with high oxygen concentration and the blue labels with hypoxia. The bar represents the amount of genes annotated to the functional term.

The boxplot represents the distribution of all genes Inside each array.

Functional profiling analysis


Time 12 hours


Time 24 hours


Time 5 days


Time 10 days

Overall, our analysis showed that hESC grown under hypoxia exhibit a remarkable increase in expression levels of genes associated with development, differentiation, regulation of cell proliferation, extracelular matrix organization and biogenesis.

Nevertheless in normoxia, the significant terms associated with genes overrepresented in this conditions are terms related to metabolism: macromolecule metabolic process, primary metabolic and genes include in cell cycle, response to endogenous stimulus (DNA repair) (data no shown).

Conclusions

The results obtained in this study could be used to: i) improve conditions for *in vitro* growth of undifferentiated hESC; ii) drive differentiation of hESC; iii) understand the mechanisms of cell signaling pathways activated by hypoxia, and iv) examine the processes that prevents cellular damage.

References:

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http://www.babelomics.org