^{1}H-NMR Metabolic Profiling of the *In Vitro* Hypoxic Response of H9 Human Embryonic Stem Cells

D. A. MacIntyre[1], S. Prado Lopez[2], B. Jiménez[1], D. Melguizo[2], R. Moreno Palanques[2], M. Stojkovic[2] and A. Pineda Lucena[1].

[1]Structural Biology Laboratory, [2]Cellular Reprogramming Laboratory, Centro de Investigación Príncipe Felipe, 46012, Valencia, Spain.

Human embryonic stem cells (hESCs) are exposed to hypoxic environment *in vivo*[1]. Evidence suggests that this environment promotes and maintains their ability to self-renew[2] whilst inhibiting spontaneous differentiation[3]. Despite this, the underlying role of hypoxia in stem cell differentiation and renewal is poorly characterised. The aim of this study was to use ^{1}H-Nuclear Magnetic Resonance (NMR)-based metabolomics to examine changes in metabolic profiles of hESCs associated with hypoxic conditions *in vitro*. Specifically our objectives were to identify and quantify metabolites that change in accordance with a hypoxic environment. To achieve this H9 hESCs were grown in normoxic (21%) or hypoxic (5% and 1% O_2) conditions using a feeder free culture system (n=5 for all sample groups). Media and cells were collected during a time course (0, 12, 24 and 72 hrs). Water-soluble and lipid-soluble cellular metabolites were extracted and ^{1}H-NMR spectra acquired via a Bruker 600 MHz spectrometer using a customized CPMG-1D pulse sequence. Spectra were integrated by rectangular bucketing using AMIX (V3.8, Bruker) and the resulting data analyzed using Principal Components Analysis (PCA) and Partial Least Squares Discriminatory Analysis (PLS-DA). Identification of metabolites of interest was performed using the Bruker NMR Metabolic Profiling Database and reference to the literature. Marked changes in the metabolic components of hESC culture media could be detected after 12hrs at both 5% and 1% O_2 concentration. These changes included decreased levels of glucose and increased levels of lactate, formate and acetate. Major differences between the profiles of water-soluble and lipid-soluble metabolites from hESCs in normoxic or hypoxic conditions were also detected at all time points. Our results clearly show that ^{1}H-NMR based metabolomics is a useful tool for assessing changes in the metabolic pathways of hESCs *in vitro*. The metabolic changes identified in this study may reflect functional metabolic pathways underpinning the role of hypoxia in maintaining and promoting hESC renewal.

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