560c Effects of Oxygen on Murine Embryonic Stem Cell Energetics and Growth

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Introduction: Prior to vascularization *in vivo*, embryonic stem (ES) cells and many of their differentiated progeny exist in an environment with an oxygen partial pressure (pO_2) that is often far less than 40 mmHg. Most *in vitro* culture of these cells is done in a 5% CO_2 / 95% air incubator that produces a gas phase pO_2 of 142 mmHg. As an initial step to test whether culture pO_2 significantly affects ES cell growth and differentiation, we studied the proliferation and cellular energetics of undifferentiated CCE and D3 murine ES cells maintained in different known pO_2 conditions. **Methods:** ES cells were grown attached to gelatin coated culture dishes using DMEM containing 1000 units/ml leukemia inhibitory factor. Gas phase pO_2 was controlled by placing culture dishes in small airtight containers purged with premixed gas containing 5% CO_2 and either 0, 1, 5, or 40% oxygen, resulting in environments with pO_2 values of 0, 7, 36, or 286 mmHg, respectively. Culture in a standard incubator was used as a control. We measured cell growth rate, oxygen consumption rate (OCR) immediately following cell detachment and reoxygenation, lactate production rate (LPR), cell specific lactate dehydrogenase (LDH) activity, and ATP content. We estimated the cell surface pO_2 by equating oxygen diffusion across the culture medium to the measured OCR. ATP production rate (APR) by aerobic and anaerobic metabolism, respectively, was estimated by assuming 6 mol of ATP were produced per mol of O_2 consumed and 1 mol of ATP was produced per mol of lactate formed. Cells were verified to be undifferentiated by flow cytometric analysis of Oct-4 and SSEA-1 expression. **Results:** ES cell specific growth rates were about 0.6 day^{-1} at a pO_2 of 0 mmHg, increased to about 1.15 day^{-1} in the range of 40-142 mmHg, and decreased to 0.9 day^{-1} at 286 mmHg. OCR decreased from about 2.9 nmol/10^8 cell sec at 142 mm Hg to 2.4, 1.7, and 1.0 nmol/10^8 cell sec at 36, 7, and 0 mm Hg, respectively. Over the same pO_2 range there were concomitant increases in LPR and LDH activity (about 2-fold). The estimated APR was constant down to 7 mmHg, followed by a 20% decrease under complete anoxia. There was no significant decrease in intracellular ATP content or cell viability over the entire range of pO_2 values studied if sufficient medium was used to prevent excessive waste accumulation. Anaerobic metabolism accounted for 40% of the total ATP produced at 286 and 142 mm Hg, and 50, 70, and 100% at 36, 7, and 0 mmHg, respectively. **Conclusions:** ES cells utilize anaerobic metabolism for a significant fraction of their total energy production, even after having been grown at high pO_2 conditions (142 mmHg) for extended periods of time. With decreasing pO_2, ES cells further down-regulate aerobic and up-regulate anaerobic metabolic pathways well before oxygen becomes limited. Our results imply that precise oxygen control is not critical in bioprocess development for the culture of undifferentiated stem cells. Maintaining a pO_2 of 40 mm Hg or above is probably preferable to minimize lactate production, but the cells are not sensitive to either higher or lower pO_2 conditions. As long as glucose and other nutrients and growth factors are present, and excessive amounts of wastes do not accumulate, ES cells are tolerant of any pO_2 less than about 300 mm Hg. Further work needs to be done to determine whether human ES cells behave in a similar manner.