Metabolic Changes of Human Embryonic Stem Cells During Cardiomyocyte Differentiation

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Introduction

Cell transplantation using derivatives of adult, embryonic stem cells (ESCs) or induced pluripotent stem cells (iPS) is a promising therapeutic approach for heart failure. Despite positive preliminary findings, graft size and degree of cardiac repair are insufficient to restore heart function. Limited oxygenation and energy substrate delivery to the infarcted area cause depletion of high energy phosphates (ATP, PCr) and death of transplanted cardiomyocytes. ATP is the major form of chemical energy in cells. ATP is produced via two pathways: oxidative phosphorylation of nutrients (aerobic pathway) and glycolysis, a biochemical conversion of glucose into pyruvate (anaerobic pathway). In this study we evaluated changes in the energy metabolism of human ESCs during differentiation from the pluripotent (undifferentiated) phase to energetically competent, contracting cardiomyocytes. Understanding ESCs energetics during differentiation will help to optimize transplantation conditions, techniques and timing.

Materials and Methods

Human embryonic stem cells (huESCs) were maintained in mouse embryonic fibroblast-conditioned media supplemented with bFGF (5 µg/ml). Beating cardiomyocytes were generated from huESCs using the directed differentiation protocol [1]. Oxidative phosphorylation and glycolysis rates were measured in cell culture using a Seahorse XF24 analyzer. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were normalized to cell number; results are represented per 20,000 cells. High resolution 31P NMR spectroscopy on live cells was performed using a Bruker 600 MHz spectrometer. Cells were placed in a 10mm NMR tube in the oxygenated cell growth media, temperature was maintained at 37°C. huESCs were studied at 2 time points at the pluripotent stem cell phase and one month after initiation of differentiation (beating cardiomyocytes phase). 31P spectrum was acquired using a block pulse sequence with 100 ppm sweep width, 8770 data points and 2048 signal averages. Comparison of 31P compounds were made by measuring the integrals of the peaks that had a 15-Hz line broadening.

Results

Our data show that undifferentiated huESCs are metabolically active and generate ATP mainly through oxidative phosphorylation (OCR 495±95 pMoles/min for 20,000 cells) as well as by glycolysis (ECAR 2.16±0.13 mpH/min). Inhibition of the mitochondrial F1Fo-ATP synthase with oligomycin (2.5 µM) decreased respiration of undifferentiated huESCs by 10-fold to 45±11 pMoles/min (figure 1, injection point A). Uncoupling oxidative phosphorylation from ATP synthesis with CCCP (0.5 µM; figure 1, injection point B) is expected to induce maximal respiration without ATP production; the OCR of undifferentiated huESCs increased to 285±48 pMoles/min, but did not reach the baseline level, reflecting no reserve respiratory capacity in pluripotent huESCs. The rate of glycolysis for undifferentiated huESCs, measured by extracellular acidification rate (ECAR), was 2.16±0.13 mpH/min and did not increase significantly in response to mitochondrial stress (figure 2). Differentiated huESC-derived cardiomyocytes demonstrated opposite trends. OCR at the baseline conditions was 189±85 pMoles/min for 20,000 cells, which was 2-folds lower than for the same number of undifferentiated cells; however maximal oxidative capacity of differentiated cardiomyocytes (respiration reserve) was 5-folds higher than for undifferentiated huESCs. The basal rate of glycolysis (ECAR) in differentiated huESC-cardiomyocytes was 3.43±0.66 mpH/min, which was higher than in undifferentiated cells. ECAR increased to 5.49±1.53 mpH/min after inhibition of mitochondrial respiration with oligomycin and increased 2-fold (to 6.27±0.39 mPh/min) after uncoupling of membrane potential with CCCP (figure 1). 31P NMR spectroscopy demonstrated high ATP content in fully differentiated beating huESC-derived cardiomyocytes, but no phosphocreatine (PCr), which is expected since PCr is not synthesized in cardiomyocytes (figure 3). The largest peak on the spectrum represents inorganic phosphate (Pi) content in the cell media.

Discussion and Conclusions:

Despite previous studies showing that anaerobic glycolytic metabolism is sufficient for mouse ESCs homeostasis [2], we demonstrated that undifferentiated human ESCs have active mitochondrial metabolism reflected by a high respiratory rate. These results are in agreement with other studies showing that huESCs generate their ATP through oxidative phosphorylation [3]. Our data also demonstrate greater metabolic flexibility of differentiated cardiomyocytes characterized by a quick shift of ATP production from respiration to glycolysis in conditions where mitochondrial ATP production is impaired. Measurement of respiration and glycolysis rates by extracellular flux analysis using and assessment of high energy phosphates by 31P NMR spectroscopy are complementary to each other for evaluation of metabolic activity on live cells.

Figure 1. Average OCR vs Time (normalized to 20,000 cells)

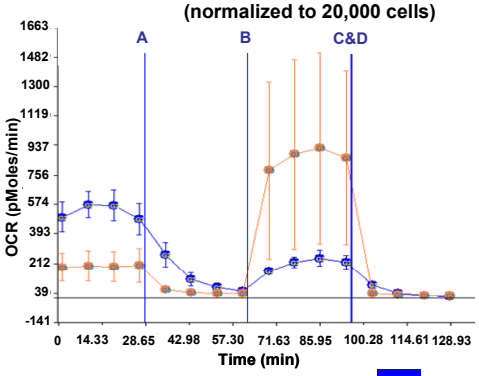


Figure 2. Average ECAR vs Time (normalized to 20,000 cells)

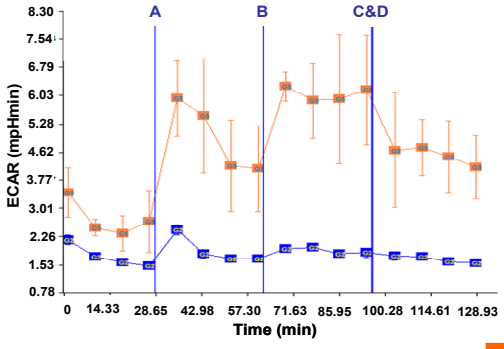
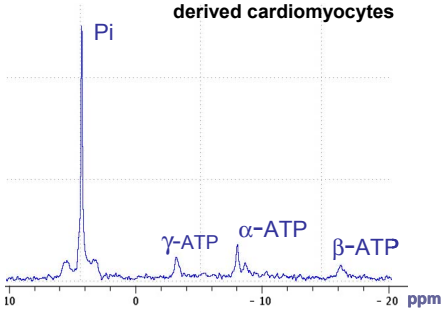


Figure 3. 31P spectrum of beating hESC-derived cardiomyocytes



Undifferentiated hES cells are marked blue Differentiated hESC-derived cardiomyocytes are marked orange

References:

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