Primary Human Cardiomyocyte Culture: A Model for Assessment of Cardiovascular Disorders

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Abstract

**Background:** Animal model systems have long been used to simulate and study human cardiac physiology and pathophysiology. However, application from the laboratory setting to human clinical studies has proven to be difficult to replicate. The purpose of this research is to establish an *in vitro* cardiac system using primary human cardiomyocyte cultures. We hypothesized that the neurotransmitter, norepinephrine (NE), and hormone, endothelin-1 (ET-1), will contribute to human cardiomyocyte growth and development of the contractile apparatus.

**Methods and Results:** Primary human cardiomyocyte cultures (PHCC) were obtained from Celprogen, San Pedro, CA. PHCC of the 6th passage were grown in the presence of NE (1, 10, 100 mmol) in T-25 flasks for 72 hours. Cells were harvested to measure percent viability post-treatment. Protein expressions of contractile proteins were analyzed via immunocytochemistry and western blot. Analysis of confocal images showed a significant decrease in nuclear area in all three treatment groups. NE (10 mmol) stimulated significant increases in fluorescent intensities of the contractile proteins, f-actin, troponin and tropomyosin. Immunoblot analysis revealed no significant changes in protein expressions of a-actin, tropomyosin, and myosin heavy chain (MYH11). The protein expression of myosin light chain (MYL3) was observed significantly lower with increasing concentrations of NE.

PHCC of the 5th passage were grown in the presence of ET-1 (1, 10, 100-nmol) in T-25 flasks for 72 hours. Analysis of confocal images showed a significant increase of nuclear area in all three treatment groups. In addition, the average cell area increased in all groups, which is characteristic of ET-1 as a hypertrophic agent. In contrast to the NE data, significant decrease of fluorescent intensities was observed in the contractile proteins, f-actin, troponin and tropomyosin.

**Conclusion:** The 5th and 6th passage of PHCC were 78 - 95% viable and appear healthy on visual inspection suggesting that these cells can be maintained in our laboratory conditions. The cells were found 60% confluence following 72-hours of incubation in the growth media. The PHCC with or without treatment of NE or ET-1 exhibit the presence of f-actin, troponin I, tropomyosin and MLC suggesting that PHCC possess abundant contractile proteins to support contractile function of cardiomyocytes. ET-1 appeared to exhibit a decrease on the expression of contractile proteins in PHCC. NE 10 mmol provided the most promising data in regards to increasing development of contractile proteins. Therefore, NE 10 mmol may be a useful additive to the growth media for assessment of contractile function in future studies.

**Recommended Citation**