Effect of T3 on human induced pluripotent stem cell-derived cardiomyocyte maturation

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Purpose: Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) hold great potential for regenerative medicine and in vitro screening. However, the phenotype is immature and more closely resembles foetal/neonatal cardiomyocytes. The aim of this study is to drive hiPSC-CM to a more mature state using thyroid hormone, triiodothyronine (T3), which has a role in cardiomyocyte maturation during development.

Methods: hiPSC-CM (Cellular Dynamics) were treated with 30nM T3 for up to 4 weeks. Expression of β1 adrenergic receptor (β1AR) was assessed by qPCR (Taqman). Beating rates were determined using a Nikon Eclipse TE2000E microscope with Digital sight camera and NIS Elements3.2 software video tracking. Ca2+ dynamics were studied using Fluo-4AM loaded cells imaged with a Nikon Eclipse T6200 and Redshirt NeuroCMOS camera at 0.5KHz with 1Hz, 15V, 5ms field stimulation. Comparative proteomic analysis (4 week T3) was performed by mass spectrometry using isobaric tags. Data expressed as mean ± SEM, statistical significance determined by student’s t-test (n=3).

Results: 4 week T3 treatment resulted in a 1.5-fold increase in basal beating rate. T3 increased β1AR gene expression and remained stable for up to 2 weeks (p<0.05); CGP 20712A (10μM), a β1AR inverse agonist, decreased the beating rate of T3-treated cells (39 ± 9bpm) to basal levels in untreated cells (25 ± 9bpm). Ca2+ transient analysis revealed alterations in Ca2+ handling. 4 week T3 treatment resulted in decreased time to transient peak from 244 ± 40 ms to 91 ± 15 ms (p<0.0006). Time to 50% decay also decreased from 309 ± 6 ms to 287 ± 3 ms (p<0.05) and transient duration (start to 75% decay) decreased from 495 ± 6 ms to 537 ± 3 ms (p<0.002). Proteomic analysis revealed increases in proteins involved in regulating sarcoplasmic reticulum (SR) function including phospholamban (17%), SERCA (27.6%) and calsequestrin2 (303.8%). In addition increases in mitochondrial enzymes, including ATP5B (32.7%), and αβ-MHC ratio were detected along with decreases in slow skeletal troponin I (-43.7%) and C (-36.2%) isoforms.

Conclusions: T3 treatment induced alterations in expression of proteins involved in SR function, mitochondrial metabolism and cytoskeletal composition. In addition, T3 treatment improved Ca2+ handling and increased β1AR function, with increases in beating rate possibly driven by constitutive β1AR activity. Collectively these data provide evidence for a shift towards a more mature phenotype and indicate that this may be an effective strategy to drive maturation of hiPSC-CM.