CARDIAC DIFFERENTIATION OF EMBRYONIC STEM CELLS: FUNCTIONAL PROPERTIES AND MODULATING FACTORS

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Cardiomyocytes (CM) derived from human embryonic stem cells (ESC) constitute a promising cell source for the regeneration of damaged hearts. The assessment of their *in vitro* functional properties is mandatory to envisage appropriate cardiac cell-based therapies. Electrophysiological characterization over a three-month period revealed that I_{to1} and I_{K1} potassium currents are present in differentiated CM only, whereas I_{Kr}, I_f and $I_{Ca,L}$ currents can be recorded both in undifferentiated ESC and CM. Most of the currents underwent developmental maturation in CM, as assessed by modifications in current density, properties and levels of channel transcripts. Moreover, action potential parameters and response to pharmacological tools underwent modifications according to time of differentiation. In particular action potential morphology indicated that ventricular cells are present only after 2 months of culture. These data suggest that human ESC-derived CM mature over time during *in vitro* differentiation, approaching an adult phenotype. Identification of factors responsible for cardiac differentiation of ESC is a major challenge to obtain a suitable cell source for transplantation. We focused our attention on differentiation medium and proved that ESC spontaneously differentiate into the cardiac lineage if grown in medium containing Fetal Bovine Serum (FBS). Cardiac differentiation of ESC is regulated by endoderm-secreted factors driving the downregulation of pluripotential markers, promotion of mesodermal specification and appearance of spontaneous beating activity. This process is impaired by using Serum Replacement in place of FBS, thus suggesting that FBS enables endodermal-dependent cardiac differentiation of human ESC. Another issue which dragged our attention is the elevate content (≈5 μM) of serotonin in medium selected for cardiac differentiation of murine ESC. During heart development, serotonin acts as morphogen via type 2 receptors ($5HT_2R$). Interestingly, we found that mouse $5HT_2R$ transcripts are selectively expressed in differentiated CM, being absent in undifferentiated ESC. In ESC-derived CM $5HT_2R$ resulted to be functionally coupled to intracellular calcium handling, which modulates frequency of spontaneous firing. Importantly, $5HT_2R$ proved to play an important role in cardiac specification of the ESC. In fact application of selective $5HT_2R$ antagonists during cardiac differentiation reduced occurrence of pulsating activity as well as mRNA expression of cardiac transcription factors and of ventricular myosin light chain.