Enhancement of cardiac differentiation of mouse pluripotent stem cells by β3 adrenoceptor stimulation

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Identification of factors and signalling mechanisms able to enhance cardiac differentiation of stem cells is important to envisage cardiac stem cell therapy. Previous studies have shown that β1 and β2-adrenoceptor (R) stimulation enhances cardiomyogenesis of mouse embryonic stem cells (mES) (Lehmann et al. 2013) as well as survival and proliferation of cardiac progenitor cells (CPCs) (Khan et al., 2012). Despite the expression of β3-R has been detected in mES, no information are available on the role they play during cardiac development and maturation. Conversely, a number of studies have defined a distinctive role of β3-R in the in adult heart, that diverge from that of β1 and β2-R (Belge et al., 2007). Interestingly, recent clinical evidence have showed a positive effect on left ventricular ejection fraction (LVEF) in patients with severe chronic heart failure treated with mirabegron, a β3-Rs agonist (Bundgaard H et al. 2017).

CGR8 mES were differentiated into the cardiogenic lineage in control conditions (Ctr) and in presence of BRL37344 (7μM) or SR59230A (10μM), selective β3-R agonist or antagonist, respectively. Western-blot analysis showed that throughout cardiac differentiation β1-R was highly expressed in the early phase and significantly declined thereafter; by contrast, both β2 and β3 have a rather stable expression throughout the process. Development of spontaneous contractile activity in differentiating mES was enhanced or suppressed by β3-R selective stimulation or inhibition, suggesting a possible developmental role of these receptors in the cardiogenic process. Gene expression analysis revealed that compared to Ctr, β3-R stimulation significantly increased expression level of cardiogenic genes, including mesodermal marker (at day 5: brachyury ≈+760%), precardiac (at day 5: Wnt3A ≈+25000%, Wnt11 ≈+20000% and β-catenin ≈+725%), first heart field (at day 7: Mef2C ≈+1360%, Hand1 ≈+1000%) and late cardiac markers (at day 7: Gata4 ≈+60%, Nkx2.5 ≈+62%, Tbx5 ≈+44%, Tbx20 ≈+150%), with negligible effects on second heart field markers. Oppositely, β3-R blockade severely blunted the same genes, reducing significantly the expression of mesodermal (at day 5: brachyury ≈-50%, Mesp1 ≈-96%), precardiac (at day 5: Wnt3A ≈-99%, Wnt11 ≈-88%), first (at day 7: Mef2C ≈-90%, Hand1 ≈-95%) and second (at day 7:hand2 ≈-99%, Isl1 ≈-84%, Tbx1 ≈-71%, Fgf10 ≈-90%) heart field and late (at day 7: Gata4 ≈-90%, Nkx2.5 ≈-95%, Tbx5 ≈-95%, Tbx20 ≈-95%) cardiac markers. Interestingly, markers of pluripotency and neurogenic phenotype were significantly upregulated by β3-Rs blockade (at day 2 Oct4 ≈+193%; at day 14 Msl1 ≈+100%), while the endodermic marker Sox17 was suppressed (at day 14 ≈-60%), suggesting that disruption of β3-R signalling likely shifts differentiation toward the neurogenic lineage. Furthermore, diameter of embryo body grown with the β3-R agonist resulted significantly longer in the early differentiation phase compared to Ctr (at day 7 from 917±60 680±20 □M), thus suggesting the involvement of β3-Rs with signalling regulating myogenic cell proliferation and/or hypertrophy. Moreover, frequency of spontaneous contraction was significantly enhanced by β3-R stimulation (at day 12 from 27±0.6 to 37±1.4bpm), suggesting a possible modification of electrogenic mechanisms driving mES spontaneous beating by β3-R

signalling. We further explored this point by analysing expression of genes involved in sinoatrial node formation: compared to Ctr at day 7 Tbx3, Tbx18, and Shox2 were upregulated (≈1100%, ≈+7000% and ≈+4200%, respectively). Accordingly, pacemaker channels were also robustly increased: at day 8 HCN1 ≈+7000% and HCN4 +1300%. Other important markers of cardiac differentiation were also increased (at day 7 cTnI≈+5700%, Cav1.3≈+5700%) and displayed a preferential shift toward the atrial phenotype (at day 7 mlc2a≈+3100% and mlc2v≈+280%).

In conclusion, β3-Rs are functionally expressed in mES undergoing cardiogenic differentiation and mediate an important signal to promote cardiac specification of undifferentiated cells.

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