overexpressing mESC-CMs expressed higher levels of Hcn4 than did control mESC-CMs. HCN4-overexpressing mESC-CMs showed significantly more rapid beating than did control mESC-CMs (Hcn4-overexpressing, 87.4±11.9 /min; control, 43.1±4.8 /min). The beating rate of HCN4-overexpressing mESC-CMs decreased in response to ivabradine (no treatment, 105±18.9 /min; 3 μM ivabradine, 74.0±14.7 /min; 30 μM ivabradine, 37.5±4.1 /min) and increased in response to isoproterenol (no treatment, 93.0±8.9 /min; 1 μM isoproterenol, 124±6.5 /min).
Conclusions: We established HCN4-overexpresssing mESC-CMs that show rapid spontaneous beating and responses to drugs in vitro. The results indicate the possibility of application of these cells as a biological pacemaker.

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Key differences in hypertrophic signalling between hESC- and hIPSC-derived cardiomyocytes

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The potential of stem cell-based disease modelling is enhanced by the realisation that cardiomyocytes from human embryonic stem cells (hESC-CM) and induced pluripotent stem cells (hiPSC-CM) can be obtained with disease-specificity. Hypertrophy is a high priority target because of its central role in the transition to heart failure. Strikingly, here we found that hiPSC-CM are relatively unresponsive to major hypertrophic signals compared to hESC-CM. We show that the normal alpha-adrenergic receptor 1A subtype (ADRA1A) is not expressed robustly in either cell type. ADRA1A is reversibly silenced during differentiation, accompanied by up-regulation of ADRA1B, resulting in a distinct gene profile from that in adult human cardiomyocytes. Loss of ADRA1A is more pronounced in hiPSC-CM, due to greater epigenetic silencing and more marked up-regulation of HIF-1α, but ultimately both cell types differ from adult in their reliance on active ADRA1B rather than ADRA1A. ADRA1B up-regulation is sufficient in hESC-CM for hypertrophic changes such as cell size, cell volume and ANF. However, in hiPSC-CM, additional decreased G-protein signalling and tonically inhibitory pathway networks suppress the effect of alpha-adrenoceptor stimulation on growth. Superficial similarities between hESC-CM, hiPSC-CM and adult cardiomyocytes may mask complex differences in signalling. These data raise serious questions regarding the hiPSC-CM as a valid model system for certain aspects of cardiac disease.

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Administration of regulatory T cells ameliorates myocardial inflammation in experimental myocarditis

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Background: Myocarditis is an inflammation of the heart mostly caused by viral infection, mainly the coxsackievirus B3 (CVB3). We investigated whether regulatory T cells (Tregs) could modulate and prevent immune-mediated myocardial damage in experimental viral myocarditis.
Methods: Male C57Bl6j mice were infected with CVB3. CD4+CD25+FoxP3 Tregs (nTregs) were isolated by magnetic separation from Foxp3-GFP-mice and injected intravenously 3 days after CVB3 infection. On day 7 post infection myocardial function was examined by conductance catheter technique and the expression of cytokines and profibrotic parameters in the heart and spleen was analysed with RT-PCR.
Results: Myocardial expression of TNFa was reduced by 40% (p=0.0289), IFNg by 50% (p= 0.0239) and Ccl2 by 40% (p=0.006) by Tregs compared to PBS. Tregs led to decreased expression of TGFβ (20%-reduction, p=0.01), collagen I (50%-reduction, p=0.006) and collagen III (50%-reduction, p=0.006) in the heart. This was associated with 40% higher myocardial contractility in the Tregs-treated mice compared to PBS-treated mice (dP/dtmax, p=0.001). Furthermore, Tregs were able to reduce the myocardial viral load by 50% compared to PBS-treatment (p=0.003). Effects of Tregs in the heart were associated with a 40% higher expression of IL-10 as an effector molecule of regulatory t cells in the spleen compared to PBS-treated mice (p=0.0025).
Conclusion: The application of regulatory t cells during the acute phase of viral myocardial inflammation is able to modulate myocardial inflammation and improve cardiac function.

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MicroRNA133 and microRNA499 exert synergistic effect on cardiac differentiation

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Purpose: It has been demonstrated that miR1 and miR133 are involved in cardiac development and stem cell differentiation, while miR499 enhances the differentiation of embryonic and adult stem cells into cardiomyocytes (CMC). However, it is currently unknown if these miRNA may act synergistically to improve differentiation efficiency.
Methods: Mouse P19 cells transduced with a viral vector expressing GFP under the control of a cTnI promoter were primed with 0.5% of DMSO and used as cardiogenic differentiation model (P19 CTRL). MiR499 was transiently overexpressed in P19 cells individually or together with miR1 or miR133. Moreover, we differentiated P19 cells with pre-miR precursors without DMSO. Cardiac differentiation was quantified by: 1) count of spontaneous beating areas; 2) qPCR of the cardiac markers Nkx2.5, GATA4, MEF2c, MLC2v, Cx43 and cTnT; 3) Western Blot and ICC of cTnT and Cx43; 4) measure of inducibility of RyR-mediated Ca++ transients in P19 exposed to caffeine; 5) recording of mechanical activity after Ca++ depletion and in the presence of nifedipine or ryanodine.
Results: The overexpression of miR499 alone increased the number of beating CMC by 1.7 fold compared with P19 CTRL ($p<0.05$). The association of miR499+133, but not miR499+1, exerted synergistic effects further increasing the number of beating CMC (2.02 fold vs. P19 CTRL, $p<0.01$). qPCR showed that miR499+133 enhance the expression of GATA4, Nkx2.5, Cx43 and cTnT compared with P19 CTRL ($p<0.01$). WB and ICC for Cx43 and cTnT confirmed these findings. Caffeine responsiveness was increased 2.6 fold by miR499+133 compared with P19 CTRL (32.5 vs 12.5%, $p<0.05$). Cyclic contractions were reversibly abolished by extracellular Ca++ depletion and by both nifedipine and ryanodine.
Strikingly, the percentage of responsive cells to caffeine was significantly higher in P19 cells treated with pre-miR499+133 but no DMSO compared with P19 CTRL ($p<0.05$). PCR and WB demonstrated that the over-expression of early and late cardiac genes in P19 cells treated with miRNA precursors and not exposed to DMSO and ICC showed the expression of Cx43 and cTnT proteins, SERCA2a and Cav1.2.
Conclusions: Our results show that miR133 and miR499 play synergistic effect in cardiac differentiation to the point that no chemical trigger is required. Our findings may contribute to improve the efficiency of stem cell therapy for cardiac repair.

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Modeling of Friedreich Ataxia related iron overloading cardiomyopathy using patient-specific induced pluripotent stem cellsCellp

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Background: Friedreich ataxia (FRDA), a recessive neurodegenerative disorder commonly associated with hypertrophic cardiomyopathy, is due to GAA repeats expansion within the first intron of the frataxin (FXN) gene encoding the mitochondrial protein involved in iron-sulfur cluster biosynthesis. The triplet codon repeats leads to heterochromatin-mediated gene silencing and loss of frataxin. Nevertheless, inadequate of existing FRDA-cardiac cellular model limited the cardiomyopathy studies. We tested the hypothesis that iron homeostasis deregulation accelerates reduction in energy synthesis dynamics which contribute to impaired cardiac calcium homeostasis and contractile force.
Methods and results: Silencing of FXN expressions occurred both in somatic FRDA-skin fibroblasts and two of the iPSC clones; a sign of stress condition was shown in FRDA-iPSC-cardiomyocytes with disorganized mitochondrial network and mitochondrial DNA (mtDNA) depletion; hypertrophic cardiac stress responses were observed by increased in α-actinin-positive cell sizes revealed by FACS analysis as well as elevation in brain natriuretic peptide (BNP) gene expression; the intracellular iron accumulated in FRDA-cardiomyocytes might be due to attenuated negative feedback response of transferring receptor (TSFR) expression and positive feedback response of ferritin (FTH1);energy synthesis dynamics, in terms of ATP production rate, of FRDA-iPSC cardiomyocytes were prone to iron overload condition which retarded calcium reuptake to sarcoplasmic reticulum (SR) responses to adrenergic stimulation.
Conclusion: Our data showed for the first time that FRDA iPSCs cardiac derivatives represent promising models to study cardiac stress response due to impaired iron-homeostasis condition and mitochondrial damages. Cardiomyopathy phenoptype was accelerated in iron-overloaded condition early in calcium homeostasis aspect.