SIGNAL TRANSDUCTION IN CARDIAC DISEASE

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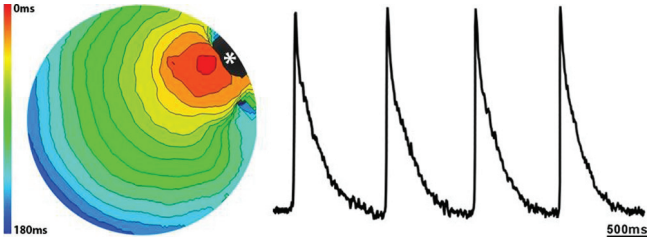
Massive expansion of native human atrial cardiomyocytes by immortogenetics

N. Harlaar[1], L. Volkers[1], J. Liu[1], P.R. Van Gorp[1], A.A. Ramkisoensing[1], T.J. Van Brakel[2], D.A. Pijnappels[1], A.A.F. De Vries[1]. *[1]Leiden University Medical Center, Cardiology, Leiden, Netherlands; [2]Leiden University Medical Center, Cardiothoracic Surgery, Leiden, Netherlands*

Background: Preclinical cardiac research has been largely based on animal-derived cellular models, thereby hampering clinical translation. While upcoming human pluripotent stem cell technology seems to decrease this gap between bench and bedside, its complex/multi-step protocol to produce cardiac muscle cells, its required expertise, and its trouble to produce large numbers of phenotypically homogeneous cardiomyocytes so far has limited broad application.
Purpose: We aimed to immortalize native human atrial cardiomyocytes to produce natural and standardized lines of these cells by gaining full control over their proliferation and differentiation with a common cell culture agent.
Methods: Human fetal atria (gestational age 18 weeks) were dissociated and transduced with a lentiviral vector directing myocyte-specific and doxycycline-inducible expression of simian virus 40 large T antigen (here defined as immortogenetics). Addition of doxycycline to the culture medium pushed cardiomyocytes towards a proliferative phenotype. Sixty proliferating clones were isolated, expanded and screened for their cardiomyogenic differentiation capacity upon doxycycline removal. Selected clones were characterised using various molecular biological and electrophysiological assays.
Results: Upon doxycycline removal (i.e. under differentiation conditions), cells spontaneously reacquired a cardiomyocyte-like appearance as judged by phase-contrast microscopy. Simultaneously, these cells stopped proliferating, which was accompanied by a drop in large T level, loss of Ki67 expression and the development of sarcomeres with striated α-actinin and troponin T staining patterns. These cells were tagged conditionally immortalised human atrial cardiomyocytes (hereinafter called hiAMs). Optical voltage mapping of hiAM monolayers (clone 7L#12) revealed the presence of excitable cells showing homogeneous spreading of action potentials at 7.6±1.2 cm/s following 1-Hz point stimulation. The mean APD80 of these cells was 491±89 ms and could be shortened to 119±20 ms in response to the KATP/Kir6.x channel opener P1075. Single-cell patch clamp recordings in current-clamp mode confirmed their excitability with a resting membrane potential of -60.9 mV, peak potential of 31.8 mV and APD80 of 361 ms.



Optical mapping of differentiated hiAMs

Conclusion: We have generated first-of-a-kind lines of natural human atrial cardiomyocytes through immortogenetics, allowing massive cell expansion under proliferation conditions and robust formation of cross-striated and excitable cardiomyocytes after differentiation. Thereby, a user-friendly, clinically-relevant and much-anticipated research model has been produced, which application could range from drug response studies to disease modelling and myocardial regeneration.

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Cardiac injury-induced regulation of clock genes: lessons from mice and patients

Y. D'Alessandra[1], S. Gioffre'[1], M. Chiesa[1], L. Piacentini[1], F. D'Avila[1], C. Ruggeri[1], C. Vavassori[1], V. Ricci[1], L. Castiglioni[2], A. Scopece[1], G. Milano[1], G. Marenzi[1], G. Pompilio[1], G.I. Colombo[1]. *[1]Cardiology Center Monzino IRCCS, Milan, Italy; [2]University of Milan, Milan, Italy*

Background: The circadian clock is an evolutionarily conserved timekeeper that adapts body physiology to diurnal/nocturnal cycles of about 24 hours. The cardiac circadian mechanism orchestrates rhythms in heart rate, blood pressure, cardiac contractility, and gene expression. Notably, perturbations in this pathway, which relies on the oscillatory expression of a bunch of core clock genes (e.g. CLOCK, ARNTL, PER, and CRY), increase cardiovascular disease risk and can exacerbate cardiac damage-induced remodelling. Several years of studies yielded an extensive knowledge about the effects of circadian rhythms on cardiovascular diseases. Curiously, though, very little is known about the impact of heart damage on clock genes expression.
Purpose: We investigated the impact of cardiac injury on the expression of selected clock genes in a murine model of doxorubicin-induced cardiac damage and in the whole blood of patients suffering from acute myocardial infarction (AMI).
Methods: Heart damage was induced to C57BL/6 female mice by administration of a 24 mg/kg cumulative dose of doxorubicin (dox) or saline during 2 weeks, followed by a recovery period. Cardiac function was evaluated by echocardiography at the end of treatment (T14), and after 1 week (T21) and 1 month (T42) of recovery. We observed a functional dox-induced impairment in left (LV) and right (RV) ventricles and atria at every time point, and collected the tissues (4 mice/group) to assess clock genes expression by RT-qPCR. All genes were also investigated in blood samples collected from patients (n=40) suffering from AMI, and compared with 10 healthy donors (HD). The research involving human subjects conformed to the principles of the Declaration of Helsinki of the World Medical Association, and all patients signed a written informed content. The animal studies were conducted following the principles of laboratory animal care and according to the national law. Two- and One- way ANOVA tests followed by post hoc analyses were used to assess statistical significance ($p<0.05$).
Results: The in vivo experiments showed increasing dysregulation for 7 clock genes (Arntl, Clock, Ciart, Dbp, Per2, Per3, Tef) in all cardiac districts of dox-treated animals, starting from T14 up to T42. In particular, Arntl and Clock were upregulated, while the other 5 genes presented a downregulation (vs. controls, $p<0.05$). In the human setting, CLOCK and CRY1 were significantly decreased in AMI vs. HD subjects ($p<0.05$), while PER1 showed an increased expression ($p<0.05$).
Conclusions: Our results indicate that cardiac damage influences clock genes expression in vivo. Moreover, we showed, for the first time, a potential specific impact of AMI on the circulating levels of human circadian genes.
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Smooth muscle cell specific SDF-1/CXCL12 KO mice display severe cardiac hypertrophy and vascular defects

S.K. Ghadge[1], M. Messner[1], H. Seiringer[1], A. Wimmer[1], T. Zeller[2], D. Boernigen[2], G. Poelzl[1], M.M. Zaruba[1]. *[1]Innsbruck Medical University, Department of Internal Medicine III, Innsbruck, Austria; [2]University Heart Center Hamburg, Department of General and Interventional Cardiology, Hamburg, Germany*

Background: The chemokine SDF1/CXCL12 plays a central role in homing of (stem) cells from the bone marrow to the ischemic area after myocardial ischemia. Stabilization of the SDF-1/CXCR4+ axis has been shown to provide beneficial effects on myocardial repair. Moreover, SDF-1 and its receptors CXCR4 & CXCR7 play significant roles during embryonic development, cardiogenesis, vascularization and valve morphogenesis. Nevertheless, detailed mechanisms of the cell specific role of SDF1 (CXCL12) are poorly understood. Therefore, we aimed to analyze cell specific SDF-1 expression utilizing SDF-1EGFP reporter mice. Since SDF-1-EGFP lineage tracking revealed high expression of SDF-1 in smooth muscle cells, we aimed to investigate the cell specific role of SDF-1 in cardiogenesis and vasculogenesis by generating a conditional smooth muscle cell specific SDF-1 (SM-SDF-1 KO) knockout mouse model.
Methods: SDF-1 expression was analyzed utilizing genetically tagged SDF-1-EGFP mice. Smooth muscle cell specific knock-out of SDF-1 was achieved using Cre/LoxP method (SM22a-Cre; SDF-1fl/fl). The genotypes were determined through PCR. Morphology was analysed with immunohistochemistry and immunofluorescence. Cardiac function was assessed utilizing VEVO-cardiac ultrasound and Millar Tip-catheterization. Whole transcriptome analysis, qRT-PCR and western blotting were performed. Further, apoptotic index and cell proliferation were quantified by TUNEL assay and PH3 immunostaining, respectively.
Results: SDF-1-EGFP lineage tracking revealed a high expression of SDF-1 in in smooth muscle cells and very moderately in perivascular cells. Conditional SM-SDF-1 KO mice showed a high pre- and perinatal mortality (50%). Immunohistochemistry in surviving adult SM-SDF-1 KO mice revealed a severe cardiac hypertrophy phenotype, associated with increased cardiac fibrosis and apoptotic cell death. SM-SDF-1 KO mice revealed very thin and dilated arteries. VEVO-Ultrasound measurement revealed concentric hypertrophic cardiac walls, narrowed chambers and decreased stroke volume reflecting restrictive hypertrophic cardiomyopathy. Immunohistochemistry confirmed pronounced hypertrophy of cardiomyocytes. Additionally, we found evidence for enhanced proliferation markers in cardiomyocytes of SM-SDF-1 KO mice. Transcriptome analyses from KO hearts vs. non-ablated littermates identified over 150 significantly up- and down-regulated genes. Westernblot analysis for HIF-1α, AKT and ERK cell-signalling pathways were significantly elevated in SM-SDF-1 KO mice. qRT-PCR analysis of SDF-1 isoforms showed that specifically the SDF-1β isoform was downregulated in mutant aortas and hearts.
Conclusion: Our data suggest that smooth muscle cell specific expression of SDF-1 plays a prominent role in cardiovascular development and cardiac hypertrophy in adult animals. Our data further suggest that particularly the SDF-1β isoform is expressed in smooth muscle cells and might be involved in the observed phenotype.
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