Abstract: P1885

Cardiomyocyte-specific STAT3 deficiency alters the epigenetic program of cardiac progenitor cells from endothelial towards adipocyte priming

Authors:
E Stelling¹, M Ricke-Hoch¹, AK Bergmann², S Erschow¹, M Scherr³, S Hoffmann⁴, J-L Balligand⁵, D Hilfiker-Kleiner¹, ¹Hannover Medical School, Department of Cardiology and Angiology - Hannover - Germany, ²Hannover Medical School, Department of Human Genetics - Hannover - Germany, ³Hannover Medical School, Department of Hematology, Oncology and Stem Cell Transplantation - Hannover - Germany, ⁴Fritz Lipmann Institute for Age Research, Computational Biology - Jena - Germany, ⁵Universite Catholique de Louvain, Institut de Recherche Experimentale et Clinique (IREC), Pole of Pharmacology and Therapeutics (FATH) - Brussels - Belgium,

Topic(s):
Basic Science - Cardiac Diseases: Heart Failure

Citation:
Funding Acknowledgements:
Volkswagen Stiftung, REBIRTH

Background: Cardiac STAT3 expression decreases with age and is reduced in failing hearts. Male mice with a cardiomyocyte-restricted knockout of STAT3 (aMHC-Cretg/+; STAT3floxflox, CKO) show age-related heart failure with reduced capillary density associated with diminished Erythropoietin (EPO) production and subsequent reduction of the endothelial differentiation potential of Sca-1+ cardiac progenitor cells (CPC).

Purpose: We hypothesized that reduced cardiomyocyte STAT3 expression impairs the cardiomyocyte secretome and thereby changes the epigenetic priming of CPC.

Methods: Freshly isolated CPC (MACS) from 14 weeks old CKO and WT male mice as well as 2 clonally expanded CPC cell lines were used. Genome-wide methylation profiling was performed on freshly isolated CPC by reduced representation bisulfite sequencing. 3T3-L1 preadipocytes were incubated with conditioned supernatants of HL-1 cardiomyocytes with a lentiviral knockdown of STAT3 (STAT3-KD). Mice (age: 3 months) were injected weekly (i.p.) with NaCl or with a low-dose of an EPO derivative (3 µg/kgBW) for 3 months. Cardiac fat content was analyzed by triglyceride measurement and Oil Red O staining. Using qRT-PCR, immunoblotting and FACS, the expression of (pre-)adipocyte markers was measured.

Results: Clonally expanded CPC could be differentiated in endothelial cells or in adipocytes indicating that both cell types can derive from the same CPC cell. CKO-CPC showed a 2-fold increase in adipocyte formation after 4 weeks in culture (Oil Red O measurement, enhanced mRNA levels of FABP4, CEBPA and OLR1, *P<0.05) compared to WT-CPC. Epigenetic analysis revealed 568 differentially methylated regions between CKO- and WT-CPC. Zfp423, a transcription factor controlling preadipocyte determination, was found to be less methylated in CKO-CPC. QRT-PCR confirmed higher expression of Zfp423 in CKO-CPC compared to WT-CPC. EPO treatment (10 ng/ml) of CKO-CPC cultures reduced Zfp423 expression and adipocyte formation. Cultivation of 3T3-L1 with conditioned media from STAT3-KD HL-1 cardiomyocytes significantly increased adipocyte formation (Oil Red O absorbance, increased mRNA levels of FABP4, AdipoR1 and CEBPA, decreased mRNA levels of Pref-1 and PDGFra) compared to cultivation with control media, which could also be attenuated by EPO supplementation. Left ventricular fat content and adipocyte number were increased in hearts of 6-month-old CKO mice (2.5-fold compared to WT, *P<0.01), which could be reduced by EPO treatment.

Conclusion: Age-related heart failure in CKO mice is associated with an epigenetic shift in the differentiation
potential of CPC from endothelial cells to adipocytes. This is in part caused by diminished EPO secretion by STAT3-deficient cardiomyocytes into the cardiac microenvironment and changes in the epigenetic pattern of adipogenesis related genes. EPO administration could reduce enhanced adipocyte formation in hearts of CKO mice.