Novel Insights in Intercellular Communication within the Heart

Session held on 8 July 2016

doi:10.1093/cvr/cvw134

17  
LRP5 transcription and activation of the canonical Wnt signalling are protective  

signals in the myocardium after infarction  
M. Borrell-Pages; G. Valiur; C. Romero; L. Cañas; L. Badimon  
Barcelona Cardiovascular Research Center (CIRC-CCG), IIB-Sant Pau, Hosp Sant Pau, UAB, Barcelona, Spain  

Background: LDL receptor-related protein 5 (LRP5) triggers the canonical Wnt pathway which participates in cell function regulation, including lipoprotein metabolism, macrophage mobility and phagocytosis, but its function in the heart is unknown.  

Purpose: The aim of this study was to investigate LRP5 and the canonical Wnt signalling pathway in myocardial injury after acute-mycardial infarction (MI).  

Methods: MI was induced in WT and LRP5-/- mice by coronary ligation. Infarct size, LRP5 and Wnt signalling proteins were measured. LRP5 and the different metabolic pathways involved in myocardial damage post-MI were analyzed in isolated cardiomyocytes, myofibroblasts and endothelial cells.  

Results: LRP5-/- mice have significantly larger infarcts than WT mice (20.8±9.9 vs 0.5±0.9) suggesting a protective role of LRP5/Wnt in injured myocardium. Furthermore, administration of a GSK3 inhibitor that activates the Wnt pathway downstream LRP5, induced smaller infarcts in LRP5-/- mice indicating that an active Wnt pathway plays a protective role in the myocardium. Hypoxia induced LRP5 over-expression in isolated cardiomyocytes and endothelial cells indicating that a defensive and protective expression of LRP5 is triggered in both cell types. Induction of MI in WT and in LRP5-/- hypercholesterolemic animals, common risk factor in patients with ACS, induced larger infarcts in both genotypes. In isolated cardiomyocytes, LDL induced LRP5 overexpression and Wnt pathway activation whereas LRP5-silencing blocked the pathway.  

Conclusions: LRP5 and the canonical Wnt pathway activation is a defensive pro-survival process triggered to protect the ischemic myocardium against different injury triggers, such as hypoxia and hypercholesterolaemia to favour and restore cell viability.

18  
FGF10 is required to promote cardiomyocyte proliferation after myocardial infarction  
R. Surry; S. Payan; R.G. Kelly; F. Rochais  
Aix-Marseille University, IBDM CNRS-UMR 7288, Marseille, France  

In mammals, cardiomyocyte proliferation decreases after birth resulting in severely limited regenerative capacities in the adult heart. Understanding the developmental processes controlling cardiomyocyte proliferation may thus identify new therapeutic targets to modulate the cell cycle activity of cardiomyocytes in the adult heart. We recently identified FGF10 as a regulator of regional cardiomyocyte-autonomous proliferation in the fetal heart and showed that overexpression of Fgf10 promotes cell cycle reentry of adult cardiomyocytes. These results identify FGF10 as a potential clinically relevant target for promoting adult cardiomyocyte cell cycle reentry after cardiac injury.  

Using an experimental mouse model of myocardial infarction (MI), together with Fgf10 loss of function mouse models, we investigated the role of FGF10 in pathological conditions. We first demonstrated that myocardial infarction leads to increased Fgf10 expression levels in the injured ventricles. Using an Fgf10-Luc:2 enhancer trap mouse line, we showed that Fgf10 is upregulated in cardiomyocytes, suggesting a role for FGF10 in pathological conditions. In order to investigate a potential protective role of FGF10 under pathological conditions, adult transgenic mice with reduced Fgf10 expression were subjected to myocardial infarction. Three weeks after myocardial infarction, cardiomyocyte hypertrophy, cell proliferation and heart function were evaluated. Immunofluorescence experiments revealed that while altered Fgf10 expression as no impact on cardiomyocyte hypertrophy, it significantly decreases post-MI cardiomyocyte proliferation. In addition, preliminary echocardiography experiments indicate that post-MI cardiac function is further impaired when Fgfo10 levels are reduced. Together these results suggest that under pathological conditions FGF10 may play a protective role by promoting cardiomyocyte proliferation. FGF10 is thus as a potential target to improve the limited innate regenerative capacities of the myocardium after injury.

19  
A new role for transcription factor EB (TFEB) in mouse epicardial development  
E. Astauni; G. Doronzo; D. Cora; F. Neri; D. Välderbrink; G. Sarini; S. Oliviero; A. Ballabio  
1University of Turin, Department of Oncology, Turin, Italy; 2Human Genetics Foundation (HuGeF), Turin, Italy; 3Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy  

Epicardium is the source of smooth muscle cells of coronary vessels (vSMCs) and intracardiac fibroblasts. Several pathways promoting epithelial to mesenchymal transition (EMT) of epithelial cells and their differentiation in vSMCs and fibroblasts have been identified, but little is known about the factors limiting these processes. A member of MTF-TFE family of transcription factors, TFEB is now extensively being studied due to its ability to increase lysosomal biogenesis and autophagy in response to the lack of nutrients. Although other MTF family proteins are known to regulate differentiation of several lineages, TFEB involvement in embryo development has not yet been studied. Our goal was to investigate the role of TFEB in mouse embryo development. Once we discovered Tfeb expression in epicardium, we aimed to understand its function during epicardial development. To study Tfeb expression in the embryo, we used a transgenic mouse that expresses fusion protein TFEβ-GFP. To evaluate the role of TFB in epicardial development, we generated mice with epidermis-specific TFB overexpression (Gata5-Cre; Tfeb-3xflag) and knock-out (Gata5-Cre; Tfeb flox/flox). Primary epicardial cells were cultured without or in the presence of vSMC differentiation-stimulating factor BMP4. Epicardial mesothelial cell line EMC was used for Tfeb silencing and overexpression experiments. Acquisition of myofibroblast (MF) phenotype was defined by the increased expression of vSMC (PDGFRb, a-SMA, transgelin) and fibroblast (PDGFRb, fibronectin) markers evaluated by immunostaining, Real-Time PCR and Western blot and increased cell migration capacity measured in wound healing assay. ChIP-Seq analysis was performed in EMCs overexpressing constitutively active TFBβ. TFBβ is expressed in mouse epicardium at 11.5 and 13.5 dpc and is not detectable after 15.5 dpc. TFBβ is present in epicardial cells, but not in epidermally derived cells (EPDCs), vSMCs and fibroblasts. The prolonged epicardial overexpression of TFBβ leads to embryonic lethality at dpc 15.5. Epicardium of transgenic embryos develops normally, but differentiation and migration of EPDCs are severely inhibited. Tfeb overexpression promotes cardiomyocyte proliferation in culture but fail to differentiate in vSMCs under BMP4 stimulation. Mice with epicardium-specific deletion of TFBβ are viable. Mutant epicardocytes in culture easily transform in MFs in absence of BMP4 stimulus, when wild type cells maintain epithelial phenotype. Accordingly, in EMCs Tfeb silencing promotes transformation to MFs already in basic conditions, while TFBβ overexpression inhibits MF differentiation induced by TGFβ. ChIP-Seq analysis revealed about 2000 TFBβ targets, among these functional groups of TGFβ and Notch pathways components are enriched. Our results suggest a new role for TFBβ in restriction of epicardial EMT. More should be done to individuate TFBβ targets crucial for this process and understand how TFBβ expression in epicardium is regulated.