Cardiobiology

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Regulation of cardiac cell growth – implications for heart disease

Our group investigates mechanisms whereby receptor tyrosine kinases regulate hypertrophic and hyperplastic cell growth in the heart. To this end we use primary neonatal rat heart cultures and several *in vivo* mouse models of cardiac disease. During the past reporting period we have investigated models of high-fat diet-induced cardiomyopathy and aortic-constriction-induced cardiac hypertrophy to mimic the pathophysiology associated with obesity and valve disease, respectively. In addition, we are establishing a model of anthracycline-induced cardiomyopathy, relevant to the interdisciplinary field of oncology and cardiology. Our focus at the cellular level is on protein turnover mechanisms, which to a large extent depend on available energy resources together with hormone and cytokine levels. We have recently directed our attention to the ErbB family of receptor tyrosine kinases activated by neuregulin-1β (NRG-1), while also extending our earlier work on the insulin/IGF-I/Akt/mTOR pathway.

Neuregulin-1β

NRG-1 regulates cell growth and differentiation in various tissues including the heart. After myocardial infarct or anthracycline therapy, NRG-1 is released by the endothelium and activates multiple signaling pathways leading to distinct biological effects depending on the expression levels and dimerization of its receptors ErbB2, ErbB3 or ErbB4 on neighboring cells in the heart. NRG-1 is cardioprotective in a range of animal models of cardiac disease. While its therapeutic use is being tested in heart failure, the hormone risks to enhance tumor growth. A better understanding of the mechanisms whereby NRG-1 acts in cardiac versus cancer cells is important.
We reported that NRG-1 causes glucose uptake in neonatal cardiomyocytes by triggering translocation of GLUT4 to the plasma membrane via ErbB2/ErbB4 (Fig. 1). Using Seahorse, we showed that NRG-1 also enhances glycolysis. Like for insulin and IGF-I, the mechanism involves PI3Kα, Akt and AS160 (Pentassuglia *et al.,* 2016; Heim *et al.,* 2020). Under stress conditions cardiomyocytes switch from fatty acids to glucose as energy source. We hypothesized that the NRG-induced glucose uptake contributes to cardiomyocyte contractility e.g. under ischemic conditions or in insulin-resistant states. However, our experiments demonstrated that NRG-1 does not activate this cardiac glucose uptake pathway in adult models. We are currently testing whether the metabolic effects of NRG-1 provide the means for cell cycle activation and proliferation observed in neonatal cardiomyocytes (Fig. 2). While our studies reveal mechanisms that contribute to normal physiological cardiac growth and differentiation observed in neonatal hearts, they also aim to provide fundamental insights that may contribute to cardiac regenerative approaches.

Function of mTORC2 in the heart

To perform its function as biological pump that provides oxygen and nutrients to our whole body, the heart consumes large amounts of energy. A tight regulation of the available resources, including cellular proteins, becomes critical in disease states where metabolism must increase to maintain cardiac performance, e. g. in hypertensive or valve disease. We previously reported that the metabolic regulator mTOR is essential for cardiac function when part of mTORC1: its cardiomyocyte-specific ablation causes heart failure rapidly followed by death of the mice, a phenotype explained by mTORC1's role in protein synthesis as well as mitochondrial metabolism. We next demonstrated that aortic constriction-induced pressure overload significantly increases rictor (an essential mTORC2-specific protein) and PKCβII and PKCδ phosphorylation in control mice, but not in cardiac rictor knockout mice. Whereas pressure-overload causes hypertrophy with maintained func-

tion in controls, it leads to systolic dysfunction in rictor-deficient hearts without having any effects on cardiac weight or fibrosis. These data suggest that mTORC2 regulates metabolism and contractility of the heart via PKCβII and PKCδ and is not implicated in its hypertrophic growth (Shende *et al.,* 2016; Xu *et al.,* 2016).

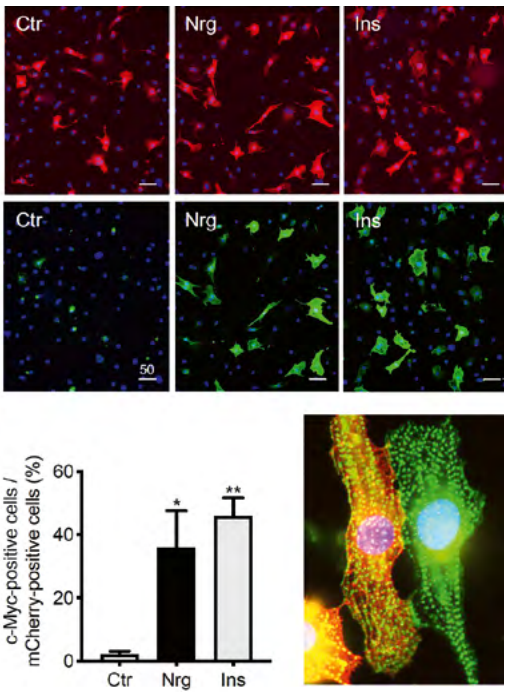

Fig. 1: NRG-1 causes translocation of GLUT4 to the plasma membrane. pLenti-myc-GLUT4-mCherry transfected cardiomyocytes from neonatal rats were stimulated with NRG-1 (Nrg, 10 ng/mL), insulin (Ins, 13 nM) or vehicle (Ctr) and fixed with 4 % paraformaldehyde after 30 min. The GLUT4-transfected cells are revealed based on the mCherry label (red, top pictures). Translocated GLUT4 was detected on the surface of the fixed non-permeabilized cells using a c-Myc-specific antibody followed by an Alexa 633-labeled secondary antibody (green). The bottom picture identifies the the left mCherry-positive cells (red) as cardiomyocytes after staining with antibodies specific for sarcomeric actinin (green), The nuclei were stained with DAPI (blue).

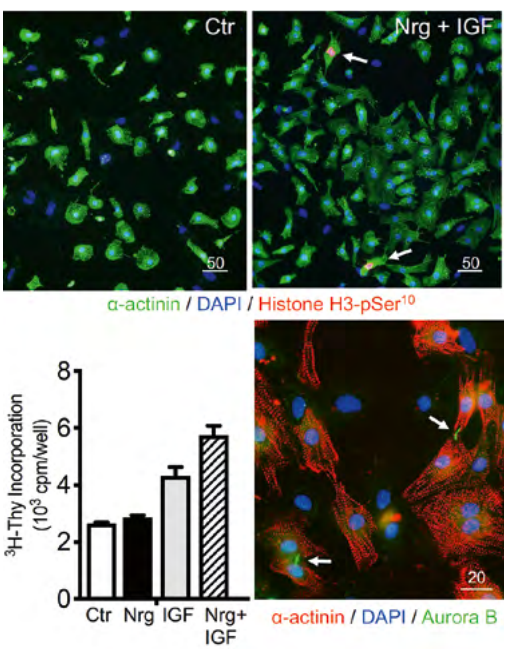

Fig. 2: Effects of IGF-I and NRG-1 on cardiomyocyte cell cycle activation. Cardiomyocyte cultures prepared from neonatal rats were stimulated with NRG-1, IGF-I or both factors together for 24 h. Cell cycle activation was evaluated by measuring ^{3}H-thymidine incorporation and by immunofluorescent labeling with antibodies to phosphorylated Histone H3 (top). To confirm cytokinesis in cardiomyocytes, double labelling with antibodies to α-actinin and Aurora B was performed (Bottom micrograph). Bar size indicated in µm.

Connection to Clinical Practice

ErbB4 agonists

For the translational side of our work on NRG-1, we collaborate with teams of the University of Antwerpen (Profs Gilles de Keulenaer and Vincent Segers) and Moscow (Profs Anastasia Shchendrygina and Yuri Belenkov), funded by a European ERA.Net RUS Plus grant.

Multiple lines of evidence demonstrate that the cardiac NRG-1/ErbB4 system is activated in chronic heart failure, exerting disease mitigating and regenerative effects. NRG-1 is developed as a drug for heart failure and clinical trials have progressed to stage III. In addition, there is solid evidence from animal studies that the NRG-1/ErbB4 pathway is involved in other chronic diseases, such as diabetic nephropathy, pulmonary hypertension, atherosclerosis and fibrotic disorders. All of these are common chronic disorders, and potential therapeutic targets for NRG-1. In the project together with Antwerpen and Moscow, a multi-disciplinary approach is taken to identify novel potent and selective agonists of the ErbB4 receptor, to test these in validated rodent models of heart failure, and to define specific patient populations in the heterogeneous field of cardiovascular diseases that could benefit from ErbB4 agonists.

Selected Publications

Heim P, Morandi C, Brouwer GR, Xu L, Montessuit C and Brink M (2020). Neuregulin-1 triggers GLUT4 translocation and enhances glucose uptake independently of insulin receptor substrate and ErbB3 in neonatal rat cardiomyocytes. Biochim Biophys Acta Mol Cell Res 1867, 118562.

De Keulenaer GW, Feyen E, Dugaucquier L, Shakeri H, Shchendrygina A, Belenkov YN, Brink M, Vermeulen Z and Segers VFM (2019). Mechanisms of the multitasking endothelial protein NRG-1 as a compensatory factor during chronic heart failure. Circ Heart Fail 12, e006288.

Pentassuglia L, Heim P, Lebboukh S, Morandi C, Xu L and Brink M (2016). Neuregulin-1beta promotes glucose uptake via PI3K/Akt in neonatal rat cardiomyocytes. Am J Physiol Endocrinol Metab 310, E782–794.

Xu L and Brink M (2016). mTOR, cardiomyocytes and inflammation in cardiac hypertrophy. Biochim Biophys Acta 1863, 1894–1903.

Shende P, Xu L, Morandi C, Pentassuglia L, Heim P, Lebboukh S, Berthonneche C, Pedrazzini T, Kaufmann BA, Hall MN, Rüegg MA, Brink M (2016). Cardiac mTOR complex 2 preserves ventricular function in pressure-overload hypertrophy. Cardiovasc Res 109, 103–114.