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Reduced scar maturation and contractility lead to exaggerated left ventricular dilation after myocardial infarction in mice lacking AMPK α 1

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Objective: Cardiac fibroblasts (CF) are crucial in left ventricular (LV) remodelling after myocardial infarction (MI). They predominantly express the α 1 catalytic subunit of AMP-activated protein kinase (AMPK α 1), while AMPK α 2 is the major catalytic isoform in cardiomyocytes. AMPK α 2 is known to protect the heart by preserving the energy charge of cardiac myocytes during injury, but whether AMPK α 1 interferes with maladaptive heart responses remains unexplored. In this study, we aim at further substantiating the role of this AMPK isoform in the pathogenesis of post-MI LV remodelling and more particularly in the regulation of fibrotic properties of CF.

Methods: AMPK α 1 knockout (KO) and wild type (WT) mice were subjected to permanent ligation of the left anterior descending coronary artery to mimic MI. Cardiac fibrosis was monitored using qRT-PCR analysis, histology and immunohistochemical staining. LV function and remodelling was assessed by echocardiography.

Results: In the absence of AMPK α 1, the CF proliferative response was increased in infarcted myocardia. It resulted in elevated levels of fibrotic factors but did not lead to excessive matrix deposition or degradation in KO infarcts. While CF proliferation was increased, expression of the myodifferentiation marker α -smooth muscle actin was decreased. This faulty maturation of myofibroblasts might derive from down-regulation of the transforming growth factor- β 1/p38 mitogen-activated protein kinase pathway in KO infarcts. Although infarct size was similar in KO and WT hearts subjected to MI, these changes resulted in defective scar collagen maturation. This was associated with an exacerbated adverse remodelling as indicated by increased LV diastolic dimension 30 days after MI.

Conclusion: Our data genetically demonstrate the centrality of AMPK α 1 in post-MI scar formation and highlight the specificity of this catalytic isoform in cardiac fibroblast/myofibroblast biology.

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A screening strategy to identify modulators of the mitochondrial ADP/ATP translocase (ANT) for cardioprotection

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In many pathological conditions, a sudden lack of energy, accompanied by an increased reactive oxygen (ROS) level and calcium overload, accelerates cell death with necrotic features. As the mitochondrion is the main source of cellular ATP and ROS, it has become an attractive therapeutic target for human pathology such as cardiac, neuronal and hepatic ischemia-reperfusion injury. In this context, we and others have shown that pharmacological modulation of ANT can prevent three acute pathologies in mice¹ and recently, we hypothesized that activation of ANT might be an innovative strategy for cardioprotection. Here, we present the design and the implementation of enzymatic, cellular and subcellular assays and their optimization for the screening of modulators of ANT (i.e. inhibitors and/or activators). We miniaturized the ADP/ATP exchange measurement in cardiac inter-fibrillar and subsarcolemmal isolated mitochondria from rat heart, evaluated its robustness (Z-factor) in 96 well-microtiter plates and screened an *in silico*-generated library of ANT-potential ligands and small

molecules, i.e. about 100 molecules. Cytotoxicity was evaluated in rat H9c2 and human HT29 cell lines and non-cytotoxic compounds (i.e. with LD50 >200 μ M) were selected for further studies. To confirm potential hits activity directly on ANT, we purified the native protein from rat hearts in the presence of Triton X-100, confirmed its purity by western-blot and incorporated it into small unilamellar vesicles (100 nm of diameter) as described.² Validation of a novel ANT-containing proteoliposome bioluminescent assay is currently on-going to develop a method to quantify the ability of molecules to modulate ADP/ATP exchange efficacy in dose-response and calculate EC₅₀. If robust, this assay will be adapted to our high throughput screening platform. For a therapeutic perspective, identified molecules will be evaluated for their toxicity, druggability, and for their ability to influence the disease outcome in animal models as a prerequisite for future clinical studies.

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Glucagon-like peptide 1 or GLP-1 counteracts NADPH oxidase activation during hyperglycemia through an AMPK-dependent pathway in adult cardiomyocytes

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Background and objective: Exposure to high glucose (HG) stimulates NADPH oxidase (NOX2) dependent-ROS production in cardiomyocytes. NOX2 activation is not triggered by an increased glucose metabolism but results from a glucose transport through a sodium-glucose co-transporter (SGLT). The aim of this work is to identify potential therapeutic approaches to counteract glucotoxicity.

Methods and results: Primary cultures of adult rat cardiomyocytes were exposed to high glucose concentration (HG, 21mM). AMP-activated protein kinase (AMPK) activation by A769662 or Phenformin nearly suppressed ROS production under hyperglycemia. GLP-1, a new anti-diabetic drug, exerted a similar effect, blocking hyperglycemia-mediated ROS production. Interestingly, GLP-1 treatment induced a AMPK activation, the maximal activation being observed at 100 nM. α 2AMPK, the major isoform expressed in cardiomyocytes (but not α 1AMPK) was activated in response to GLP-1. Anti-ROS properties of AMPK were not related to change in glucose uptake or glycolysis. Using *in situ* proximity ligation assay technology, we demonstrated that AMPK activation prevented p47phox translocation to the caveolar structure after high glucose concentration exposure, whatever the AMPK activators used. NOX2 activation by α -methyl-D-glucopyranoside, a glucose analog exclusively transported through SGLT, or by Angiotensin 2 was also counteracted by GLP-1. The crucial role of AMPK in limiting glucotoxicity was demonstrated by overexpressing a constitutively active form of AMPK using adenoviral infection. This overexpression prevented NOX2 activation in response to HG. Finally, in mice cardiomyocytes, GLP-1 did not exert protective action in the absence of α 2AMPK.

In conclusion, GLP-1 induces α 2AMPK activation and blocks HG-induced p47phox translocation to the plasma membrane, limiting glucotoxicity.

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The estrogen receptor alpha C451 palmitoylation site is absolutely required for vascular membrane-initiated action of estrogens in mice

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