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Reduced scar maturation and contractility lead to exaggerated left ventricular dilation after myocardial infarction in mice lacking AMPKα1

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Objective: Cardiac fibroblasts (CF) are crucial in left ventricular (LV) remodelling after myocardial infarction (MI). They predominantly express the α1 catalytic subunit of AMP-activated protein kinase (AMPKα1), while AMPKα2 is the major catalytic isoform in cardiomyocytes. AMPKα2 is known to protect the heart by preserving the energy charge of cardiac myocytes during injury, but whether AMPKα1 interferes with maladaptive heart responses remains unexplored. In this study, we aim at further substantiating the role of this AMPK isoform in the progression of post-MI LV remodelling and more particularly in the regulation of fibrotic properties of CF.

Methods: AMPKα1 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 immunohistofluorescent staining. LV function and remodelling was assessed by echocardiography.

Results: In the absence of AMPKα1, 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 infarcts. While CF proliferation was increased, expression of the myodifferentiation marker a-smooth muscle actin was decreased. This faulty maturation of post-MI scar formation and highlight the specificity of this catalytic isoform in cardiac fibroblasts/myofibroblast biology.

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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 an AMPK activation, the maximal activation being observed at 100 nM. eAMPK, the major isoform expressed in cardiomyocytes (but not α1AMPK) 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 ligassation 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 eAMPK.

In conclusion, GLP-1 induces eAMPK 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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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 ischaemia-reperfusion injury. In this context, we and others have shown that pharmacological modulation of ANT can prevent three acute pathologies in mice 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 intercellular and subcellularly 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 Hep2 cells and human HT29 cell lines and non-cytotoxic compounds (i.e. with LDS0 >200 μM) 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. 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.
Estrogen Receptor alpha (ERα) activation functions AF-1 and AF-2 classically mediate gene transcription in response to estradiol (E2). A fraction of ERα is targeted to plasma membrane and elicits membrane-initiated steroid signalling (MISS), but the physiological roles of MISS in vivo are poorly understood. We therefore generated a mouse with a point mutation of the palmitoylation site of ERα (C451A-ERα) to obtain membrane-specific loss-of-function of ERα.

The abrogation of membrane localization of ERα in vivo was confirmed in primary hepatocytes, and it resulted in female infertility with abnormal ovaries lacking corpora lutea and increase in luteinizing hormone levels. In contrast, E2 action in the uterus was preserved in C451A-ERα mice and endometrial epithelial proliferation was similar to wild-type. However, E2 vascular actions such as rapid dilatation, the acceleration of endothelial repair and endothelial NO synthase phosphorylation were abrogated in C451A-ERα mice. A complementary mutant mouse lacking the transactivation function AF-2 of ERα (ERα-AF2°) provided selective loss-of-function of nuclear ERα actions. In ERα-AF2°, the acceleration of endothelial repair in response to estrogen-dendrimer conjugate, which is a membrane-selective ER ligand, was unaltered, demonstrating integrity of MISS actions. In genome-wide analysis of uterine gene expression, the vast majority of E2-dependent gene regulation was abrogated in ERα-AF2° whereas in C451A-ERα it was nearly fully preserved, indicating that membrane-to-nuclear receptor crosstalk in vivo is modest in the uterus.

Thus, this work is the first to genetically segregate membrane versus nuclear actions of a steroid hormone receptor and to demonstrate their in vivo tissue-specific roles.