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Renal papillary tip extract stimulates BNP production and excretion from cardiomyocytes

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Background: Brain natriuretic peptide (BNP) is an important biomarker for patients with cardiovascular diseases including heart failure and cardiac hypertrophy. It is known that BNP levels are relatively higher in patients with chronic kidney disease with no heart disease; however, its mechanism remains unclear.
Purpose: Our purpose is to determine whether a substance in the renal papillary tip is associated with BNP or not.
Methods: We developed a BNP reporter mouse, pBNP-tdTomato transgenic (Tg) mice, with the DNA fragments of mouse BNP 1.1k promoter into the expression vector of tdTomato and six lines of Tg mice were screened. To evaluate the activation of BNP promotor in aged mice, we examined the expression of tdTomato in each of organs from pBNP-tdTomato Tg mice. We attempted to establish a primary culture of the pBNP-tdTomato-positive cells from kidneys of BNP reporter mice. We investigated the effects of an extract of the papillary tip of rat on the expression of BNP in cultured rat neonatal cardiomyocytes with the use of northern blot and ELISA measurement. The blood pressure, serum BNP and urine cGMP were measured in stroke-prone spontaneous hypertensive rats (SHR-SP) after the extract from the papillary tip was injected intraperitoneally. Ligation of the rats' left anterior descending coronary artery was performed, and the effects of extracts from the papillary tips of kidneys of heart failure rats were examined five days after the induction of myocardial infarction.
Results: In addition to the expression of tdTomato in cardiomyocytes, we occasionally found that the BNP promoter was activated specifically in the papillary tip of the kidneys and was not accompanied by BNP mRNA expression. pBNP-tdTomato-positive cells appeared to be interstitial and were not observed in any area of the kidneys except for the papillary tip. No evidence was observed to show the existence of BNP isoform or other nucleotide expression than BNP from around the BNP promotor region. Unexpectedly, both the expression and the secretion of BNP increased in the primary cultured neonatal cardiomyocytes after the treatment with the extract of the renal papillary tip. We were able to culture papillary tip tissue containing tdTomato-positive cells for more than 2 months, but no proliferation was observed. Intraperitoneal injection of the extract of the papillary tips reduced blood pressure accompanied by increasing serum BNP and urinary cGMP production in SHR-SP. Furthermore, the induction of BNP by the papillary extract from rats with heart failure due to myocardial infarction was increased in cardiomyocytes.
Conclusions: These results suggest the interstitial cells in the renal papillary tip possess a substance that can stimulate BNP production and secretion from cardiomyocytes in vivo and in vitro.
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FGF23 - and angiotensin II signaling cross-talk in cardiomyocytes

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Heart failure (HF) manifestation and progression are driven by systemic activation of neuroendocrine signaling cascades, such as the renin-angiotensin aldosterone system (RAAS). Fibroblast growth factor 23 (FGF23), an endocrine hormone, is linked to HF and cardiovascular mortality. It is also a mediator of left ventricular hypertrophy (LVH). FGF23 is proposed to trigger pathological signaling by involving Ca2+-regulated transcriptional pathways. In vivo, high circulating levels of FGF23 are associated with an altered systemic RAAS response. In the present study, we investigated Ca2+-dependent signaling of FGF23 in ventricular cardiomyocytes and its association with angiotensin II (ATII).
Neonatal rat ventricular cardiomyocytes (NRVMs) were isolated and cultured for 5 days. ATII (1μM) or FGF23 (25ng/ml) were used as hypertrophy stimuli. Cell surface area and ATII levels were studied by immunostaining. Gene expression was accessed by qPCR. Ca2+ transient (CaT) amplitude (F/F0peak at 1Hz; Fluo-4 AM) and CaT area under the curve (AUC) were quantified in cytosol and nucleus. A subset of NRVMs was treated with losartan (1 μM), Aminoethoxydiphenyl borate (2-APB, 5μM) 30 min before agonist stimulation. Mass spectrometry analysis was carried out to detect secreted ATII.
In neonatal rat ventricular myocytes (NRVMs), both ATII and FGF23 induced hypertrophy as reflected by cell area and hypertrophic gene expression. In Ca2+ imaging experiments, an increase of cytoplasmic (2.4folds±0.3) and nuclear (1.9folds±0.3) CaT amplitude was observed on acute treatment with FGF23 ($p<0.01$) similar to ATII. CaT AUC too was augmented significantly by both the treatments in cytoplasm and nucleus. The study with inositol 1, 4, 5-triphosphate (IP3) inhibitor 2-APB showed that FGF23-like ATII-induced IP3-dependent Ca2+ release from the nucleoplasmic Ca2+ store, associated with cellular hypertrophy.
Interestingly, ATII receptor antagonist losartan significantly attenuated FGF23-induced changes in Ca2+ homeostasis and cellular hypertrophy suggesting the involvement of ATII receptor-mediated signaling. Furthermore, FGF23 increased intracellular ATII expression (4.2folds±0.5) at 24h as well as on acute (90mins) treatment (2.2folds±0.1) vs. control ($p<0.01$), suggesting ATII involvement. Moreover, mass spectroscopy of the supernatant collected from FGF23 treated NRVMs clearly showed the presence of ATII peptide (peak at m/z 1046.562), confirming ATII secretion, whereas the ATII peak was absent in the supernatant collected from untreated NRVMs.
In conclusion, FGF23 and ATII share a common mechanism of IP3- and Ca2+-dependent cardiomyocyte hypertrophy. FGF23-mediated cellular hypertrophy is associated with increased production and secretion of ATII by cardiomyocytes. These findings indicate a pathophysiological role of the cellular angiotensin system in FGF23-induced hypertrophy in ventricular cardiomyocytes.

MOLECULAR MECHANISMS OF CARDIAC DISEASE

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Pharmacological inhibition of the mitochondrial NADPH oxidase 4/PKC/Gal-3 signaling pathway attenuates adverse cardiac fibrosis, following myocardial infarction

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Background: The antifibrotic mechanism of metformin is not completely understood. Our aim, therefore, was to characterize the possible involvement of NADPH oxidase 4 (mitoNox), PKC and Gal3 in this mechanism, given that each has been separately related with myocardial fibrosis and they could represent therapeutic targets.
Methods: Rats were subjected to MI by permanent ligation of the anterior descending coronary artery, and randomized into four experimental groups: (1) placebo-treated sham group; (2) placebo-treated MI; (3) Metformin-treated MI and (4) Metformin-treated sham group. Rats received MET (250 mg/kg/day) or normal saline for 4 weeks. An experimental model of biomechanical strain and a coculture, to allow cross talk between primary cultures of cardiomyocytes and cardiac fibroblasts (all obtained from C57BL6J mice using a Langendorff system), were established to characterize the underlying molecular mechanisms involved in the MET antifibrotic actions. The role of AMPK was determined via siRNA-mediated knockdown while those of mitoNox and PKCα were determined using the inhibitors GKT137831 and Chelerythrine, respectively. The mRNA and protein expression of different markers were measured by quantitative RT-PCR and Western blot, respectively.
Results: Long-term metformin treatment (4 weeks) following MI was associated with: i) a reduction in myocardial fibrosis, Gal3 mRNA and protein levels, as well as macrophage infiltration; ii) an increase in AMPK a1/a2 levels ($p<0.001$), and an inhibition of both mRNA expression and enzymatic activities of mitoNox and PKCa ($p<0.001$, in all cases). Following MI, the increase in expression and activity of both enzymes was associated with an increase in the accumulation of the superoxide anion ($p<0.001$) which positively correlated with mitoNox and PKCα activities ($rs=0.362$, $p=0.018$), as well as an increase in the lipid peroxidation ($p<0.001$) and the activation of the apoptotic death program characterized by the activation of initiatory procaspase 9 ($p<0.001$) and effectory procaspase 3 ($p<0.001$). MET therapy was associated with lower superoxide accumulation ($p<0.001$), lipid peroxidation ($p<0.001$) and caspases activation ($p<0.001$). These findings were replicated using a biomechanical strain model. The silencing of AMPK expression

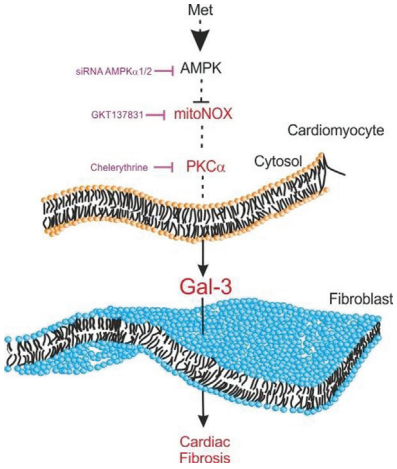

Figure 1

(siRNA) blocked the ability of metformin to protect cardiomyocytes from biomechanical strain in terms of mitoNox and PKCa activities, Gal3 levels, cells viability and proliferation, and reactive oxygen species levels ($p<0.001$, in all cases). The use of specific inhibitors supported the idea that PKC is downstream of mitoNox, and the activation of this pathway results in Gal-3 up-regulation. The Gal-3 secreted by cardiomyocytes has a paracrine effect on cardiac fibroblasts, inducing their activation.

Conclusion: A metformin-induced increase in AMPK improves myocardial remodeling post-MI, which is related to the inhibition of the mitoNox/ PKCa/Gal-3 pathway.

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Direct monitoring of cAMP at cardiac ryanodine receptor reveals altered beta2-adrenoceptor dependent regulation in hypertrophy

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3',5'-cyclic adenosine monophosphate (cAMP) is an ubiquitous second messenger which regulates cardiac excitation-contraction coupling by cAMP-dependent protein kinase (PKA) mediated phosphorylation of several calcium handling proteins. cAMP is confined to subcellular microdomains as for example in the vicinity of the cardiac ryanodine receptor (RyR), which is responsible for systolic calcium release from the SR. In cardiac disease, dissociation of calstabin and phosphodiesterase 4 (PDE4) can lead to hyperphosphorylation of the cardiac RyR and diastolic calcium leak which can provoke live threatening arrhythmias. Although these chronic disease-associated molecular changes have been well established, direct monitoring of cAMP near RyR2 capable of detecting early disease driven alterations has been not performed due to the lack of appropriate imaging techniques.

Here, we designed the first targeted Förster resonance energy transfer (FRET) based cAMP biosensor which can be used to directly monitor local cAMP dynamics in the RyR2 microdomain. Using this sensor in transgenic mice subjected to aortic banding, we could observe dramatic pathology-associated changes in the microdomain-specific PDE effects which was presumably due to subcellular redistribution of PDE2, 3 and 4. Furthermore, in healthy cardiomyocytes, β1-AR but not β2-AR stimulation strongly increases local cAMP levels in the vicinity of RyR measured by FRET. However, in cardiac hypertrophy, β2-AR/cAMP signal can cross the cleft and reach RyR microdomain due to subcellular redistribution of phosphodiesterases, in particular because of a dramatic loss of the local pool of PDE4. Importantly, local β2-AR-induced rise of cAMP at RyR2 could be linked to a significant increase of diastolic calcium leak triggering calcium sparks and calcium waves similar to the effects of β1-AR stimulation under healthy conditions. Indeed, diseased but not healthy myocytes showed increased calcium leak in response to β2-AR stimulation, as measured by confocal calcium spark imaging. This occurs despite only a moderate increase of cell contractility and relaxation and might serve as an important mechanism to increase arrhythmia susceptibility in response to normally cardioprotective β2-AR subtype. This might have a direct impact on RyR phosphorylation and arrhythmia susceptibility.

In conclusion, early cardiac disease leads to changes in PDE composition of the RyR microdomain which causes increased local β2-AR/cAMP signaling which leads to arrhythmias originating from this normally cardioprotective adrenoceptor subtype.

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P934
Gene-gene interaction in ischemic cardiopathy by MDR: beyond logistic regression

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Introduction: Multiple genetic variants have been identified in GWAS as being associated with ischemic cardiopathy (IC). To better analyze the gene-gene interaction, new computational and statistical methods emerged beyond logistic regression.

Objectives: Study the best gene-gene interaction model and predictor of coronary disease (CAD), using new data mining methods such as Multifactor Dimensionality Redution (MDR).

Methods: We included 2888 participants (mean age 53±7.9 years, 77.8% male), namely 1566 coronary patients documented by angiography with one or more epicardial stenoses greater than 75% and 1322 controls adjusted for age and gender. Taqman SNP genotyping (Applied Biosytems) was used and then a gene-to-gene analysis was performed between 33 variants associated with CAD. MDR was applied to obtain the best genetic predictor for IC, by using the 10 most significant variants.

Results: In the one-gene model, the MDR projected the TCF21 gene as the most significant genetic risk factor for CAD. The model with two genes demonstrated synergistic interaction between the TCF21 and APOE. The genetic bivariate model of TCF21 and APOE was the best predictive model with an OR of 1.51 (95% CI: 1.29–1.76; $p<0.0001$) and with adequate cross-validation (10/10), with no evidence of overfitting model. The acuity of the best G-G predictor model of CAD was 0.55. A reasonable sensitivity (60%) and specificity (50%) were obtained from this model.

G–G interaction models by MDR including 10 genetic variants that revealed significance in the univariate analysis

Best models	Training		Testing		CV Consistency
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value	
TCF21	1.27 (1.09 – 1.49)	0.002	1.13 (0.71 – 1.80)	0.606	8/10
TCF21; APOE	**1.51 (1.29 – 1.76)**	**<0.0001**	**1.51 (0.94 – 2.40)**	**0.085**	**10/10**
TCF21; CDKN2B; APOE	1.62 (1.39 – 1.89)	<0.0001	1.22 (0.77 – 1.94)	0.404	7/10

MDR – Multifactor dimensionality reduction; CI – Confidence interval; CV – Cross validation. Genetic variants: LPA, TCF21, APOE, CDKN2B, PON55, PHACTR1, FTO, Locus9p21, ADIPOQ, ZC3HC1. Statistically significant for p<0.05.

Conclusions: In our population, the interaction between the genetic variants TCF21 (cell axis) and APOE (lipid axis) showed consistent association with IC. An in-depth investigation of this interaction may lead to the identification of new therapeutic targets in IC.

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Specific invalidation of AMP-activated protein kinase alpha1 in cardiac fibroblasts exacerbates left ventricular remodeling following ischemia in mice

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Purpose: Following myocardial infarction (MI), the necrotic area is replaced by a fibrotic scar and this is associated with deleterious left ventricular (LV) remodeling. Cardiac fibroblasts (CF) are crucial components of the fibrotic healing following ischemia. We have previously shown that AMPKα1 is a key regulator of CF properties. The aim of the study was to investigate whether CF AMPKα1 is a central player in the functional and structural adaptation of the heart in response to cardiac injury.

Methods: A transgenic mouse strain in which AMPKα1 is specifically deleted in CF (CF-KO) was generated and animals were subjected to MI (left anterior descending coronary artery permanent ligation). Control mice were sham operated. Cardiac function of CF-KO and -WT mice was assessed by 2D-echocardiography at baseline as well as 14, 30 and 90 days post-MI (Vevo 2100, VisualSonics). Mice were sacrificed 14 or 90 days post-surgery and hearts were collected to analyze fibrosis and to characterize LV remodeling by histology, polarized light / electron microscopy and mRNA / protein expression.

Results: At basal state, no phenotype differences were observed between CF-KO and -WT hearts. While infarct size did not differ between the two genotypes, echocardiographic analysis showed that CF-KO mice exhibit exacerbated LV remodeling, as manifested by augmented diastolic (KO:205.0±3.8μl, WT:119.4±10.7μl; $p<0.001$) and systolic (KO:175.7±26.0μl, WT:95.4±10.3μl; $p<0.01$) volumes, 30 days post-MI. Furthermore, a Kaplan-Meier curve demonstrated increased mortality of CF-KO animals, 90 days post-MI (KO:34.8%, WT:59% survival). Histological analysis showed a significantly augmented fibrosis in the infarct area of CF-KO mice compared to -WT, 14 days post-surgery (KO:69.5±3.7%, WT:58.8±1.8%; $p<0.05$). Polarized light as well as electron microscopy data revealed a more organized and denser collagen matrix in CF-KO, in striking contrast to -WT hearts. Immunostaining and qPCR analysis demonstrated that myodifferentiation of CF was 2-fold increased in CF-KO infarcted hearts. Mechanistically, we showed that the expression of connexin 43 (Cx43), a protein involved in CF-cardiomyocyte junctions and cardiac fibrosis development, was drastically suppressed in CF isolated from infarcted CF-KO hearts (KO:0.27±0.19AU, WT:1±0.07AU; $p<0.05$). This result was confirmed in vitro, in human CF transfected with a specific siRNA targeting AMPKα1. Our recent data provide evidence that a post-transcriptional control of Cx43 by microRNAs is involved in this effect.

Conclusion: Our results illustrate that CF AMPKα1 plays an essential role in cardiac remodeling, as its specific invalidation leads to a deleterious phenotype following MI. This phenotype is characterized by robustly increased CF myodifferentiation and fibrosis, exacerbated LV dilatation and increased mortality following MI. These noxious effects are potentially mediated via a post-transcriptional regulation of Cx43 by microRNAs.