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Functional Genomics of Cardioprotection by Ischemic Conditioning and the Influence of Comorbid Conditions: Implications in Target Identification

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Abstract: Ischemic heart disease including myocardial infarction develops on the basis of several risk-factors and co-morbidities such as obesity, diabetes, hypertension, and hypercholesterolemia. Ischemic heart disease is the leading cause of mortality worldwide, therefore, identification of novel drug targets for cardioprotection is of great importance. Ischemic preconditioning, postconditioning, and remote conditioning trigger endogenous cardioprotective mechanisms that render the heart more resistant to lethal ischemic-reperfusion injury. However, major cardiovascular co-morbidities such as hyperlipidemia, diabetes, and their co-medications interfere with these cardioprotective mechanisms thereby limiting the efficacy of cardioprotective ischemic conditioning maneuvers. Ischemia reperfusion injury and cardioprotection by conditioning have been shown to affect global myocardial gene expression profile at the transcript level. Further understanding and the comprehensive analysis of the cardioprotective gene expression fingerprint in normal, protected, and in comorbid conditions may lead to identification of novel molecular targets for cardioprotection.

Keywords: Microarray, microRNA, miRNA, mRNA, postconditioning, preconditioning, proteomics, risk factors, sequencing, system biology, transcriptomics.

1. ISCHEMIC CONDITIONING OF THE HEART

Ischemic conditioning leads to remarkable cardioprotection against I/R injury (see for extensive reviews: [1, 2]). Ischemic conditioning has 3 major forms, preconditioning, postconditioning, and remote conditioning. In ischemic preconditioning, brief episodes of ischemia/reperfusion are applied before a prolonged ischemic event that would otherwise lead to significant irreversible tissue injury. Ischemic preconditioning is the most effective cardioprotective mechanism that evokes cardioprotection [3] immediately after triggering ischemia/reperfusion cycles and in a delayed manner (appearing 12-24 hours after the triggering ischemia/reperfusion cycles and lasting for approximately 72 hours) the latter termed as the second window of protection [4]. In case of ischemic postconditioning, very brief episodes of ischemia/reperfusion are applied immediately after the prolonged ischemic event. In remote ischemic conditioning, brief cycles of ischemia/reperfusion are applied to an extracardiac organ before, during, or after the prolonged ischemic event. The cardioprotective effect of pre-, post-, and remote conditioning has been well established in preclinical models as well as in clinical studies (see for reviews: [2, 5, 6]), however, its effectiveness is limited by the presence of several risk factors, comorbidities, and co-medications (see for extensive reviews: [1, 7, 8]). Moreover, in spite of 3 decades of intense research on the cellular mechanism of conditioning, it is still not completely understood. Therefore, no drug targets have been identified so far that led to the development of a cardioprotective drug until market launching. This fact urges to find novel approaches to increase the translational value of preclinical studies (see for review [9]) and to find valid drug targets and novel therapeutic approaches for cardioprotection (see for review: [1, 10] and Table **1**).

In this review, we describe how assessment of changes in cardiac gene expression profile triggered by conditioning may facilitate the development of cardioprotective drugs that mimic the remarkable cardioprotection achieved by ischemic conditioning maneuvers.

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Table 1. Examples for potential targets revealed by functional genomics approaches.

Target	Role	Reference
Ischemic conditioning		
MAPKAP kinase 3	signaling	[11]
cathepsin G	protease	[11]
PDE9A1	phosphodiesterase	[12]
peroxredoxin 4	antioxidant defence	[18]
PDGFR	signaling	[18]
ZAC1	transcription factor	[20]
microRNA-139-5p	unknown	[17]
microRNA-125b*	unknown	[17]
let-7b	unknown	[17]
microRNA-487b	unknown	[17]
Hyperlipidemia and obesity		
ATF3	transcription factor	[25]
NADH-ubiquinone oxidoreductase	energy metabolism	[27]
Hsp86	stress response	[27]
procollagen	structural proteins	[27]
argininosuccinate synthetase	Arg metabolism	[28]
Hsp70	stress response	[28]
cytochrome C oxidase	energy metabolism	[30]
MMP-9	remodeling	[30]
microRNA-25	oxidative stress	[33]
microRNA-27b	hypertrophy	[34-36]
Diabetes		
vanilloid receptor 1	signaling	[64]
histone deacetylase 2	gene regulation	[64]
apolipoprotein B	lipid metabolism	[64]
MMP-13	remodeling	[64]
microRNA-208a	hypertrophy	[54, 55]
microRNA-24	vascularization	[54, 55]
microRNA-21	hypertrophy	[54, 55]

2. GENE EXPRESSION OF THE HEART IN CARDIOPROTECTION BY ISCHEMIC CONDITIONING

There is a continuously increasing interest in ischemic conditioning of the heart as shown by the increasing number of papers in PubMed. Although there are a total of more than ten thousand papers in the literature on cardioprotection by ischemic conditioning, less than 1% of these studies assessed global gene expression profile of the heart in response to ischemic conditioning. These studies have shown that preconditioning and postconditioning significantly affect the gene expression profile of the ischemic heart at the transcript level. The first studies in the literature showing that early and late ischemic preconditioning affect gene expression profile of the heart using 1, 2 or 3 cycles of 5 min ischemia/5 min perfusion in rabbits and in isolated rat hearts, respectively, are more than 10 years old [11-13]. In rabbit hearts, 35 genes with significantly altered expression patterns in response to preconditioning were discovered including upregulated MAPKAP kinase 3 and cathepsin G genes in the preconditioned region, and GTP exchange factor, Na^+/K^+-ATPase, Zn finger protein 35, cytochrome c oxidase, mitogen-responsive phosphoprotein, and Ran-binding protein in the non-ischemic region [11]. In rat hearts, oligoadenylate synthase, chaperonin subunit epsilon, a cGMP phosphodiesterase (PDE9A1), a secretory carrier membrane protein, an amino acid transporter, and protease 28 subunit genes were changed in response to preconditioning [12].

These early studies have been confirmed by several papers showing that both ischemic preconditioning and postconditioning trigger a cardioprotective gene expression profile in the heart at the transcript level [14-17]. In line with these findings, changes in the expression of the post-transcriptional regulators of gene expression, i.e. microRNAs have been recently shown by preconditioning and postconditioning in rat hearts [18]. Whether remote ischemic conditioning may also affect global gene expression profile of the heart has not been specifically studied so far. Nevertheless, the early study of Simkhovich *et al.* [11] showing that the gene expression profile of the non-ischemic remote area was changed, suggests that remote conditioning also leads to a cardioprotective transcriptomic program. Accordingly, one paper has reported cardiac gene expression profile alterations following remote ischemic preconditioning; e.g. up-regulation of genes involved in oxidative stress (e.g. peroxiredoxin 4, platelet-derived growth factor receptor) 15 min or 24 hours after 6 cycles of 4-min femoral artery occlusion/reperfusion in mice [19]. In line with this assumption, extracellular vesicles, potential carriers of microRNAs, have been shown to mediate the cardioprotective effect of remote ischemic preconditioning induced by 3 × 5-5 min ischemia/reperfusion cycles in rat hearts [20].

Identification of global gene expression profile of the heart in response to ischemic conditioning may help to identify key cellular pathways of ischemia/reperfusion injury and cardioprotection by ischemic conditioning. The transcription factor ZAC1 gene has been identified as potential target for gene silencing as this gene was significantly down-regulated by preconditioning and postconditioning [21]. Similarly, by a systematic comparison method looking at the direction of microRNA expression changes after ischemia/reperfusion injury with or without preceding preconditioning or subsequent postconditioning, respectively, potential cardioprotective microRNA targets have been identified and termed "protectomiRs", e.g. mimics of microRNA-139-5p, -125b*, let-7b, and antagomiR of microRNA-487b in rat hearts [18].

These preclinical results show that by assessing the cardioprotective gene expression profile of the heart subjected to ischemia/reperfusion with or without cardioprotection elicited by ischemic conditioning, novel targets for cardioprotection can be identified. However, no data on the cardioprotective gene expression fingerprints in the human heart are available so far.

3. INFLUENCE OF CO-MORBIDITIES ON CARDIAC GENE EXPRESSION PATTERN

The mechanism by which the remarkable cardioprotective effect of ischemic conditioning is attenuated or abolished in the presence of major cardiovascular risk factors and comorbidities as well as their routine drug treatments is not exactly known (see for extensive reviews: [1, 7, 8]). Therefore, for successful cardioprotective target identification and validation, it is of great importance to assess the effect of major cardiovascular comorbidities and co-medications such as dyslipidemia, diabetes, hypertension, obesity, aging etc (see for review: [1]). Accentuated myocardial oxidative stress has been reported in the presence of major comorbidities. Therefore, it is plausible to speculate that redox signaling-dependent global gene expression changes contribute to the pathological phenotypes. The redox-sensitive modulation of gene expression may occur both by direct oxidative/nitrative modification of the transcription factor itself or by posttranslational modifications (i.e. mainly phosphorylation/dephosphorylation] due to alterations in redox-regulated intracellular signaling cascades (e.g. p38 MAPK) [22]. So far limited amount of data have been accumulated in the literature regarding the effect of hyperlipidemia, obesity, and diabetes on cardiac gene expression profile, while there are still negligible data on other cardiovascular risk factors and comorbidities. In this chapter we will review the current data available in the literature on the effect of major cardiovascular comorbidities on cardiac gene expression profile, focusing on metabolic diseases such as hyperlipidemia, obesity and diabetes.

3.1. Hyperlipidemia, Obesity and Statins

Hyperlipidemia and obesity are amongst the most important risk factors for cardiovascular diseases due to their steeply increasing prevalence worldwide. Moreover, hyperlipidemia was the first risk factor to be associated with the loss of cardioprotection by ischemic preconditioning in rabbits and rats on chronic cholesterol-enriched diet ([23, 24], see for the first extensive review [8]). Since then, it has been well established that most of the other major risk factors and their medications may modify cardioprotective signaling [1, 7]. Therefore, to better characterize the effect of these pathologies on the heart and to uncover novel therapeutic targets, several research groups have performed systematic analyses of cardiac gene expression in various disease models.

A few publications aimed to decipher how the composition of consumed excess fat influences gene expression in the heart. Lockridge and colleagues hypothesized that exposure to various fatty acids alone have differential effect on the gene expression pattern of isolated cardiomyocytes. They compared effects of fatty acids considered cardioprotective and cardiotoxic by cDNA microarrays and revealed that 24 h treatment of saturated fatty acids tended to induce endoplasmic reticulum- and oxidative stress markers (e.g. activating transcription factor 3 or growth arrest and DNA-damage-inducible protein α) more than a treatment with unsaturated fatty acids [25]. The acute effects of unsaturated fatty acids on gene expression in the heart were studied in-vivo as well. Mice were fed with triglycerides comprising specific fatty acids, and 6 hour later mRNA expression profiles of the hearts were determined. Similarly to the experiment on isolated cells, a differential modulation of gene expression was shown, where linolenic acid (C18:3) modulated the expression of most genes, the majority of which was related to the PPARα signaling pathway (e.g. heme oxygenase 1 or angiopoietin-like 4 protein) [26]. These studies clearly demonstrate that even a short term overload of dietary fats might modulate cardiac gene expression, and that these effects are specific to fatty acid moieties and most likely not a direct consequence of the cellular energy imbalance.

Long term effects of hyperlipidemia and obesity on cardiac gene expression has been studied in several papers. In an early study, Puskas *et al* reported that in the hearts of rats on cholesterol-enriched chow for two months expression of numerous genes were modulated, including members of energy metabolism (e.g. NADH-ubiquinone oxidoreductase), heat shock proteins (heat shock protein 86), ion channels (sodium/potassium ATPase), and structural proteins (procollagen, type III, α1) [27]. Later, Sarkozy *et al* described differences in gene expression between Zucker diabetic fatty and control lean rats including several metabolic enzymes (e.g. argininosuccinate synthetase), stress response proteins (e.g. heat shock protein 70, 1A) and ion channels (e.g. sodium/potassium ATPase β4 polypeptide) [28]. Several of these genes have not been implicated in cardiovascular diseases before. Tissue specific gene expression changes were also compared in obese animals [29]. In high-fat chow fed mice expression of several hundred genes is altered, although only a fraction of them is common between tissues. In the heart most robust changes were detected in members of lipid metabolism (e.g. major urinary proteins 2, 3 and 5), but in contrast, in the adipose tissue expression of several genes of the immune system were altered (e.g. CD84 antigen). In addition, a profound modulation of PPAR signaling in most tissues is shown in this publication, similarly to others, e.g. [26].

Large animal models have been rarely used for cardiac transcriptomics studies. Rouet and colleagues investigated dogs after 9 weeks of high fat diet that lead to approximately 25% weigh gain and arterial hypertension. This treatment resulted in a profound alteration of atrial and ventricular gene expression [30]. They identified several metabolic enzymes (e.g. cytochrome C oxidase), ion channels (e.g. sodium/potassium ATPase), genes involved in protein synthesis and remodeling (e.g. matrix metalloprotease 9, or nuclear factor erythroid 2–related factor 2). Interestingly, most gene expression changes have been detected in the atria and not the left ventricle. For example, they assessed a decrease in phospholamban and sarco-endoplasmic reticulum Ca^{2+}-ATPase-2 (SERCA-2) mRNA level only in the atria. In a following study, they studied temporal changes in gene expression as well. Dogs were fed with high-fat chow for 9-24 weeks and left ventricular mRNA levels were assessed by

microarrays [31]. In these experiments they identified a time-dependent decrease in the ventricular expression of phospholamban and SERCA-2. At 24 weeks they also found a decrease in myostatin expression, and an up-regulation in the expression of several genes related to the TGF-β pathway, which are also implicated in obesity-related cardiac pathologies, such as hypertrophy and excessive fibrosis.

The effect of human obesity or hyperlipidemia on cardiac gene expression patterns is not well studied. Philip-Couderc *et al* investigated mRNA transcript levels from right atrial appendages of obese and lean patients. They reported that obesity modulates the expression of almost 400 genes in the heart, and showed that the most affected signaling pathway was the down-regulated Wnt system, which is involved in cardiac hypertrophy [32]. Although this report shows that the gene expression pattern of the human atrium is severely altered in obesity, so far data is unavailable from ventricular samples. Given that the overlap in reported gene expression changes between species and models seem to be low, studies on obesity- and hyperlipidemia-modulated gene expression is still warranted.

During the last decade an increasing number of researchers directed their focus on the systematic analysis of miRNAs in the cardiovascular system. We recently reported that expression of several miRNAs is altered in the ventricles of rats fed a cholesterol-enriched chow [33]. One of the most robust down-regulation was found in miR-25, which might be responsible for the increased oxidative- and nitrosative stress observed in this model, since one of the putative targets of miR-25 is NOX4, a major source of reactive oxygen species. The role of miRNAs in hyperlipidemia- and obesity-induced changes in gene expression was also studied in the liver of mice on high-fat diet [34]. They found that hepatic expression of miR-27b is elevated in obese animals. The study concluded that miR-27b is a key regulator of hepatic lipid metabolism since the expression of its several target genes were also altered in hyperlipidemia. One might suggest that cardiac lipid metabolism can also be influenced by miRNAs through the circulation. Indeed, it has been reported that the overexpression of miR-27b leads to cardiac dysfunction and hypertrophy in mice [35], however, elsewhere it was identified as an anti-hypertrophic miRNA [36]. As in case of mRNA transcriptomics studies, data on cardiac expression of miRNAs in hyperlipidemic or obese humans is missing.

Statins, as inhibitors of 3-hydroxy-3-methyl glutaryl coenzyme A reductase, are potent cholesterol-lowering drugs that are widely used in clinical practice for primary and secondary prevention of coronary heart disease. Statins can exert pleiotropic effects aside from lowering cholesterol and blood pressure through several different pathways including the induction of endothelial nitric-oxide synthase expression, anti-inflammatory actions, and antioxidant activity [37]. We have previously shown in isolated rat hearts that lovastatin interferes with the anti-ischemic effect of ischemic pre- and postconditioning [38]. However, only a handful of preclinical studies investigated the effects of statin treatment on cardiac gene expression pattern. In a comparative study different types of statins such as atorva-, prava-, pitava-, and rosuvastatin were administered orally to normal C57Bl/6 mice for 4 weeks [39]. The authors found altogether 49 genes to be up- or down-regulated by statin treatments in a drug-specific manner, from which tissue inhibitor of metalloproteinases-3 showed the most robust increase in response to atorvastatin treatment. Others reported on cardiac expression changes of 93 genes after 4-week atorvastatin [15 mg/kg/day) treatment in normal Wistar Kyoto rats, whilst cardiac mRNA level of 60 genes has been altered by the same treatment in stroke prone spontaneously hypertensive rats [40]. The altered genes included several ones involved in general metabolic pathways (glucose, fatty acid, and cholesterol biosynthesis, etc.), cell division, signaling, motility, cell/organism defense, regulation of protein expression, oxidative stress, and inflammatory processes. Despite the abundance of clinical studies investigating the effects of statins, comprehensive studies on the effect of statins on cardiac gene expression profiles in the healthy and ischemic human myocardium are still missing.

3.2. Diabetes

Diabetes-related metabolic and molecular alterations ultimately lead to complex and serious cardiovascular complications, involving accelerated progression of atherosclerosis and deterioration of cardiac function [41). Diabetic myocardial dysfunction and diabetic cardiomyopathy is the factor that is responsible for higher cardiac morbidity and mortality in diabetics [42]. It is well established now that multiple factors contribute and promote to the development and evolution of diabetic cardiomyopathy (these include hyperglycemia and subsequent insulin resistance, increased fatty acid metabolism, microcirculatory changes, sympathetic dysfunction, myocardial inflammation, oxidative/nitrative stress, remodeling and fibrosis), however the exact molecular events that initiate and fuel these pivotal pathological mechanisms are not entirely clear [43]. To understand the underlying biological processes and identify new molecular targets, detailed characterization of gene expression in these hearts with systems biological tools is of high importance.

Investigations in pre-diabetic models are clinically essential, due to the enormously increasing incidence and overlapping prevalence of metabolic syndrome and pre-diabetes [44]. There is also evidence for the deterioration of myocardial function in models of pre-diabetes [45-47], in line with these, recently we have reported marked global gene expression alterations in the heart Zucker diabetic fatty rats [28], a model that is characterized by main features of pre-diabetes and metabolic syndrome (obesity, hyperglycemia, hyperinsulinemia, and hypercholesterolemia). Among the 14921 transcript assessed, 10244 showed detectable expression in the heart. Altogether 85 transcripts showed significant alterations (up- or down-regulation), when compared to the lean control rats. The detailed analysis (involving functional clusterization and gene ontology analysis) showed alterations in transcripts of lipid metabolism (e.g. 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2; argininosuccinate synthetase; 2-amino-3-ketobutyrate-coenzyme A ligase), extra- and intracellular structural proteins (e.g. myosin IXA; aggrecan1), signal transduction (e.g. activating transcription factor 3; phospholipase A2) stress response (e.g. heat shock 70kD protein 1A; heat shock protein 60; glutathione S-transferase Yc2 subunit), ion channels and receptors (e.g. ATPase, Na^+/K^+ transporting, beta 4 polypeptide; ATPase,

H^+/K^+ transporting, non-gastric, alpha polypeptide). These profound modifications in the cardiac transcriptome might be involved in the development of cardiac pathological abnormalities induced by pre-diabetes and metabolic syndrome.

Similar comprehensive studies were carried out in recent years in different models of overt diabetes, either using genetic models of the disease (Goto-Kakizaki diabetic rats, Otsuka Long-Evans Tokushima fatty rats) or the streptozotocin-induced type I. diabetes model. Karakikes *et al.* revealed significant divergence in myocardial gene expression profile in the hearts of the diabetic Otsuka Long-Evans Tokushima fatty rats when compared to the non-diabetic controls [48]. Out of the 20500 transcripts surveyed, 838 showed divergent expression (272 up-regulations and 566 down-regulations). Their subsequent functional analysis indicates that diabetic cardiomyopathy in this type 2 diabetes model is associated with changes in transcripts involved in immunity, development, intracellular signaling, cell proliferation and transcription regulation.

There is also evidence for the profound effect of diabetes on deteriorated post-infarction remodeling. The adverse remodeling process in diabetes is in association with profound myocardial gene expression alterations. The study by Song *et al.* suggest that in type 1 diabetic rats transcriptomic changes are responsible for altered myocardial metabolic substrate utilization (affected fatty acid metabolism, carboxylic acid synthesis, steroid metabolisms), that may account for the exaggerated progression of post-infarction remodeling processes [49].

Gestational diabetes is a specific diabetic condition, affecting both the health of the-mother and the baby. Although, there is a three- to four fold increase in the incidence of congenital heart defects caused by maternal diabetes [50], the exact molecular mechanisms that contribute to the teratogenic effects of the diabetic milieu are not known. A recent study investigated gene expression profiles of developing hearts of embryos, aiming to shed light on the mechanisms of cardiac malformation development [51]. The study revealed several differentially expressed genes in healthy and diabetic embryonic hearts. Based on the functional categorization of the altered transcripts it is hypothesized that genes involved in lipid metabolism, cell cycle and transcription regulation have important role in cardiac embryopathy.

Although, transcriptomic tools have revealed several important gene expression alterations in the heart in diabetic conditions, several limitations have to be noted. In the last years it became evident that the fate of the mRNA is determined by presence of other regulatory non-coding RNA species (e.g. microRNAs). In addition the importance and extent of alternative splicing events in cardiac pathologies are now recognized [52] that are impossible to assess with conventional microarray-based tools. In line with these, reactivation of the fetal splicing gene program has been described by Verma *et al.* [53]. RNA sequencing and subsequent bioinformatics identification of splicing events revealed that several important transcripts implicated in diabetes (e.g. VEGF, Mef2a) undergo alternative splicing. In addition there is a significant similarity of splicing pattern in the diabetic and developing embryonic heart, indicating the reprogramming of fetal gene expression events.

Recent studies also indicate the importance of microRNAs in the development of diabetic myocardial pathologies. The concordant studies of the Hu lab indicate striking alterations in microRNA expression profile in the heart of streptozotocin treated rats, implicating alterations in whole gene networks [54, 55]. They identified 19 microRNAs that were dysregulated in diabetic hearts (up-regulation of miR-195, miR-199a-3p, miR-142-3p, miR-24, miR-21, miR-208a, miR-221, miR-499-3p, miR-700, and miR-705 and down-regulation of miR-1, miR-143, miR-29, miR-20a, miR-220b, and miR-373). Based on bioinformatic target prediction and partial experimental validation, it seems that the affected microRNAs have a significant fingerprint on myocardial transcriptomic profile (affected genes involve hypertrophy- and fibrosis-related targets).

In addition, accumulating evidence proves the applicability of microRNAs as diagnostic biomarkers. In a landmark study Zampetaki *et al.* determined plasma microRNA expression profiles in diabetic patients [56], revealing 5 candidate microRNAs (miR-15a, miR-29b, miR-126, miR-223, and miR-28-3p) to be used as diagnostic indicators and predictors of later onset diabetes. Another study recently addressed altered circulating miRNA levels in children with type I diabetes; here interestingly levels of miR-126 were predominantly down in urine but not plasma of patients suggesting more pathological involvement of the kidney in response to type I diabetes [57].

The prevalence of diabetic neuropathy is greater than 50% in patients with long-standing diabetes [58]. Although, several papers have been published investigating the gene expression changes in different neural structures during the development of diabetic neuropathy, it is not clear if diabetes affects cardiac gene expression due to sensory and/or motor neuropathy. Capsaicin, an essential compound of hot pepper, is used as topical treatment to alleviate severe pain in patients with diabetic neuropathy [59]. Capsaicin is a highly selective sensory neurotoxin that leads to a selective functional blockade of a primary sensory neurons [60, 61]. We have previously shown that capsaicin-sensitive sensory nerves play an important role in preconditioning in rat hearts [62]. Furthermore, we demonstrated that capsaicin-sensitive nerves are involved in the regulation cardiac NO-cGMP signaling [63], which is known to be one of the major regulatory pathways for ischemic conditioning. Moreover, we have also shown that selective chemodenervation of capsaicin-sensitive sensory nerves in male Wistar rats *in vivo*, beyond cardiac diastolic dysfunction, induced changes in myocardial gene expression [64]. In this study, among the 6400 rat genes examined by DNA microarray and/or by QRT-PCR, 47 genes exhibited significant up-regulation and 36 were down-regulated. The up-regulated genes affect neural functions (e.g. vanilloid receptor 1; GABA receptor rho-3 subunit), signal transduction (including NO signaling), cell adhesion (integrin α v subunit), gene regulation (histone deacetylase 2), and metabolic pathways particularly the lipid (apolipoprotein B, farnesyl transferase) metabolism. Among the repressed genes we have also found genes of metabolic pathways (e.g. aldehyde dehydrogenase), cell cycle regulators (cyclin-dependent kinase 4), matrix metalloproteinase 13, and interleukin 7.

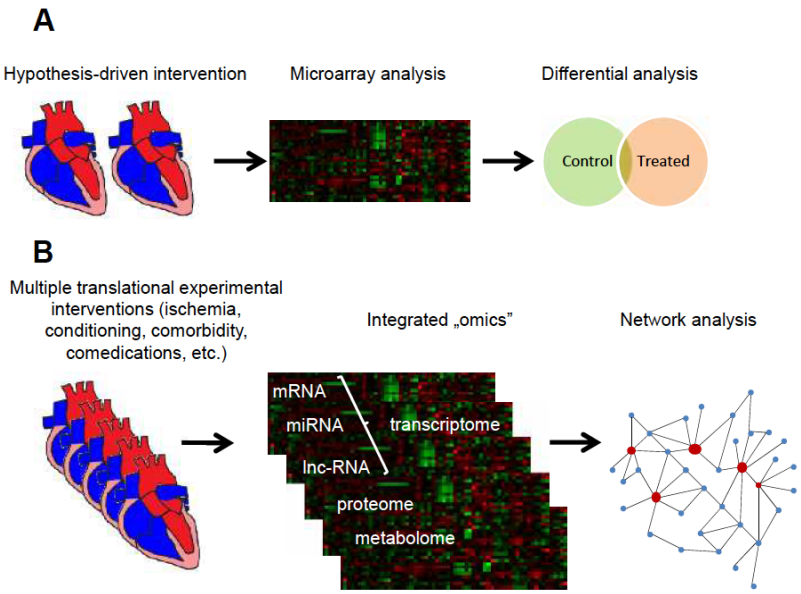

Fig. (1). Approaches of functional genomics. Panel **A** shows a conventional hypothesis-driven approach to investigate differentially expressed transcripts induced by a single experimental intervention. A more comprehensive way is to compare multiple expression patterns on different levels of functional genomics (mRNA, microRNA, long non-coding RNA) and proteomics and to integrate these "omics" data by network analysis to identify novel signalling hubs and networks. The array images were created by matrix2png algorithm [68].

CONCLUSION AND PERSPECTIVE

By assessing global cardiac gene expression profile of the ischemic heart with or without cardioprotection by ischemic conditioning, one may explore the cardioprotective gene expression fingerprint and identify novel drug targets for cardioprotection (Table **1**). However, for identification of valid drug targets, the effect of cardiovascular risk factors, comorbidities, and co-medications of ischemic heart disease should be taken into account. There is also an unmet need for studies that utilize an integrated "omics" approach (mRNA and non-coding RNA transcriptomics followed by proteomic analysis) with subsequent robust bioinformatics (e.g. network analysis) to identify key regulatory networks and signaling hubs in response to cardioprotection (Fig. (**1**) [65, 66]). This approach may provide the basis of cardioprotective gene threrapy as well [67]. So far very few studies looked at changes in the gene expression profile of the ischemic heart in response to cardioprotection and its confounding factors. However, such studies would be necessary to identify key pathways of cardioprotection that are still active in the presence of major cardiovascular comorbidities and their routine medications.

LIST OF ABBREVIATIONS

I/R	=	Ischemia/reperfusion
MAPKAP kinase	=	Mitogen-activated protein kinase-activated protein kinase
Mef2	=	Myocyte enhancer factor-2
NO-cGMP	=	Nitric oxide, cyclic GMP
NOX4	=	NADPH oxidase 4
PPARα	=	Peroxisome proliferator-activated receptor alpha
QRT-PCR	=	Quantitative real time polymerase
SERCA-2	=	Sarco-endoplasmic reticulum Ca^{2+}-ATPase-2
VEGF	=	Vascular endothelial growth factor

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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