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Periostin alters transcriptional profile in a rat model of isoproterenol-induced cardiotoxicity

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Abstract

Periostin is an extracellular matrix protein from the fasciclin family that guides cellular trafficking and extracellular matrix organization. Periostin stimulates mature cardiomyocytes to reenter the cell cycle. The molecular mechanism behind such stimulation remains to be explored. A DNA microarray technology constituting 30,429 gene-level probe sets was utilized to investigate effects of recombinant murine periostin peptide on the gene expression pattern in a rat model of isoproterenol (ISO)-induced myocardial injury. The experiment was performed on 84 adult male Sprague-Dawley rats in four groups ($n = 21$): (1) control group, (2) only periostin applied group, (3) ISO cardiotoxicity group, and (4) ISO + periostin group. The experiment was continued for 28 days, and rats were killed on days 1, 7, and 28 ($n = 7$). Microarray analyses revealed that periostin significantly altered the expression of at least $\pm$2-fold of 2474 genes in the ISO + periostin group compared to the ISO cardiotoxicity group of which 521 genes altered out of 30,429 gene-level probe sets. Ingenuity pathway analysis indicated that multiple pathway networks were affected by periostin, with predominant changes occurring in the expression of genes involved in oxidative phosphorylation, oxidative stress, fatty acid metabolism, and TNF-α NF-κB signaling pathways. These findings indicate that periostin alters gene expression profile in the ISO-induced myocardial injury and modulates local myocardial inflammation, possibly mitigating inflammation through TNF-α NF-κB signaling pathway along with a decreased Casp7 activity and apoptotic cell death.

Keywords

Cardiotoxicity, gene expression, isoproterenol, microarray, periostin, rat

Introduction

Cardiovascular diseases are the leading cause of deaths in the world, and 17.7 million people die annually, representing 31% of all deaths globally.[1] Treatment of failing myocardium following myocardial infarction (MI) is one of the biggest challenges waiting to be resolved. It is vital to explore in detail of molecular signaling pathways that regulate cardiac repair process following MI for providing more effective treatment options.

Periostin, a component of the extracellular matrix with four repetitive fasciclin domains, regulates epithelial–mesenchymal transition during cardiac development.[2,3] However, periostin is not detected in the adult myocardium, while it is reexpressed in the mature heart myocardium following pathological conditions such as MI in various species.[4,5] Periostin protein and transcription synthesis is upregulated in the hearts following MI in human and murine myocardium.[4,5] Recent studies suggest that periostin may be used as a therapeutic agent in cardiovascular diseases.[6] It has been shown that periostin treatment may

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induce endogenous cardiomyocyte proliferation and enhance regenerative potential of mature cardiomyocytes.[7] However, the mechanism of its action on cardiomyocytes and effects on cardiac genes during the post-infarction period is not fully investigated. With the development of high-density gene array technologies, it becomes possible to evaluate thousands of genes simultaneously and to compare gene expression profile under different pathological conditions.

In the present study, a rat model of isoproterenol (ISO)-induced myocardial injury was utilized to evaluate the effects of periostin on the gene expression profile and gene signaling pathways during the healing period from day 1 to day 28 of post-myocardial damage to put forward the molecular mechanisms that regulate cardiac gene expression in the pathogenesis of cardiac remodeling.

Materials and methods

Animals

Male Sprague-Dawley rats (weighing 210–220 g, aged 25–26 weeks) were obtained from a private company (Kobay D.H.L. Inc, Ankara, Turkey). All animals were housed in the animal care facility under room temperature (+21 ± 1°C) with a 12-h light:12-h dark cycle and relative humidity of 50 ± 5% in standard cages and were given free access to standard laboratory diet and water ad libitum. The animals received humane care, and the experimental design and procedures were approved by the Institutional Ethical Committee for Animal Care and Use at the Mehmet Akif Ersoy University, Burdur, Turkey (File no: 93773921-27).

Experimental design

Animals were divided into four groups with 21 animals in each group. Control group (group 1): Animals were given saline only (1 ml/kg) and served as the vehicle control group. In the present study, the administration dose of 1 μg/kg is used for the recombinant murine periostin peptide as this dosage was commonly used in experimental infarction model studies where growth factors were applied.[8,9] Only periostin applied group (group 2): Recombinant periostin peptide (Biovision, Milpitas, California, USA; Product code: 4205, 1 μg/kg/intraperitoneally (i.p.)) dissolved in saline applied to group 2 on days 1, 3, 7, 14, and 21 on the same dates with group 4. ISO cardiotoxicity group (group 3): ISO dissolved in saline (Sigma-Aldrich, St Louis, Missouri, USA; Product code: I6504, 85mg/kg/day/i.p.) applied two times with 24-h intervals. ISO + periostin group (group 4): Rats in group 4 further received recombinant periostin peptide starting one day after the final ISO administration on days 1, 3, 7, 14, and 21. Following the final application of periostin, rats were continued to feed with a standard laboratory diet and water ad libitum for further 7 days. On days 1, 7, and 28, rats ($n = 7$) were weighed and killed under mild anesthesia. After euthanization, the hearts were weighed and divided horizontally through interventricular septa and immediately immersed in RNAlater tissue storage solution 10 times the volume of the tissue and stored at −24°C.

Gene expression profiling and microarray data analysis

Total isolated RNA (100 ng) from representative rats from each group and controls ($n = 12$) were converted to complementary DNA following instructions provided in the WT Plus reagent kit (Affymetrix, Santa Clara, California, USA). Biotinylated complementary RNA (cRNA) was then synthesized using an RNA transcript labeling reagent (Affymetrix). Labeled cRNA was fragmented and hybridized onto the GeneChip® Rat Gene 2.0 ST arrays (Affymetrix) according to the manufacturer's protocol. The array scans the whole genome with over 610,400 probes constituting 30,429 gene-level probe sets. The hybridization mixture was heated at 99°C for 5 min, followed by incubation at 45°C for 5 min before the sample was injected into the microarray. Hybridization was carried out at 45°C for 16 h, mixing on a rotisserie at 60 r min^{-1}. After hybridization, solutions were removed and the probe arrays were washed and stained using the Fluidics Station 450 according to AGCC Fluidics Control Software. After washes, the fluorescence on the probe arrays was scanned using the GeneChip 3000 scanner (Affymetrix) with a high-resolution 6 g patch. The probe cell intensity files (*.CEL files) generated by Affymetrix GeneChip Command Console software were transferred into probe-level summarization files (*.CHP files) using a robust multi-array detection algorithm workflow. The *.CHP files were further analyzed using Transcriptome Analysis Console (TAC) software, version 3.1.0.5, to identify and visualize the differentially expressed genes. Each gene was represented by the use of 20 perfectly matched and mismatched (MM) control probes. The MM probes acted as specificity controls that allowed

the direct subtraction of both background and cross-hybridization signals. Furthermore, functional annotation and pathway analysis of genes altered were performed by using TAC software. Differentially expressed genes were identified through fold change screening.

Statistical analyses

Statistical analyses were performed when the number of samples was sufficient and the distribution of data acceptable. Differences between groups were analyzed using one-way analysis of variance. The threshold used to screen up- or downregulated genes was a fold change of ≥ 2 or ≤ -2 and the limit of significance was set as $p < 0.05$.

Results

Gene expression pattern of rat hearts from four groups including only saline applied control group, only periostin applied control group, ISO cardiotoxicity group, and ISO + periostin applied group which were further subdivided into three subgroups based on the dates that rats were killed on days 1, 7, and 28 were compared using hierarchical clustering and heat map. Hierarchical clustering revealed a pattern where each individual sample and groups from experimental and control ventricles clustered, differentiating the experimental from the control transcriptome (Figure 1(a)). Heat maps of 30,429 gene-level probe sets displayed their relative level of expression within each individual sample are shown in green (downregulated), red (upregulated), or black (unchanged) (Figure 1(b)). Additionally, differences in expression of genes in the experimental and control ventricles plotted as a volcano map defined the degree of significance in expression according to their p value and fold change (Figure 1(c) and (d) as well as plotted on a scatter map to visualize variations in gene expression between arrays (Figure 1(e) and (f)).

Rat gene array revealed that 521 genes of 30,429 gene-level probe sets (1.71%) were differentially expressed in the ISO cardiotoxicity group compared to the control group (group 1) at a p value of <0.05. The majority (329 of 521 genes) of these genes (63.14%) were downregulated, while 36.85% (192 of 521 genes) were upregulated (Figure 2(a)). Similar results obtained in the ISO cardiotoxicity group compared to the only periostin administered group in which 474 genes were altered (1.55%), majority of which were downregulated (65.4%; 310 of 474 genes) while 34.59% (164 of 474 genes) were upregulated. On the other hand, the number of differentially expressed genes (2474 genes; 8.13%) were significantly elevated in the ISO + periostin applied group compared to the saline applied control group. The majority of these genes (1886 of 2474 genes; 76.23%) were downregulated, while 23.76% (588 of 2474 genes) were upregulated. Similar results obtained in the ISO + periostin applied group compared to the only periostin-administered group in which 2301 genes were altered (7.56%), majority of which were downregulated (74.75%; 1720 of 2301 genes) while 25.24% (581 of 2301 genes) were upregulated (Figure 2(a)). Furthermore, changes in the number of genes were compared within groups on days 1, 7, and 28 as well as between groups on their corresponding days namely days 1, 7, and 28 (Figure 2(b)).

Pathway analysis of genes showing statistically significant change related to cardiac metabolism and vascular inflammation in only ISO and/or ISO + periostin groups is summarized in Table 1. Pathway analysis showed that no key transcript altered in the only periostin-administered group compared to the saline applied control group indicating the safety of the recombinant periostin peptide. However, significant changes occurred in other experimental groups. Pathway analysis revealed that key transcripts involved in pathways such as Delta-Notch signaling pathway ($p = 0.039$) (Figure 2(c)) and Fas pathways ($p = 0.02$) (Figure 2(d)) were altered significantly with the presence of nearly equal and limited numbers of upregulated and downregulated genes in the ISO cardiotoxicity group compared to the saline applied control group. However, only a few genes involved in Delta-Notch signaling and Fas pathways altered in the ISO + periostin group with no statistically significant difference (Table 1). On the other hand, TNF-α NF-κB signaling pathway ($p = 0.0001$) (Figure 2(e)) was significantly altered in the ISO + periostin group in which most of the genes were downregulated while a few transcripts were upregulated, compared to the saline applied control group. The number of altered genes in the TNF-α NF-kB signaling pathway was rather low in the ISO cardiotoxicity group with no statistically significant difference (Figure 2(e)). Similarly, oxidative phosphorylation ($p = 0.00001$) (Figure 2(f)), oxidative stress ($p = 0.036$) (Figure 3(a) and (b)), IL-2 ($p = 0.015$), IL-3 ($p = 0.038$), and IL-6 ($p = 0.015$) signaling pathways were significantly altered in the ISO + periostin group, while

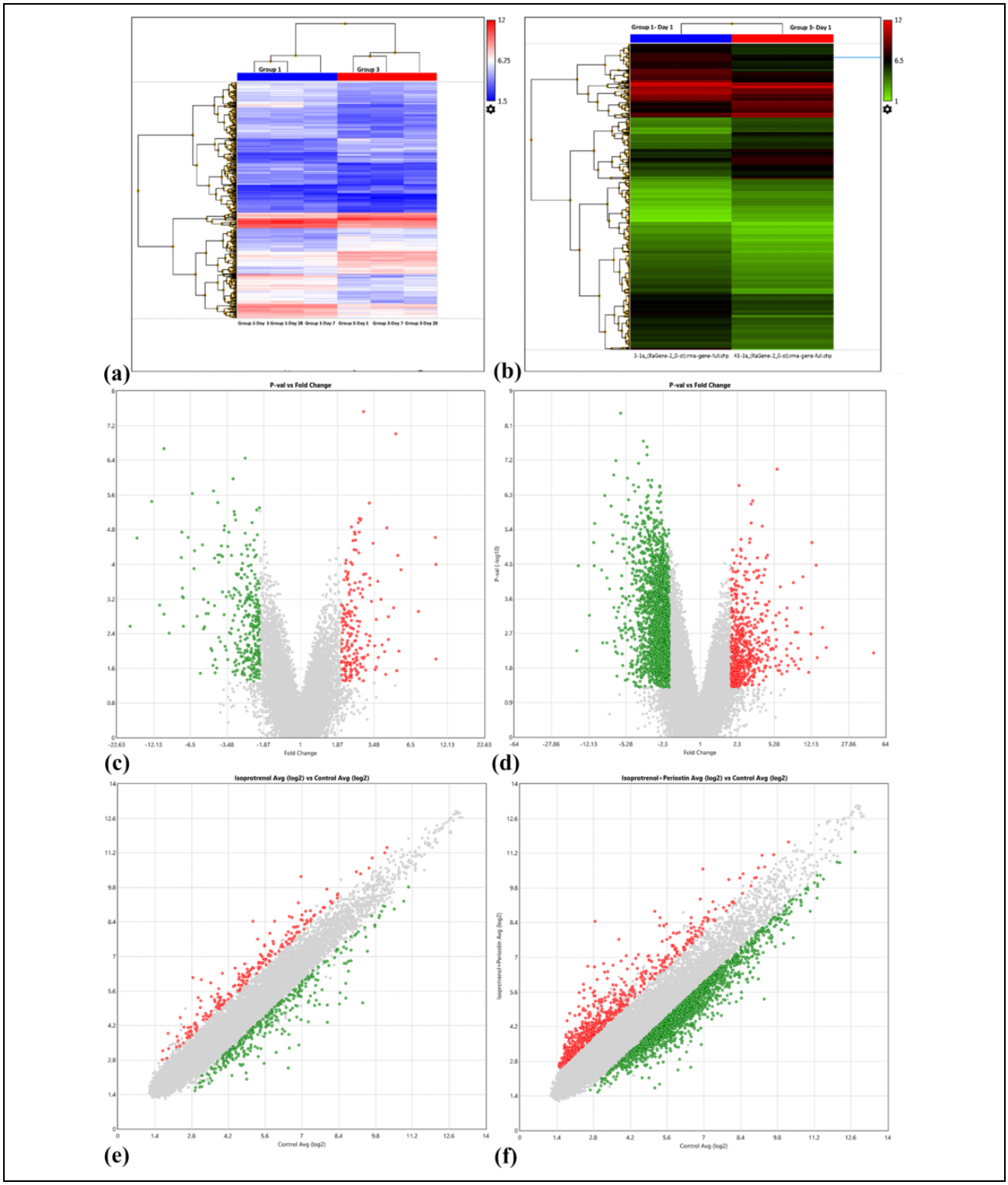

Figure 1. (a) Hierarchical clustering of ISO (group 3) versus control (group 1) group ventricles on days 1, 7, and 28. (b) Heat map of ISO (group 3–day 1) versus control (group 1–day 1) ventricles. (c) Differences in expression of genes in the ISO (group 3) versus control (group 1) ventricles plotted as a volcano map. (d) Differences in expression of genes in the ISO + periostin (group 4) versus control (group 1) ventricles plotted as a volcano map. (e) Differences in expression of genes in the ISO (group 3) versus control (group 1) ventricles plotted on a scatter map to visualize variations in gene expression between arrays. (f) Differences in expression of genes in the ISO + periostin (group 4) versus control (group 1) ventricles plotted on a scatter map to visualize variations in gene expression between arrays. ISO: isoproterenol.

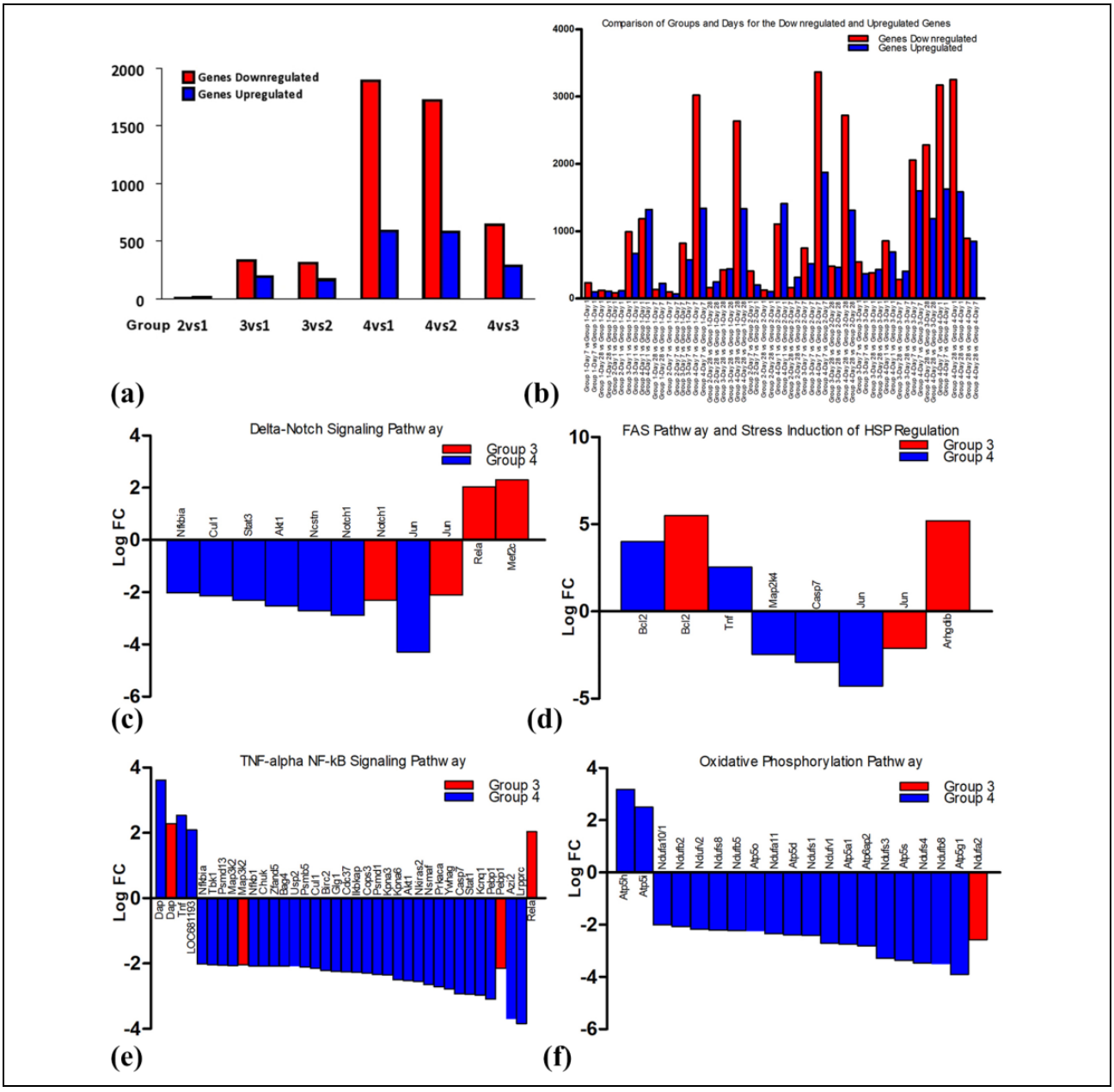

Figure 2. (a) General comparison of groups for the number of significantly upregulated and downregulated genes out of 30,429 gene-level probe sets. (b) Detailed comparison of significantly upregulated and downregulated genes out of 30,429 gene-level probe sets within groups on days 1, 7, and 28 as well as between groups on their corresponding days 1, 7, and 28. (c) Comparison of altered genes in the Delta-Notch signaling pathway in groups 3 and 4. (d) Comparison of altered genes in the Fas pathway and stress induction of HSP regulation in groups 3 and 4. (e) Comparison of altered genes in the TNF-α NF-κB signaling pathway in groups 3 and 4. (f) Comparison of altered genes in the oxidative phosphorylation pathway in groups 3 and 4.

these pathways showed only limited alteration in some transcript with no statistical difference in the ISO cardiotoxicity group (Table 1). Whereas some other pathways were altered significantly in ISO cardiotoxicity group and ISO + periostin groups compared to the saline applied control group. These pathways were mitochondrial long chain fatty acid beta-oxidation pathway ($p = 0.05$ for group 3 and $p = 0$ for group 4) (Figure 3(c)), Kelch-like epichlorohydrin-associated protein 1 (Keap1)-nuclear factor E2-related factor-2 (Nrf2) pathway ($p = 0.0009$ for group 3 and $p = 0.028$ for group 4) (Figure 3(d) and (e)), and

Table 1. Comparison of *p* values of pathway analysis results depicting significantly altered genes in the ISO cardiotoxicity group (group 3) and ISO + periostin group (group 4) related to cardiac metabolism and vascular inflammation.

Pathway	Group 3[a] (*p*-value)[b]	Group 4 (*p*-value)
Delta-Notch signaling	0.039	1
Fas and stress induction of HSP regulation	0.02	0.39
TNF-α NF-κB signaling	0.36	0.0001
Oxidative phosphorylation	0.6	0.000001
Oxidative stress	0.35	0.036
IL-2 signaling	0.33	0.015
IL-3 signaling	1	0.038
IL-6 signaling	0.67	0.015
Mitochondrial LC-fatty acid beta oxidation	0.025	0
Keap1-Nrf2	0.0009	0.028
Proteasome degradation	0.046	0.002

ISO: isoproterenol; LC: long chain; Keap1: Kelch-like epichlorohydrin-associated protein 1; Nrf2: nuclear factor E2 related factor-2.

[a]Groups 3 and 4 compared to saline applied control group (group 1). Comparison of only periostin applied group (group 2) to saline group (group 1) did not show any significant alteration in the key transcripts.

[b]$p < 0.05$ is considered statistically significant.

proteasome degradation pathway ($p = 0.046$ for group 3 and $p = 0.002$ for group 4) (Figure 3(f)).

Discussion

In this study, we have identified a large number of genes and associated pathways that are differentially expressed in both ISO cardiotoxicity and ISO + periostin groups. Altered genes were largely downregulated in the ISO + periostin group. These genes are involved in processes such as oxidative phosphorylation, oxidative stress, fatty acid metabolism, inflammation, and apoptosis pathways.

Pathway analysis of transcripts revealed a significantly reduced presentation of genes related to proteasome degradation pathway in both ISO + periostin applied group and ISO cardiotoxicity group. Cellular proteasomes play a significant role in degrading aberrant protein aggregation.[10] The effects of proteasome inhibition on the heart functions remain controversial. Indeed, proteasome function insufficiency is reported in models of heart disease, including MI, myocardial ischemia–reperfusion, and pressure-overloaded hypertrophy.[11] Similarly, isolated rat hearts perfused with proteasome inhibitors before ischemia was associated with the reduced cardiac function.[12] Furthermore, it has been reported that proteasome function insufficiency can occur in mice myocardial ischemia–reperfusion model induced by coronary artery ligation.[13] However, some other studies depicted that the use of proteasome inhibitors reduced cardiac modeling,[14] infarct size,[15] and decreased apoptosis.[16] This dispute possibly originated from the systemic use of proteasome inhibitors which are also having anti-inflammatory effects, such as inhibition of NF-κB signaling pathway[17] and leukocyte infiltration.[18] It is known that TNF-α stimulates an inflammatory cascade through the activation of nuclear factor B (NF-B), and its chronic stimulation through the enhancement of oxidative stress promotes the development of heart failure.[19] Interestingly, in our study, most of the genes related to TNF-α NF-kB signaling pathway were significantly depleted in the ISO + periostin applied group in addition to a significant decrease in the IL-2, IL-3, and IL-6 signaling pathways reflecting anti-inflammatory effects of periostin. Tian et al.[13] pointed out that heart vasculature and immune system also potentiate protective effects of proteasome inhibitors. Additionally, Tian et al.[13] speculated that systemically inhibiting the proteasomes rather than targeting only cardiomyocytes would be more efficient for the protective effects of proteasome inhibitors. Furthermore, it is well-known that the immune system and inflammatory response upregulated during the heart diseases originate from various etiological factors.[20] Elevated expression of inflammatory cytokine TNF-α has been demonstrated in humans with heart failure[20] as well as in the spontaneously hypertensive rats with heart failure.[21] Additionally, oxidants have been shown to activate a number of transcription factors, including NF-kB, which serves as a signaling integrator to regulate gene expression programs downstream of oxidative stress. Activation of NF-kB has been shown to regulate cell survival and cardiac hypertrophy.[22] In the present study, our microarray data indicated downregulation of TNF-α NF-kB signaling pathway, and another inflammatory signaling of IL-2, IL-3, and IL-6 pathways following periostin administration further supports anti-inflammatory moiety of periostin.

Mitochondrial oxidative phosphorylation is the primary energy source in the myocardial cell. Mitochondrial oxidative phosphorylation complex proteins generate reactive oxygen species that mediate

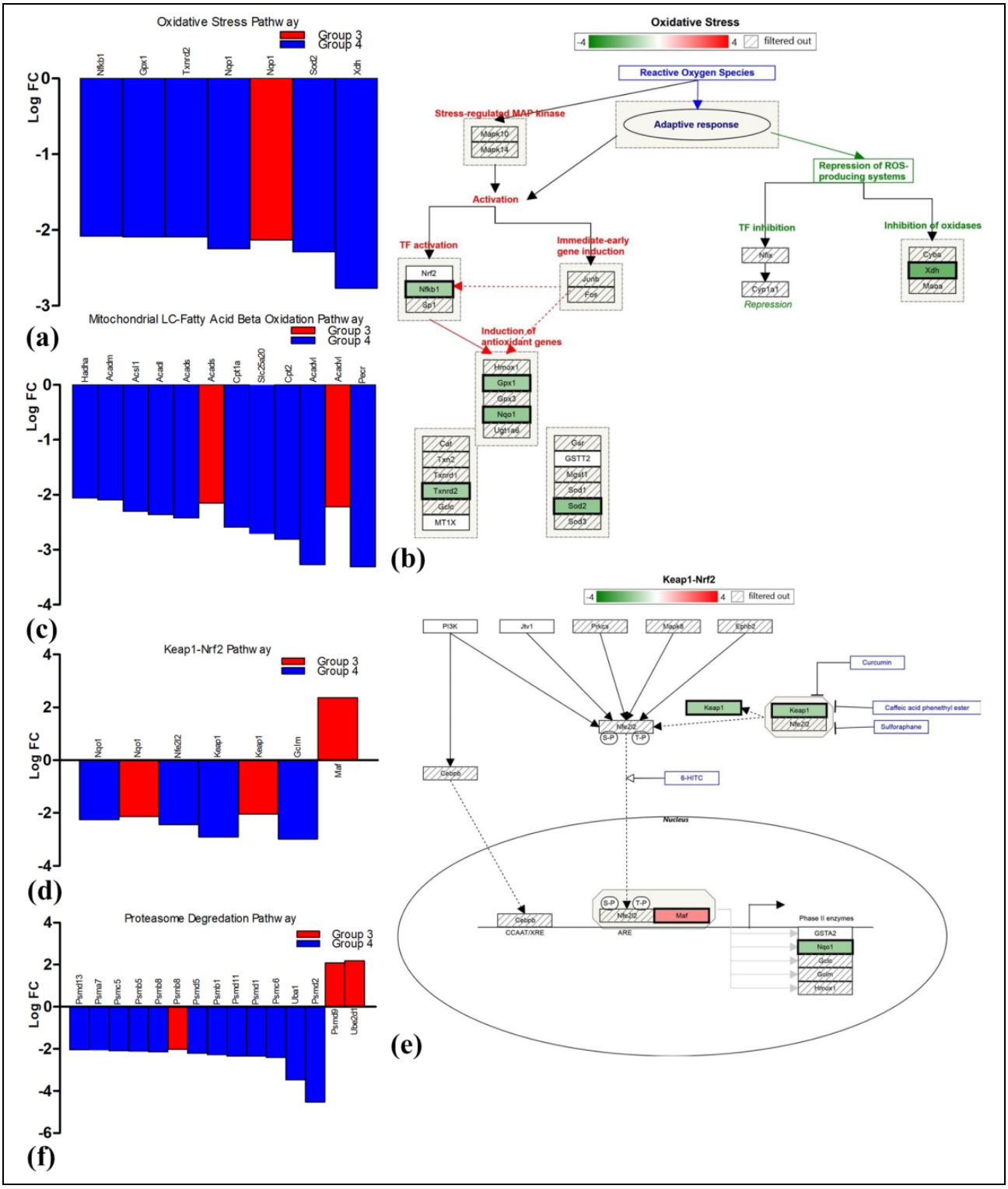

Figure 3. (a) Comparison of altered genes in the oxidative stress pathway in groups 3 and 4. (b) Diagram of oxidative stress pathway highlighting altered genes in group 4. (c) Comparison of altered genes in the mitochondrial LC-fatty acid beta oxidation pathway in groups 3 and 4. (d) Comparison of altered genes in the Keap1-Nrf2 pathway in groups 3 and 4. (e) Diagram of Keap1-Nrf2 pathway highlighting altered genes in group 4. (f) Comparison of altered genes in the proteasome degradation pathway in groups 3 and 4. LC: long chain; Keap1: Kelch-like epichlorohydrin-associated protein 1; Nrf2: nuclear factor E2-related factor-2.

ischemia–reperfusion injury in the heart.[23] The decrease of oxidative phosphorylation proteins[24] and fatty acid metabolism[25] were common findings in the failing hearts, cardiomyopathies, and aging hearts,[26] where there is a shift in mitochondrial substrate utilization from primarily fatty acid β-oxidation (FAO) in the healthy heart to glycogen, lactate, and glucose oxidation in the diseased heart.[27] Although this metabolic change considered contributing to the failing heart phenotype, others[24] suggested that switch from fatty acid to glucose metabolism is an adaptive response that allows for the preservation of adenosine triphosphate (ATP) generation during times when mitochondrial oxidative phosphorylation is reduced. Our study showed that 20 genes involved in mitochondrial oxidative phosphorylation were differentially expressed, 17 of which were significantly downregulated in the ISO + periostin group and one gene from the ISO cardiotoxicity group, while two mitochondrial ATP synthase genes (Atp5h, Atp5i) related to energy production were upregulated in the ISO + periostin group. These changes may reflect abnormalities in mitochondrial oxidative phosphorylation or indicate decreased oxidative phosphorylation complex proteins. Furthermore, downregulated mitochondrial oxidative phosphorylation gene may suggest alterations in cardiac energy metabolism in addition to downregulated mitochondrial FAO metabolism. These downregulated genes in both pathways were possibly indicating a general decline in the expression of genes involved in cardiac energy metabolism, fatty acid metabolism, and mitochondrial metabolism, irrespective of the specific role in glucose metabolism. Interestingly, in human end-stage dilated cardiomyopathies, pathways related to oxidative phosphorylation and fatty acid metabolism were upregulated,[28] while these pathways downregulated in a canine model of tachycardia-induced heart failure.[29]

In cardiac myocytes, oxidative stress activates the mitochondrial death pathway.[30] Mitochondrial death pathway activation is possibly influenced by the genes and proteins expressed in the myocytes before or during the insult.[31] It has been known that oxidative stress is associated with the activation of intracellular signaling pathways known to influence gene and protein expression. In the present study, the ISO + periostin group showed oxidative stress with downregulation of Nfkb1, Gpx1, Txnrd2, (NAD(P) H dehydrogenase quinone (Nqo1), Sod2, and Xdh levels. A decrease in the mRNA for the antioxidant enzymes Sod and catalase was reported in the infarcted rat hearts.[32] Although impaired antioxidant capacity augments oxidative stress in the infarcted hearts, Wang et al.[33] reported that plant-derived remedies of salvianolic acid and tanshinone exert cardioprotective properties by downregulating oxidative stress–related genes in addition to downregulation of G-protein-coupled receptor activity and apoptosis in acute MI model of rats.

The transcription factor, Nrf2, functions as a cellular defense system against oxidative stress and is negatively regulated in the cytosol by Keap1, which facilitates the degradation of Nrf2 through ubiquitinated proteosomal degradation.[34] The Keap1-Nrf2 pathway is an important antioxidant defense mechanism and is closely associated with oxidative stress–mediated cardiac remodeling in cardiovascular diseases.[35] Previously it was stated that, in antioxidant administrated fructose-fed diabetic rats, cardiac Keap1 protein expression was decreased along with elevated Nrf2 expression which may result of enhanced endogenous antioxidant activities through nuclear translocation of Nrf2.[36] This is possibly in response to oxidative stress; Nrf2 dissociates from its repressor protein-Keap, translocates into the nucleus, binds to antioxidant response elements, and transactivates the genes of both detoxifying and antioxidant enzymes.[37] Another study revealed opposite findings; it was stated that incubation of H9C2 rat cardiac myoblasts with doxorubicin (DOX) caused a decrease of both Keap1 protein and mRNA levels through autophagy.[38] Similarly, DOX significantly reduced Keap1 protein but increased nuclear Nrf2 level, and these changes were further potentiated by α-linolenic acid administration in rats with DOX-induced cardiotoxicity.[39] In our study, genes functioning in Keap1-Nrf2 antioxidant response pathway were involved and significantly altered in the both ISO cardiotoxicity and ISO + periostin groups. However, the statistical difference was higher in the ISO group compared to the ISO + periostin group. In both groups, Keap1 transcript was constitutively degraded possibly by autophagy for the maintenance of redox homeostasis.[40] Generally, in response to DOX-induced oxidative stress, Nrf2 protein, but not mRNA, is induced to compensate low antioxidant capacity in the heart.[38] Similar to the findings of Nordgren and Wallace,[38] in our study, Nrf2 transcript levels did not alter in both groups while Nrf2-regulated downstream gene (NAD(P) H dehydrogenase quinone (Nqo1) was downregulated in

contrast to Nordgren and Wallace.[38] These findings indicate that ISO administration activated the Keap1-Nrf2 pathway and autophagy to help survival by removing damaged proteins in the heart.

The Delta-Notch signaling pathway is essential for the heart development by promoting vascular and myocardial growth and associated with cardioprotection in cardiac injury.[41] The role of Notch signaling pathway in cardiac disease is rather complex. Notch system could be activated under conditions of chronic cardiac stress and limit pathological cardiac remodeling.[42] Notch signaling may also enhance key features of diastolic dysfunction, including fibrosis, extracellular matrix remodeling, and microvascular inflammation.[43] In the present study, only ISO administration significantly affected the transcription of several components of the Delta-Notch signaling pathway (reduction of Notch1 and Jun and increase of Rela and Mef2c transcripts). However, ISO + periostin group showed only reduced transcriptional activity (reduction of Nfkbia, Cul1, Stat3, Akt1, Nctsn, Notch1, Jun) with no statistical significance. In a recent study, Matsuda et al.[44] showed that reducing Notch signaling pathway in human cardiac stem cells resulted in better cardiomyogenic differentiation and therapeutic potential in a rat acute infarction model. Furthermore, Notch is also an important modulator of inflammation, and the use of Notch inhibitors reduces the inflammatory response in different animal models.[45] Notch signaling stimulates systemic and local macrophage chemotaxis and prolongs the recruitment of inflammatory cells to the wound.[46] Inhibiton of Delta-Notch signaling pathway decreases cytokine expression and reduces macrophage infiltration in rats following MI.[47] In the present study, although not statistically significant, the general decrease in the transcripts of Delta-Notch signaling pathway in the ISO + periostin group along with reducing NF-kB signaling pathway[48] may alleviate cytokine inflammatory response in the cardiomyocytes causing a state of decreased inflammation in the heart that may alleviate myocardial injury.

Apoptosis in cardiomyocytes is one of the critical pathological mechanisms in the development of heart failure and is recognized as a predictor of adverse outcomes in patients with cardiac diseases or heart failure.[49] Previous studies showed that oxidative stress causes cardiomyocyte apoptosis in ISO-induced myocardial infarcted rats.[50] Fas-mediated apoptosis pathway is largely involved in the ISO-induced myocardial injury.[51] The "extrinsic" Fas receptor-dependent (type I) pathway and "intrinsic" mitochondria-dependent (type II) apoptotic pathways are two major pathways that directly trigger cardiac apoptosis. Bcl2 is one of the key molecules involved in intrinsic apoptosis pathway which has an antiapoptotic function.[52] In the present study, only ISO administration showed a statistically significant change in the transcription level of the Fas-mediated apoptosis pathway and stress induction of heat shock protein (HSP) regulation (elevated Bcl2 and decreased Jun and ARHGDIB transcripts). However, ISO + periostin group showed generally reduced transcriptional activity (reduced Map2k4, Casp7, Jun and elevated Bcl2 and TNF transcripts) with no statistical significance. In the present study, no significant difference was found in Bcl2 expression level between ISO and ISO + periostin groups. But, slightly higher expression of Bcl2 gene was detected in the ISO + periostin group compared to the ISO group. Casp7 belongs to the caspase family of proteases that play a key role in the apoptotic process and the downregulation of Casp7 may inhibit cell apoptosis.[53] Casp7 is an executioner caspase and is essentially the effector protein for cellular apoptosis. Preventing Casp7 activation is a strong indicator of anti-apoptotic capacity. Malkapuram et al.[54] demonstrated that prevention of Casp7 activation protects H9C2 cells from DOX-induced apoptosis. Our findings showed that periostin application downregulated myocardial expression of apoptotic executioner gene Casp7 from caspase family in the ISO-induced myocardial injury model in rats, which may reflect antiapoptotic function of periostin. These findings indicate that periostin can modulate intrinsic and extrinsic pathways of myocardial apoptosis induced by ISO. Since apoptosis plays a significant role in the pathogenesis of MI, the inhibition of caspases activation could be an effective target for therapeutic intervention in the myocardial injuries resulting in heart failure.

These findings indicate that periostin alters gene expression profile in the ISO-induced myocardial injury and modulates transcripts involved in oxidative phosphorylation, oxidative stress, fatty acid metabolism, and TNF-α NF-kB signaling pathways. In conclusion, comprehensive transcript profile and pathway analysis revealed that gene expression patterns are markedly different between the ISO and ISO + periostin applied groups, providing important data regarding critical pathways and molecular adaptations

that characterize cardiac toxicity associated with exposure to ISO and periostin.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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