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Cardiac troponin T (TNNT2) mutations in Chinese dilated cardiomyopathy patients

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Objectives: Dilated cardiomyopathy (DCM) is one of the leading causes of heart failure with high morbidity and mortality. Although more than 40 genes have been reported to cause DCM, the role of genetic testing in clinical practice is not well defined. Mutations in the troponin T (TNNT2) gene represent an important subset of known disease-causing mutations associated with DCM. Therefore, the aim of the present study was to determine the genetic variations in TNNT2 and the associations of those variations with DCM in Chinese patients.
Methods: An approximately 4 kb fragment of the TNNT2 gene was isolated from 103 DCM patients and 192 healthy controls and was analyzed by DNA sequence analysis for genetic variations.
Results: A total of 6 TNNT2 variants were identified in 99 patients, including a G321T missense mutation (Leu84Phe) and 5 novel intronic variants. Alleles of two novel SNPs (c.192+353 C>A, OR=0.095; 95% CI: 0.013-0.714, P=0.022; c.192+463 G>A, OR=0.090, 95% CI: 0.012-0.675, P=0.019) and SNP rs3729843 (OR=1.889, 95% CI: 1.252-2.852; P=0.002) were significantly correlated with DCM.
Conclusions: These results suggest that variations in the TNNT2 gene might be associated with DCM in the Chinese population.

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Angiotension II upregulates the Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel in Neonatal Rat Cardiomyocytes via a redox mechanism

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Objectives: To identify the effects of exogenous Angiotension II (AngII) on the Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel (HCN) current and its mechanisms in Neonatal Rat Ventricle Cardiomyocytes (NRVM).
Methods: NRVM from 1- to 3-day-old Wister rats were prepared by collagenase digestion, and incubated in 37°C, 95% CO_2 for If current by using of patch-clamp recording, HCN channel protein expression was detected by western-blotting analysis.
Results: exposure (~20 min) of NRVM to AngII (100 μmol/L) markedly increased I_f density (4.7±0.6 pA/pF vs. 11.7±1.1 pA/pF) along increased conductance (G_{max}: 48.7±5.6 pS/pF vs. 192.6±64.1 pS/pF), a shift in activation voltage ($V_{1/2}$) to positive potentials (-81.2±1.6 mV vs. -64.7±2.0 mV) and increase rate of activation (tact) (523.4±24.7 ms vs. 337.5±24.9 ms). Moreover, stimulation by AngII was largely inhibited by the non-specific tyrosine kinase blocker genistein (1μmol/L) or the c-Src-specific inhibitor PP2 (10 μmol/L). Augmented tyrosine phosphorylation of HCN2 channels with AngII treatment by determined by AngII Western blot using the phosphotyrosine specific antibody 4G10. Furthermore, the augmented I_f current was inhibited by pre-treatment with Trx receptor inhibitor (Auranofin 10nmol/L; 13-cis-retinoic acid 1 μmol/L). On the other hand, I_f current of NRVMs was also increased by treated with non-specific PTP inhibitors, phenylarsine oxide (PAO 1 μmol/L) or Na-orthovanadate (Na_3VO_4 10 μmol/L).
Conclusions: These data suggest that the c-Src family of tyrosine kinase mediate the augmentation of I_f density by oxidant agent AngII via a redox mechanism involving the Trx system. The studies will provide new insights into the relation of oxidative cell damage and ion channel remodeling.

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Anti-peroxynitrite Treatment Ameliorated Vasorelaxation of Resistance Arteries in Aging Rats: Involvement with NO-sGC-cGKs Pathway

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Objectives: Declined vasorelaxation function in aging resistance arteries is responsible for aging related multiple organ dysfunctions. The aim of the present study is to explore the role of peroxynitrite ($ONOO^-$) in aging resistance arterial vasorelaxation dysfunction and the possible mechanism.
Methods: In the present study, young (3-4 months olds) and aging (20 months olds) male SD rats were randomly randomized to receive vehicle (Saline) or FeTMPyP ($ONOO^-$ scavenger) for 2 weeks. The vasorelaxation of resistance arteries was determined in vitro; NOx level was tested by a colorimetric assay; the expression of nitrotyrosine (NT), soluble Guanylate Cyclase (sGC), vasodilator-stimulated phosphoprotein (VASP) and phosphorylated VASP (P-VASP) in resistance arteries was detected by immunohistochemical staining.
Results: In the present study, endothelium dependent dilation in aging resistance arteries was lower than young rats (young *vs.* aging: 68.0%±4.5% *vs.* 50.4%±2.9%, $P<0.01$). And the endothelium independent dilation remained constant. Nitrative stress was increased in aging resistance arteries, evidenced by elevated NOx level in serum from aging rats (nmol/ml; young *vs.* aging: 3.3±1.4 *vs.* 5.3±1.0, $P<0.05$) and increased NT level ($P<0.05$). $ONOO^-$ was responsible for the vasorelaxation dysfunction, evidenced by normalized vasorelaxation after inhibit $ONOO^-$ or its sources ($P<0.05$) and suppressed NT expression after FeTMPyP treatment ($P<0.05$). The expression of sGC was not significantly different between young and aging resistance arteries, but the P-VASP/VASP ratio (biochemical marker of NO-sGC-cGKs signaling) decreased, which was reversed by FeTMPyP treatment in vivo ($P<0.05$).
Conclusions: The present study suggested that $ONOO^-$ is responsible for the decline of endothelial dependent vasorelaxation in aging resistance arteries, and this effect likely involves the dysfunction of the NO-sGC-cGKs pathway.

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Silk fibroin/chitosan nanofibers based adipose tissue-derived mesenchymal stem cell patches prevent myocardial remodeling after myocardial infarction in rat

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Objectives: To fabricate novel biocompatible silk fibroin/chitosan (SF/CS) nanofibers seeded with adipose tissue-derived mesenchymal stem cells (AD-MSCs) for cardiac tissue regeneration.
Methods: Silk fibroin with good elasticity and chitosan as hydrophile were assembled onto the cellulose electrospun mat via layer-by-layer method. The micro-structure of the nanofibers was characterized by X-ray photoelectron spectroscopy (XPS) and scanning electron microscope (SEM). AD-MSCs were isolated from Fluc-EGFP transgenic mice constitutively expressing both firefly luciferase (Fluc) and enhanced green fluorescent protein (EGFP). Rat model of acute myocardial infarction (AMI) was induced by ligation of the left anterior descending coronary artery. The SF/CS nanofibrous patches ($9\times9mm^2$) seeded with or without ADMSCs (seeding density: 2×10^5 cells/patch) were adhered onto the epicardium of the infarcted region. Wild type Sprague Dawley rats (male, 120-140g) were randomized into four groups (each n=10) : Sham group, MI group, MI/SF/CS group and MI/ADMSC/SF/CS group (MI hearts with implantations of SF/CS and ADMSC/SF/CS patches respectively). Three days post-operation, cardiomyocyte apoptosis and the paracrine factors in the peri-infarct area was determined by TUNEL staining and by ELISA assay respectively. The viability of engrafted AD-MSCs was tracked using longitudinal bioluminescence imaging (BLI) and cardiac function was measured by transthoracic echocardiography (TTE) 1, 7, 14 and 28 days post-operation. Four weeks after AMI operation, H&E, Masson's Trichrome, Troponin I, CD68 and CD31 immunofluorescence stainings were performed to evaluate myocardium fibrosis, tissue regeneration, neovascularization and inflammatory reaction.
Results: BLI showed AD-MSCs were detectable until four weeks after transplantation. By TTE, ADMSC/SF/CS patches improved left ventricular ejection fraction (LVEF) (sham: 73±2%, MI: 24±3%, MI/SF/CS: 38±3%, MI/ADMSC/SF/CS: 53±4%; n=10 per group; P<0.05, four weeks post-operation). The implantation of ADMSC/SF/CS patches decreased cardiomyocyte apoptosis (P<0.05) and increased the secretion of paracrine factors (P<0.05). Four weeks after operation, the patches in both groups were intactly adhered on the MI zones with minor inflammatory as compared with MI group (P<0.05), while angiogenesis was improved (P<0.05) and fibrosis size was reduced (sham: 0, MI: 23±4%, MI/SF/CS: 15±2%, MI/ADMSC/SF/CS: 9 ±3 %; n=10 per group; P<0.05). Furthermore, EGFP+ cardiomyocytes (cTn I-positive) and endothelial cells (CD31-positive) could be identified in MI/ADMSC/SF/CS group.
Conclusions: This study demonstrated that the SF/CS nanofibers provide three-dimensional microenvironments and scaffold to support the retention and viability of engrafted ADMSCs, synergistically promote the therapeutic efficiency to alleviate cardiac fibrosis, attenuate ventricular remodeling and induce angiogenesis and cardiomyocyte regeneration.

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Rb1 protects endothelial cells from hydrogen peroxide-induced cell injury by modulating PAI-1 expression

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Objectives: Oxidative stress can induce endothelial cell injury. Plasminogen activator inhibitor-1 (PAI-1), a serine protease inhibitor, accelerates thrombus formation upon ruptured atherosclerotic plaques. However, it is not known whether or not ginsenoside Rb1 inhibits PAI-1 production induced by H_2O_2 in endothelial cells. In present study, we investigated that Rb1 may have an inhibitory effect on PAI-1 production induced by H_2O_2 and have protective effects on H_2O_2-induced endothelial injury.
Methods: Primary human umbilical vein endothelial cells (HUVECs) injury was induced by H_2O_2. PAI-1 mRNA and protein expression were analyzed by real time PCR and Western blot.
Results: Rb1 was found to protect endothelial injury, as witnessed by a significant increase of cell numbers. Rb1 could markedly decrease PAI-1 mRNA expression by