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S2.1

Chloride channel remodeling in vascular smooth muscle cells and hypertension: novel targets for treatment of hypertension

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Hypertension is the major risk factor for cardiovascular and renal diseases that affect more than 1.5 billion people world-wide in the next 10 years^[1,2]. The mechanisms for hypertension involves changes in cardiac output and a gradual and sustained increase in total peripheral resistance induced by increased contraction of vascular smooth muscle and vascular remodeling^[3-6]. It has been demonstrated that the resting vascular tone is elevated, and the contractile response is enhanced in hypertensive persons and animals compared with normotensive controls^[7-13]. While many studies have demonstrated that several cation channels such as TRPV, Ca²⁺ and K⁺ channels on the plasma membrane of vascular smooth muscle cells (VSMCs) play a pivotal role in the modulation of vascular tone of small arteries and arterioles^[4], recent studies also indicated the involvement of several chloride (Cl⁻) channels in the regulation of vascular tone and arterial blood pressure^[12,14-16]. The volume-regulated CLC-3 Cl⁻ channels may play important functional role in the vascular remodeling during hypertension and stroke^[17-19]. Disruption of the Ca²⁺-activated Ano1 Cl⁻ channels caused a decrease in the systemic blood pressure while upregulation of the Ano1 channels was associated with pulmonary arterial hypertension^[20,21]. We recently found important experimental evidence that the CFTR Cl⁻ channels play a pivotal role in the high-fructose and high-salt diet (HFSD)-induced hypertension. Therefore, CLC-3, Ano1, and CFTR Cl⁻ channels may all play crucial functional roles in the regulation of arterial resistance and blood pressure. While up-regulation of Ano1 may cause the primary hypertension, down-regulation of the CFTR Cl⁻ channels may be the underlying mechanism for the HFSD-induced hypertension. These results provide compelling evidence that Cl⁻ channel subproteome in the VSMCs may be novel molecular targets and mechanisms for the treatment of primary or HFSD-induced hypertension.

S2.2

CLC-3 deficiency prevents atherosclerotic lesion development in ApoE^{-/-} mice

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Recent evidence suggested that CIC-3, encoding Cl⁻ channel or Cl⁻/H⁺ antiporter, plays a critical role in regulation of a variety of physiological functions. However, remarkably little is known about whether CIC-3 is involved in atherosclerosis. This study aims to establish the involvement and direct role of CIC-3 in atherogenesis and underlying mechanisms by using CIC-3 and ApoE double null mice. After a 16-week Western-type high-fat diet, the CIC-3^{+/-} ApoE^{-/-} mice developed widespread atherosclerotic lesions in aorta. However, the lesion size was significantly reduced in aorta of CIC-3^{-/-} ApoE^{-/-} mice. Compared with the CIC-3^{+/-} controls, there was significantly decreased ox-LDL binding and uptake in isolated peritoneal macrophages from CIC-3^{-/-} mice. Moreover, the expression of scavenger receptor SR-A, but not CD36, was significantly decreased in both CIC-3^{-/-} peritoneal macrophages and aortic lesions from CIC-3^{-/-} ApoE^{-/-} mice. These findings were further confirmed in ox-LDL-treated RAW264.7 macrophages, which showed that silence of CIC-3 inhibited SR-A expression, ox-LDL accumulation and foam cell formation, whereas over-expression of CIC-3 produced the opposite effects. In addition, CIC-3 siRNA significantly inhibited, whereas CIC-3 over-expression increased, the phosphorylation of JNK/p38 MAPK in ox-LDL-treated RAW264.7 foam cells. Pretreatment with JNK or p38 inhibitor abolished CIC-3-induced increase in SR-A expression and ox-LDL uptake. Finally, the increased JNK/p38 phosphorylation and SR-A expression induced by CIC-3 could be mimicked by reduction of [Cl⁻], by low Cl⁻ solution. Our findings demonstrated that CIC-3 deficiency inhibits atherosclerotic lesion development, possibly via suppression of JNK/p38 MAPK dependent SR-A expression and foam cell formation.

Keywords: CIC-3; atherosclerosis; foam cell; SR-A; MAPK

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S2.3

CYP 450 inhibition and cardiovascular biomarkers alteration by Radix Puerariae lobatae (Gegen): inside story from an herb-drug interaction study

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The narrow therapeutic window of warfarin makes itself the most common drug involved in drug/herb-drug interaction, which often causes safety concerns, primarily bleeding. We have conducted a series of experiments investigating herb-drug interactions between warfarin and Danshen-Gegen (DG) product, a Radix Salvia miltiorrhiza (Danshen) and Radix Puerariae lobatae (Gegen) containing Chinese medicine formula recently developed for the treatment of cardiovascular disease. Our rat *in vivo* study demonstrated that co-administration of DG at (0.15 g/kg, bid) with warfarin (0.2 mg/kg) for five consecutive days could cause significant pharmacokinetics and pharmacodynamics herb-drug interactions. Our recent three-session clinical studies with oral administrations of DG alone (750 mg twice daily for 5 d), warfarin alone (1 mg once daily for 5 d), and warfarin in combination with DG product in fourteen healthy male subjects demonstrated similar results suggesting that DG could offset the anti-coagulation effect of warfarin as demonstrated by the reduced STM. Our further herb-drug studies with single herb of Danshen and Gegen indicated that such interactions were mainly by Gegen rather than Danshen.

Radix Puerariae lobatae (Yege or Gegen) and its products have been widely used in China for the treatment of various diseases, especially for the treatment of cardio-cerebrovascular related conditions, such as angina pectoris, myocardial ischemia and cerebral infarction. Since puerarin, the major active component in *Pueraria lobata* (Gegen), has been commercially used as a cardio-tonic drug in clinical practice and therefore has high potential to be co-administered with warfarin. In order to ensure the safe use of warfarin and puerarin in clinical practice, we further conducted the studies to investigate the potential pharmacokinetics and pharmacodynamics interactions between warfarin and puerarin. Intravenously administered puerarin could alter the pharmacokinetics of warfarin significantly, along with a shortened *t*_{1/2}, decreased AUC_{0-96h}, and increased clearance of warfarin. In the meantime, warfarin could also significantly change the pharmacokinetics behavior of puerarin with a prolonged *t*_{1/2}, increased AUC_{0-96h} and decreased clearance. Both oral and intravenous administrations of puerarin could significantly induce the activities and expressions of CYP 2B1, CYP 2C6 and CYP 1A1 (*P*<0.05). In addition, co-administration of puerarin could reduce the PT of rat plasma via enhancing VKOR and inhibiting TM. In summary, our *in vivo* and *ex vivo* rat models demonstrated that puerarin could increase warfarin metabolism and offset warfarin anticoagulation via inducing CYPs, VKOR and inhibiting TM in rats.

Keywords: Radix *Puerariae lobatae*; puerarin; warfarin; liver cytochrome P450; vitamin K epoxide reductase; thrombomodulin

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S2.4

TMEM16A contributes to endothelial dysfunction by facilitating Nox2 NADPH oxidase-derived reactive oxygen species generation in hypertension

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Ca²⁺-activated Cl⁻ channels play a crucial role in various physiological processes. However, the role of TMEM16A in vascular endothelial dysfunction during hypertension is unclear. In this study, we investigated the specific involvement of TMEM16A in regulating endothelial function and blood pressure and the underlying mechanism. Reverse transcription-polymerase chain reaction, Western blotting, co-immunoprecipitation, confocal imaging, patch-clamp recordings, and TMEM16A endothelial-specific transgenic and knockout mice were used. We found that TMEM16A was expressed abundantly and functioned as a Ca²⁺-activated Cl⁻ channel in endothelial cells. Angiotensin II induced endothelial dysfunction with an increase in TMEM16A expression. The knockout of endothelial-specific TMEM16A significantly lowered the blood pressure and ameliorated endothelial dysfunction in angiotensin II-induced hypertension, whereas the over-expression of endothelial-specific TMEM16A resulted in the opposite effects. These results were related to the increased reactive oxygen species production, Nox2-containing NADPH oxidase activation, and Nox2 and p22phox protein expression that were facilitated by TMEM16A on angiotensin II-induced hypertensive challenge. Moreover, TMEM16A directly bound with Nox2 and reduced the degradation of Nox2 through the proteasome-dependent degradation pathway. Therefore, TMEM16A is a positive regulator of endothelial reactive oxygen species generation via Nox2-containing NADPH oxidase, which induces endothelial dysfunction and hypertension. Modification of TMEM16A may be a novel therapeutic strategy for endothelial dysfunction-associated diseases.

Keywords: TMEM16A; angiotensin II; endothelial cells; hypertension; NADPH oxidase; reactive oxygen species

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S2.5

WNK1 is required for the proliferation induced by hypotonic challenge in A10 vascular cells

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Hypotonic challenge evoked vascular cell proliferation through activation of volume-regulated Cl⁻ channel (VRCC), leading to the decline of intracellular Cl⁻ concentration ([Cl⁻]). We suppose that the decrease of [Cl⁻], may activate one or more Cl⁻ sensitive kinases, resulting in subsequent signal cascade. We investigated whether WNK1 is involved in VRCC-induced proliferative signal pathway in A10 cells. Bromodeoxyuridine (BrdU) incorporation and flow cytometry were used for proliferation determination, and Western blot and cDNA/siRNA transfection was used for signal pathway determination in cultured A10 cells. Hypotonic challenge induced WNK1 phosphorylation without change in WNK1 protein expression. Hypotonic-induced A10 cell proliferation was significantly increased by over expression of WNK1, and inhibited by silencing of WNK1. Mutated WNK1 was not phosphorylated and did not mediate cell proliferation. Silencing of WNK1 caused cell cycle arrest in G₀/G₁ phase and prevented the entrance from G₁ to S phase, whereas, over expression of WNK1 accelerated cell cycle transition from G₁ to S phase. Silencing of WNK1 inhibited cyclin D₁, cyclin E₁ expression and increased p27^{KIP} and p21^{CIP} expression. Whereas, over-expression of WNK1 increased cyclin D₁, cyclin E₁ expression, and reduced p27^{KIP}, p21^{CIP} expression. Furthermore, WNK1 knockdown or overexpression significantly attenuated or increased phosphorylations of Akt and PI3K induced by hypotonic challenge, respectively. VRCC activation by hypotonic challenge reduces [Cl⁻]_i evokes WNK1 phosphorylation, which mediates cell cycle transition from G₀/G₁ to S phase and proliferation through PI3K-Akt signal pathway in A10 VSMCs.

Keywords: hypotonic solution; cell proliferation; WNK1 phosphorylation; vascular smooth muscle cell

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S2.6

Ginkgolide K protects the heart against ER stress injury by activating the IRE1α/XBP1 pathway

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Here we investigated the effects and the underlying mechanism of Ginkgolide K (1,10-dihydroxy-3,14-didehydroginkgolide, GK) on cardiac ER stress. Cell death, apoptosis, and ER stress-related signalling pathways were measured in cultured neonatal rat cardiomyocytes (NRCMs), treated with the ER stress inducers tunicamycin, hydrogen peroxide, and thapsigargin. Acute myocardial infarction was established using left coronary artery occlusion in mice, and infarct size was measured by triphenyltetrazolium chloride (TTC) staining. Echocardiography was used to assess heart function and transmission electron microscopy for evaluating ER expansion. GK significantly decreased ER stress-induced cell death in both *in vitro* and *in vivo* models. In ischemic injured mice, GK treatment reduced infarct size, rescued heart dysfunction and ameliorated ER dilation. Mechanistic studies revealed that the beneficial effects of GK occur through enhancement of inositol-requiring enzyme 1α (IRE1α)/X box-binding protein-1 (XBP1) activity, which in turn leads to increased ER-associated degradation (ERAD)-mediated clearance of misfolded proteins and autophagy. In addition, GK is also able to partially repress the pro-apoptotic action of regulated IRE1-dependent decay (RIDD) and JNK pathway. GK acts through selective activation of the IRE1α/XBP1 pathway to limit ER stress injury. GK is revealed as a promising therapeutic agent to ameliorate ER stress for treating cardiovascular diseases.

Keywords: Ginkgolide K; ER stress; IRE1α; XBP1; ER-associated degradation (ERAD); autophagy

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S2.7

PARP1 negatively regulates SIRT6 activity by PARylation of SIRT6 during cardiac remodeling

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The present study aimed to observe the interaction between Poly(ADP-ribose) polymerase 1 (PARP1) and sirtuin 6 deacetylase (SIRT6) in cardiac remodeling. Both PARP1 and SIRT6 are important NAD⁺-dependent enzymes. Accumulating evidences indicated both PARP1 and SIRT6 were involved in cardiac diseases. In the present study, the relationship between PARP1 and SIRT6 during cardiac remodeling was investigated. We found PARP1 was activated and SIRT6 was inhibited in angiotensin II (Ang II)-treated cultured neonatal rat cardiomyocytes (NRCMs) and in Ang II-infused rat hearts. Overactivation of PARP1 down-regulated SIRT6 activity. Interaction between PARP1 and SIRT6 as well as the PARylation of SIRT6 was confirmed by co-immunoprecipitation (co-IP) assays. The interaction and the PARylation of SIRT6 was enhanced by Ang II treatment, and weakened by PARP1 inhibition. PARP1 inhibition also reversed the decrease of SIRT6 activity induced by Ang II *in vivo* and *in vitro*. More important, SIRT6 over-expression by transfection significantly enhanced anti-hypertrophic and anti-fibrotic effects in Ang II treated NRCMs, cultured rat cardiac fibroblasts (CFs) and SD rat hearts. It is concluded that PARP1 negatively regulated activity of SIRT6, and PARP1 suppression protected against cardiac remodeling induced by Ang II through activating the activity of SIRT6.

Keywords: PARP1; SIRT6; PARylation; cardiac remodeling

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S2.8

Luteolin inhibits intracellular Ca^{2+} overloads and provides longer heart preservation by regulating the alteration of Ca^{2+} homeostasis

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As an optimal solution for patients with end stage cardiac failure, heart transplantation has been applied in clinic for decades. However, hypothermic preservation of heart is still limited in 4 to 6 h, and calcium overload over time is known to be an important factor resulting in cell death. To provide longer and safer storage for hearts, our previous study showed that luteolin, which has been applied in traditional Chinese medicine treatment for cardiovascular diseases, could inhibit cell death and the L-type calcium currents during hypothermic preservation to provide better protection for cardiomyocytes. In this study, we further investigated the protective role of luteolin in modulating myocytes calcium cycling. Intracellular calcium overload has been implicated in hypothermia-induced dysfunction of cardiac myocytes. Using University of Wisconsin (UW) solution with 7.5, 15, and 30 $\mu\text{mol/L}$ luteolin to preserve fresh isolated myocytes at 4 °C, respectively, we showed that all three doses of luteolin attenuated calcium overload over 6 h preservation. We further observed that luteolin had a suppressive effect on accumulating mitochondria Ca^{2+} uniporter (MCU) and calmodulin (CaM) that induced by cold storage. Besides, the activation of protein kinase A (PKA) and deactivation of Ca^{2+} - Mg^{2+} -ATPase were also suppressed by luteolin. As the important regulatory proteins and enzymes for myocyte calcium circulation, MCU, CaM, PKA and Ca^{2+} - Mg^{2+} -ATPase were dramatically changed during hypothermic preservation, leading to cytotoxic calcium overload. In this study, we demonstrated that luteolin confer a cardioprotective effect in inhibiting the changes of calcium regulators during cold storage and therefore ameliorate Ca^{2+} overload in rat cardiomyocytes.

Keywords: cardiomyocytes; luteolin; calcium; hypothermic preservation

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S2.9

Hydrogen sulfide attenuates acute myocardial ischemic injury via glycogen synthase kinase-3 β / β -catenin signaling pathway

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The endogenous signaling gasotransmitter hydrogen sulfide (H_2S) can exert cardioprotective effects in acute myocardial ischemic (AMI) injury. However, the mechanisms for these effects are not completely understood. This study investigated whether sodium hydrogen sulfide (NaHS), an H_2S donor, could attenuate apoptotic injury through glycogen synthase kinase-3 β (GSK-3 β)/ β -catenin signaling. The current study utilized an *in vivo* rat model of AMI injury by occluding the left anterior descending coronary artery. NaHS (0.39, 0.78, or 1.56 mg/kg, intraperitoneally), GSK-3 β inhibitor SB216763 (0.6 mg/kg, intravenous), or 1% dimethylsulfoxide (2 mL/kg, intravenous) were administered to rats respectively. The result demonstrated that administration of medium- and high-dose NaHS and SB could significantly improve rat cardiac function, as evidenced by increased mean arterial pressure, left ventricular developed pressure, contraction and relaxation rates, and decreased left ventricular end diastolic pressure. Besides, administration of NaHS and SB could attenuate myocardial injury as reflected by reduced apoptotic cell death, serum lactate dehydrogenase concentration, and ameliorate myocardial pathological structure changes. Administration of NaHS and SB increased the concentrations of phosphorylated GSK-3 β , p-GSK-3 β /t-GSK-3 β ratio, and downstream protein β -catenin. Moreover, Western blot and immunohistochemical analysis of apoptotic signaling pathway proteins further demonstrated the cardioprotective potential of NaHS, as reflected by up-regulated Bcl-2 expression, down-regulated Bax expression, and reduced TUNEL positive staining. These findings suggest that hydrogen sulfide exerts cardioprotective effects against AMI-induced apoptosis via the GSK-3 β / β -catenin signaling pathway.

Keywords: acute myocardial ischemia; hydrogen sulfide; GSK-3 β ; SB216763; β -catenin; apoptosis

S2.10

Discovery and evaluation of the novel drugs for intervention of vascular remodeling related diseases

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Vascular remodeling (VR) is a dynamic and strictly regulated process of structural changes, including physiological compensated expansive remodeling, and pathological constrictive remodeling (reduction in vessel diameter and neointimal thickening). VR often represents as a result of a pathological trigger in many cardiovascular diseases, such as atherosclerosis, pulmonary hypertension, ischemic diseases, and restenosis. After screening by the PDGF-BB-induced VSMCs proliferation model, the natural compound, salvianolic acid A was found with anti-vascular remodeling activities. The results demonstrated that SAA inhibited the migration and proliferation of VSMCs, and regulated the VSMCs phenotype *in vitro*. SAA exerts an action on PDGF-BB-induced proliferation via cAMP/PKA/CREB pathway and blunts PDGF-BB-induced human umbilical artery smooth muscle cell (hUASMC) proliferation via p21 induction. SAA also activated CREB, including phosphorylation at Ser133, and induced its nuclear translocation. SAA inhibits VSMCs proliferation through regulating osteopontin (OPN, a kind of ECM), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) expression. In addition, SAA does not inhibit the proliferation of endothelial cells (ECs) and the synthesis of NO and eNOS protein expression. The anti-restenosis effects of SAA were evaluated using the model of vascular restenosis established by balloon injury of rat carotid arteries. The results showed that SAA prevented arteries against injury-induced neointimal hyperplasia, lumen narrowing, and thrombosis formation. The mechanism of the actions of SAA are related with upregulating p21 expression and activating CREB in the neointima of balloon-injured arteries *in vivo*. In conclusion, the SAA exerts beneficial effects in the treatment of angioplasty or in-stent restenosis, and may be developed as the in-stent drug coating or oral dosage after PTCA, for it has high efficiency in inhibiting VSMCs proliferation and migration and arterial thrombosis formation with no interference on re-endothelialization.

Keywords: vascular remodeling; salvianolic acid A; cAMP/PKA/CREB; p21

S2.11

A novel sodium current as regulator of pacemaker cardiomyocyte basal membrane potential and rhythmic firing activity

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Firing activity of pacemaker cells underlies an array of fundamental physiological processes, such as heartbeat and respiration. Key to the generation of spontaneous rhythmic activity in pacemaker cells are the ion currents that maintain their depolarized basal membrane potential. Several known contributors to this depolarizing membrane conductance include the hyperpolarization-activated current (I_h) and persistent Na^+ current (I_{NaP}). Using patch clamp studies, we identified a novel Gd^{3+} - and Co^{2+} -sensitive voltage-independent Na^+ current that is required for the depolarized membrane potential and spontaneous rhythmic firing activity of adult mouse sinoatrial (SAN) node cardiomyocytes. This current is insensitive to blockers of I_h and TRPM4 channel currents, which have previously been shown to contribute to the depolarized basal membrane potential in SAN cardiomyocytes. Expression of the Na^+ leak channel, non-selective (NALCN) gene is detected in the SAN cardiomyocytes, suggesting it as a potential molecular basis that warrant further investigation. Taken together, our findings provide the first evidence of a novel Na^+ current involved in regulating the basal membrane potential and rhythmic activity of mammalian cardiac pacemaker cells.

Keywords: pacemaker activity; Na^+ current; sinoatrial node cardiomyocytes

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S2.12**Long noncoding RNA and messenger RNA expression profile in the aortas of normal and high-fat diet fed ApoE-deficient mice**Mei-hua BAO^{1,2,3}, Guang-yi LI¹, Li-hua CHEN¹, Zhong-ping FENG^{2,*}, Hong-shuo SUN^{2,3,*}¹Institute of Neuroscience, Changsha Medical University, China; ²Department of Physiology; ³Department of Surgery, University of Toronto, Toronto, Canada

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Atherosclerosis is a chronic multifactorial inflammatory disease with high prevalence and one of the leading causes of death worldwide. Long non-coding RNAs (lncRNAs) participate in many biological and pathological processes such as carcinogenesis and cardiovascular diseases. However, the knowledge about the genome scale of lncRNAs and their potential biological functions in atherosclerosis are still unclear. The present study is to discover the lncRNA and mRNA expression profile and to predict functions of lncRNAs and mRNAs in normal and high-fat diet fed ApoE^(-/-) mice.

Homozygous male ApoE^(-/-) mice were divided into two groups. Normal control group mice were fed with normal chow, and high-fat diet (HFD) mice were fed a diet containing 20% fat and 2.5% cholesterol. Body weight was measured each week. The whole blood and aorta were collected after 8-week treatment. Blood lipid levels were measured using automatic biochemical analyzer; lncRNA and mRNA

level were tested using Agilent mouse lncRNA Microarray which contains 51302 lncRNAs and 39430 mRNAs; morphologic changes were investigated using Oil Red staining.

No significant body weight differences were found between normal diet mice and HFD mice. The LDL-C, CHOL, HDL and TC levels in the blood of HFD mice were dramatically increased. Microarray analysis found 354 differentially expressed lncRNAs, including 168 up-regulated and 186 down-regulated (≥ 2.0 folds). Simultaneously, 357 differentially expressed protein-coding mRNAs from the same samples were found. Gene ontology and KEGG pathway analysis indicated that these aberrantly expressed lncRNAs were mostly related to pathways such as metabolism and inflammation. Further analysis using hypergeometric distribution showed that the production of top 200 differentially expressed lncRNAs may be regulated by transcriptional factors such as Myod1, Rxra, Pparg, Tcf3, etc. Additional lncRNA-target-TFs network analysis for top 20 differentially expressed lncRNAs indicated Hnf4a, PPARa, Vdr, and Runx3 as the TFs most likely to regulate the production of these lncRNAs, and might play roles in inflammatory and metabolic processes in atherosclerosis. The present study identified a panel of dysregulated lncRNAs and mRNAs that may be served as potential biomarkers or drug targets relevant to the high-fat diet induced atherogenesis.

Keywords: atherosclerosis; long noncoding RNA; microarray; high fat diet

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