

**Journal of the Hong Kong College of**  
**CARDIOLOGY**



Including Abstracts of  
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Institute of Cardiovascular Science and Medicine and the  
10th Across the Strait Scientific Conference on Cardiovascular Science  
21 November 2015  
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# Investigating the Association Between Anti-Brucella Titers and the Development of Coronary Artery Disease in Middle-East

UMAYYA MUSHARRAFIEH,<sup>1</sup> ALI CHOUKAIK,<sup>2</sup> ABDUL RAHMAN BIZRI,<sup>2</sup> HALA TAMIM,<sup>3</sup> ANTOINE NASRALLAH,<sup>2</sup> SAMIR ALAM<sup>2</sup>

From <sup>1</sup>Department of Family Medicine, American University of Beirut Medical Center; <sup>2</sup>Department of Internal Medicine, American University of Beirut Medical Center; <sup>3</sup>Faculty of Health Sciences, American University of Beirut, Beirut, Lebanon

**MUSHARRAFIEH ET AL.: Investigating the Association Between Anti-Brucella Titers and the Development of Coronary Artery Disease in Middle-East. Background:** Several studies have suggested an association between certain infectious agents and coronary artery disease (CAD). A possible role for brucella in contributing to cardiovascular disease through its ability to produce a chronic inflammatory state was hypothesized. In this study, brucella was evaluated for its possible pathogenic role in significant occlusive coronary artery disease through testing for the presence of positive brucella antibody titers. **Methods and Results:** Patients referred for coronary angiography at the American University of Beirut Medical Center between January 2005 and February 2009 were tested for C-reactive protein (CRP) quantitative level and the presence of brucella antibody titers using ELISA. All participants were asked to fill a questioner relevant to demographics, risk factors for CAD, and presence of comorbidities. **Results:** 424 subjects were categorized into two groups; those with greater than 75% stenosis in at least one coronary artery and those with normal or less than 75% stenosis. Among patients with positive anti-brucella titers, 70.6% had CAD while among patients with negative anti-brucella titers 74.9% had CAD ( $P=0.514$ ). In patients with elevated CRP level ( $\geq 3$  mg/L), 14.9% had positive titer for brucella, whereas in those with low CRP level ( $< 3$  mg/L), 5.8% had positive titers for brucella ( $P=0.016$ ). **Conclusions:** The findings failed to show an association between anti-brucella titers and the development of significant CAD despite the presence of a significant correlation between brucella antibody positivity and elevated CRP. Further studies are needed to explore the role of this infectious agent with other known cardiac risk factors. (*J HK Coll Cardiol* 2015;23:68-74)

*Brucella, Coronary artery disease, C-reactive protein, Inflammation*

## 摘要

背景：多項研究表明，某些傳染性病原體和冠狀動脈疾病（CAD）相關，透過布魯氏桿菌可造成慢性炎症狀態推測其在心血管疾病中可能發揮作用。在這項研究中，布魯氏桿菌在閉塞性冠狀動脈疾病的可能致病作用透過抗體濃度效價為陽性進行評估檢測。方法及結果：在2005年1月及2009年2月，病人被轉介於美國貝魯特大學醫療中心進行冠狀動脈造影及進行C反應蛋白（CRP）定量水平和使用ELISA進行布魯氏菌抗體濃度存在測試。所有參與者均需填寫相關於人口統計問題，CAD的風險因素及存在的合併症。結果：424名受試者分成兩組：75%最少一條冠狀動脈狹窄及冠狀動脈正常或少於75%狹窄。在對抗布魯氏桿菌濃度反應陽性的病人中，70.6%患CAD，當病人對抗布魯氏桿菌濃度反應出現陰性則有74.9%患CAD（ $P=0.514$ ）。在CRP水平提高（ $\geq 3$  mg/L）的病人中，14.9%為布魯氏桿菌陽性，而低CRP水平（ $< 3$  mg/L）的病人則有5.8%為布魯氏桿菌陽性（ $P=0.016$ ）。結論：此項研究未能顯示抗布魯氏桿菌濃度與CAD的發展有明顯關聯，儘管布魯氏桿菌抗體與CRP升高存在顯著的相關性，仍需要更多研究探討這種傳染病在其他已知心臟病所佔的風險角色。

關鍵詞：布魯氏桿菌、冠狀動脈疾病、C反應蛋白、炎症

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## Introduction

Coronary artery disease (CAD) continues to be the leading cause of morbidity and mortality in both industrialized and developing countries despite advances in prevention, detection, and treatment.<sup>1</sup> Hyperlipidemia, hypertension, smoking, diabetes, family history, obesity and inactivity are well-documented risk factors for CAD.<sup>2</sup> However, in approximately half of the patients with CAD, the above mentioned risk factors are not present and thus the search for other factors is warranted.<sup>3</sup> Previous studies have suggested a possible role for infectious agents in the pathogenesis of CAD.<sup>4,5</sup> *Chlamydia pneumoniae*,<sup>6</sup> *Helicobacter pylori*,<sup>7</sup> and *Porphyromonas gingivalis*,<sup>8</sup> cytomegalovirus,<sup>9</sup> herpes simplex virus,<sup>10</sup> and hepatitis A virus<sup>11</sup> were all evaluated.

Brucellosis is a debilitating disease caused by *Brucella spp.* that can affect different organs and may if inappropriately treated lead to a chronic illness. Both the acute and chronic manifestations of brucellosis are due to inflammatory phenomena. There is current evidence that *Brucella spp.*, can infect and survive within the endothelial cells, and can induce a pro-inflammatory response that might be involved in the vascular manifestations of brucellosis. The release of adhesion molecules and pro-inflammatory chemokines during a brucella infection plays an important role in the activation of the endothelial system. This activation may be responsible for the pathogenesis of the damage in the vascular system.<sup>12</sup> The link between brucella infection and atherogenic lipid profile coupled with endothelial dysfunction may be a contributing factor in the development of coronary artery disease.<sup>13</sup>

Brucella is known to be endemic in the Middle East region with a reported incidence between 40-69 per 100,000 population in countries like Saudi Arabia, Kuwait and Jordan.<sup>14</sup>

In Lebanon, a study conducted by Araj et al included 597 persons with high risk occupations found that the overall seroprevalence of brucella titers ranged from 26-61%.<sup>15</sup> Data from the Lebanese Ministry of Public Health reveals an annual average of 220 brucella cases reported over the past 14 years.<sup>16</sup> In a recent study from Greece acute brucellosis was associated with a shift

of serum lipids, lipoproteins, and associated enzymes toward a more atherogenic lipid profile, which is not fully restored 4 months after treatment.<sup>17</sup> Along the same token, abnormal flow-mediated dilatation (FMD) measurement as reflection of endothelial function and thus the first stage of atherogenesis, might be a marker of arterial dysfunction, increased cardiovascular risk and atherosclerosis.<sup>18</sup>

The aim of the present study is to investigate the presence of any association between brucella serology positivity and the occurrence of significant coronary artery disease.

## Materials and Methods

### Study Population

Following Institutional Review Board approval, all patients admitted to the American University of Beirut Medical Center and referred for coronary angiography between January 2005 and February 2009 were approached for recruitment in the study. Written informed consent was obtained from eligible patients for drawing and using blood samples for scientific research after explaining the aim of the study and nature of the procedure.

Based on the diagnostic findings of the coronary angiography, patients enrolled were categorized into two groups. The first group included patients with normal coronary arteries and those with less than 75% luminal stenosis by angiography, the second group included patients with 75% luminal stenosis or more in at least one coronary artery. All those who have experienced myocardial infarction within the previous 6 months, valvular heart disease, or non-atherosclerotic cardiomyopathy were excluded.

### Study Design

#### *Atherosclerosis Risk Factors*

Medical records of enrolled patients were reviewed for demographics (age and gender), the presence of known risk factors for CAD including family history, diabetes mellitus (DM), hypertension (HTN), hypercholesterolemia (HCL), obesity, smoking, and dietary habits. Medical records were also reviewed for

prior history of brucellosis and intake of medications including statins and antibiotics within the previous three months.

### Laboratory Testing

Serum samples obtained from all study subjects were frozen at -70 degrees centigrade, and aliquots were thawed when needed for testing. All samples obtained were tested for brucella antibody titers using ELISA to detect brucella-specific antibodies (Anti IgG-C3d, Bioclone/Ortho-clinical Diagnostic, inc). This test was previously reported to have sensitivity, specificity, positive and negative predictive values of  $\geq 97\%$ ,  $\geq 98\%$ ,  $\geq 85\%$ , and  $\geq 97\%$ , respectively, according to the manufacturer's instructions. An antibody titer of 1:20 or greater was considered to be positive. Quantitative ELISA was used to determine serum C-reactive protein (CRP) (CRP-kit, Roche/Hitachi, Mannheim, Germany) level. A set of CRP standards was used to plot a standard curve of absorbance versus CRP concentration from which the CRP concentrations in the unknown can be calculated.

### Statistical Analysis

Statistical analysis was carried out with the Statistical Package for the Social Sciences (SPSS) version 13 (SPSS Inc., Chicago, Illinois, USA). Categorical data were analyzed by the chi squared test (Fisher's exact test for small samples), with all tests double-sided. Analyses of CRP serum level in relation to CAD and other factors were made by the unpaired t-test between different groups as a continuous variable and further adjusted using partial correlation investigation. Estimated Pearson correlation value (r) was used to indicate the strength of the relationship. The covariates considered included age, male sex, cigarette smoking, diabetes, HCL, HTN, and family history. Results for normally distributed continuous variables are expressed as mean  $\pm$  SD. A probability value of  $P < 0.05$  was considered significant.

Categorized data were analyzed by the chi squared test. Differences in means of continuous variables between groups were compared by means of the independent samples t-test and ANOVA if more than 2 groups were assessed. Logistic and linear regression

models were used to assess the independent associations of various risk factors to CAD.

### Results

A total of 424 patients were included in the study. Of these, 15.6% had taken antibiotics during the three months prior to enrollment. Among the study group 57.3% were smokers, 35.1% consumed alcohol, 63.2% were sedentary, 28.5% had DM, 55.6% had HCL, and 42.2% were on statin therapy at the time of enrollment and 12.6% had positive anti-brucella titers (Table 1).

**Table 1. Baseline characteristics of the participants**

	Number (percentage)
Gender	
Male	306 (72.2%)
Female	118 (27.8%)
Smoking history	
Yes	243 (57.3%)
Physical activity	
None	268 (63.2%)
Minimal (2-3 days/week)	74 (17.5%)
Moderate (3-4 days/week)	30 (7.0%)
Vigorous (>5 days/week)	52 (12.3%)
History of diabetes	
Yes	121 (28.5%)
Hyperlipidemia	
Yes	236 (55.6%)
Statin	
Yes	179 (42.2%)
C-reactive protein	
<3 mg/L	110 (25.9%)
>3 mg/L	314 (74.1%)
Brucella	
Positive	54 (12.6%)
Negative	370 (87.4%)
Coronary artery disease	
Yes	316 (74.5%)
No	108 (25.5%)

Among patients with positive anti-brucella titer 70.6% had significant CAD ( $\geq 75\%$  luminal stenosis), compared with 74.9% among patients with negative anti-brucella titer ( $P=0.514$ ) (Figure 1).

In patients with elevated CRP level  $\geq 3$  mg/L, 14.9% had positive anti-brucella titers, whereas in those with low CRP level  $< 3$  mg/L, 5.8% had positive anti-brucella titers ( $P=0.016$ ) (Figure 2).

### Occlusive Coronary Artery Disease

Chi-square testing was performed to compare presence of occlusive CAD with other risk factors. Those found to be significantly associated with occlusive CAD were male gender ( $P=0.004$ ), smoking ( $P=0.001$ ), hypercholesterolemia ( $P=0.008$ ), diabetes ( $P=0.010$ ), statin therapy ( $P=0.001$ ), CRP  $> 3$  ( $P=0.010$ ) and statin therapy with a CRP level  $\geq 3$  ( $P=0.002$ ). All other variables were not found to be statistically significant (Table 2).

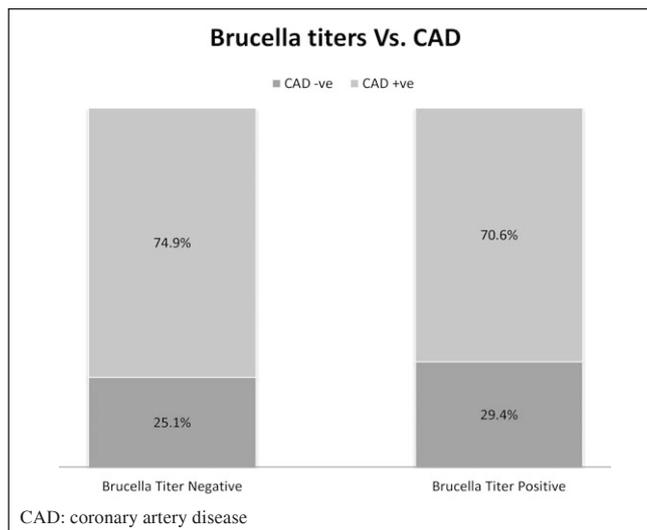
Upon performing binary logistic regression, the variables found to be predictors of occlusive CAD were male gender ( $P=0.003$ ), DM ( $P=0.005$ ), HCT ( $P=0.008$ ) and statin intake ( $P<0.001$ ). Males were twice as likely to develop occlusive CAD as compared to females (odds ratio (OR)=2.090, 95% confidence interval (CI): 1.28-

3.40). Meanwhile patients with DM had a two-fold increased risk (OR=2.217, 95% CI: 1.26-3.89) and those on statins had 2.8-fold increased risk than patients not on statin therapy (OR=2.865, 95% CI: 1.73-4.71), whereas patient with dyslipidemia had 2.4 fold increased risk (OR=2.421, 95% CI: 1.67-3.86) (Table 3).

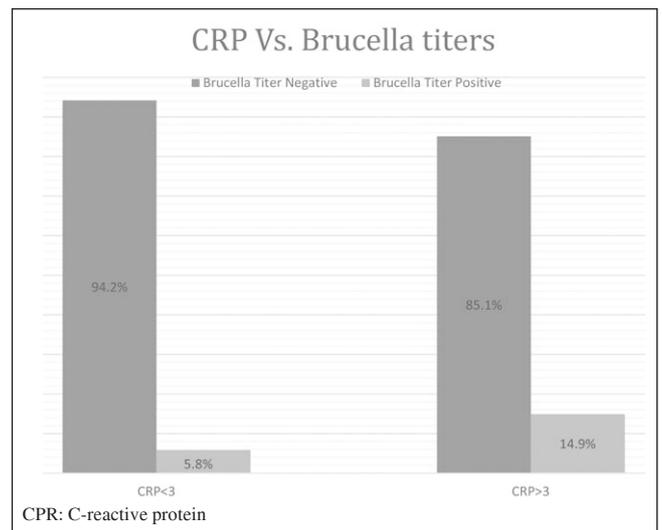
### Discussion

The current study has shown clearly the significant association of occlusive CAD and traditional risk factors including DM, smoking, hypercholesterolemia, high CRP and statin therapy. It has failed to find any association between occlusive CAD, alcohol intake, physical activity, recent antibiotic therapy and a positive brucella serology.

Few studies addressed the role of brucella infection as a risk factor for CAD. We found that positive anti-brucella titers did not weigh as a risk factor for significant disease conventional occlusive disease. Previous studies have postulated a link between certain microbial agents and cardiovascular events. Various methods, including immune serology and nucleic acid techniques, were utilized to assess the existence and



**Figure 1.** For patients positive for brucella 70.6% had CAD, and patients with negative brucella titers 74.9% had CAD ( $P=0.514$ ).



**Figure 2.** Patients with elevated CRP ( $\geq 3$  mg/L) 14.9% had positive titers for brucella whereas patients with low CRP ( $< 3$  mg/L) 5.8% had positive brucella titers ( $P=0.016$ ).

significance of such relation. Brucella, an intracellular organism that can produce a persistent infection and induce long-lasting effects on the host was mentioned in previous reports to be a possible candid pathogen. In a recent study, Togan et al suggested that the abnormal FMD observed in brucella patients might be an indicator of arterial dysfunction, increased cardiovascular risk and possibility of atherosclerosis.<sup>19</sup> The findings of the present study did not corroborate such an association. Previous reports have suggested an association between

serum antibody titers against *Chlamydia* and *Mycoplasma* with unstable angina or myocardial infarction. However, these studies were in the acute phase and not when patients were completely asymptomatic like in those evaluated in the current study.<sup>20</sup>

A recent review suggested that elevated levels of CRP, was a predictor of acute coronary syndromes.<sup>21</sup> In a study of 5248 subjects, there was a correlation between CRP levels (>10 mg/L) and the risk of

**Table 2. Association between CAD and other variables**

Variable	CAD		P-value
	Yes (n=316)	No (n=108)	
Gender			0.004*
Male	240 (75.9%)	66 (61.1%)	
Female	76 (24.1%)	42 (38.9%)	
Smoking	196 (62.0%)	47 (43.1%)	0.001*
Hyperlipidemia	194 (61.4%)	42 (38.8%)	0.008*
Statin therapy	153 (48.4%)	26 (24.0%)	0.000*
Statin therapy with elevated CRP	107 (33.8%)	18 (16.6%)	0.002*
Diabetes mellitus	101 (31.9%)	20 (18.5%)	0.010*
CRP >3 mg/L	231 (73.1%)	72 (66.6%)	0.010*
Drinking alcohol	112 (35.4%)	38 (35.1%)	0.745
Antibiotics	48 (15.1%)	17 (15.7%)	0.673
Physical activity			0.565
None/Minimal	256 (81.0%)	86 (79.6%)	
>Moderate	60 (19.0%)	22 (20.4%)	
Brucella titer >1:20	38 (12.0%)	16 (14.8%)	0.455

\*statistically significant

CAD: coronary artery disease; CPR: C-reactive protein

**Table 3. Logistic regression for CAD**

Variable	Odd ratio	95% CI for odd ratio		P-value
Gender (Male)	2.090	1.28	3.40	0.003
Diabetes mellitus	2.217	1.26	3.89	0.005
Statin therapy	2.865	1.73	4.71	0.000
Hyperlipidemia	2.421	1.67	3.86	0.008

CAD: coronary artery disease; CI: confidence interval

cardiovascular events as well as all-cause mortality.<sup>22</sup> Patients with high CRP were more likely to have more severe CAD disease than those with lower CRP levels (63.2% had at least one vessel disease greater than 75% vs. 44.3% and 45.8%) ( $P=0.001$ ) This is consistent with recent reports showing a significant correlation between serum CRP levels and severity of CAD as assessed by angiographic Gensini score.<sup>23</sup> Our results are in agreement with these reports and confirm that elevated levels of CRP are associated with at least one vessel disease with stenosis of greater than 75%, unrelated to brucella infection. Another report suggested that *Brucella species* can infect and survive within endothelial cells, and can induce a pro-inflammatory response that might be involved in the vascular manifestations of brucellosis.<sup>24</sup> Despite the findings in our report that patients with elevated CRP ( $\geq 3$ ) were more likely to have positive anti-brucella titers than those with low CRP (14.9% vs. 5.8%), it was difficult to prove the presence of any significant association as a contributory factor to occlusive CAD. Our negative findings do not negate the possible role of brucella as a preparative trigger in CAD. It has been shown that the response of the host to brucella infection and inflammation leads to an increase in oxidized lipids in the serum, and brings about LDL oxidation *in vivo*. Oxidative modification of LDL is one of the important events leading to the development of atherosclerosis.<sup>13</sup> Although the degree of inflammation induced by brucella seems to be lower than in infections caused by other organism, the chronic nature of the infection argues in favor of inflammation as a cause of tissue damage. This is due to a low degree of stimulation but incessant inflammatory tissue damaging response.<sup>24</sup>

Our results cannot evaluate the combined effect of various triggers or risk factors in the pathogenesis of occlusive disease. A weak trigger of inflammation as in a chronic brucella infection in a high risk patient may lead to a different CAD outcome when compared to a more potent trigger in a low risk patient.

It is reasonable to think that the same trigger of inflammation may lead to different outcomes in patients with different other risks for CAD. Ozbudak et al have alluded to this through a study that showed a synergistic effect of infection and cholesterol rich diet on

atherosclerosis in pulmonary arteries. The authors concluded that antibiotics and anti-inflammatory agents could be useful in prevention.<sup>25</sup>

Some published data have suggested that the aggregate effect of co-infection with multiple organisms rather than one organism may be responsible for the atherosclerotic role. This has been eluded to as the "infectious burden" or "pathogen burden".<sup>17,26</sup> In one study, over 75% of CAD patients had been exposed to at least three of five pathogens tested, suggesting a possible link between increased pathogen burden and the risk of CAD irrespective of traditional risk factors.<sup>27</sup> Thus, the contribution of infectious organisms to atherosclerosis pathogenesis is likely to involve simultaneous direct and indirect mechanisms involving multiple organisms.

### Limitation

The authors recognize the selection bias inherent in enrolling subjects from a patient population that is undergoing an invasive cardiovascular procedure. The results obtained may not be necessarily applicable to the population as a whole. Another important limitation is attempting to categorize the study population into those with and without significant occlusive disease (more than 75% stenosis) irrespective of the presence of lumen-limiting CAD or not. In addition, other risk factors assessment for CAD may not be comprehensive, in that information on hypertension, with or without anti-hypertensive therapy is lacking.

### Conclusion

The current study failed to show an association between positive brucella serology and the development of significant occlusive CAD, despite the presence of a significant correlation between brucella antibody positivity and elevated CRP.

Further studies are needed to explore any relationship between brucella infection and CAD including acute versus chronic infection, co-infection with other pathogens, and the interaction with other known risk factors.

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# Tricuspid Aseptic Endocarditis Revealing Right Endomyocardial Fibrosis During an Unrecognized Behçet's Disease: Two Cases Report

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From Ibn Rochd University Hospital of Casablanca, Morocco

**BENHALLA ET AL:** *Tricuspid Aseptic Endocarditis Revealing Right Endomyocardial Fibrosis During an Unrecognized Behçet's Disease: Two Cases Report.* We report two cases of endomyocardial fibrosis, revealed by verrucous tricuspid valvulitis extending to the right ventricular endomyocardium and complicated by a right heart failure, initially misdiagnosed and treated as infective endocarditis, during an unrecognized Behçet's disease. (*J HK Coll Cardiol* 2015;23:75-78)

*Behçet's disease, Cardiac MRI in Behçet's disease, Endomyocardial fibrosis*

## 摘要

我們報告兩例心內膜心肌纖維化，通過三尖瓣瓣膜疣狀心瓣延伸到右心室心內膜，及併發右心衰竭，透示最初不能辨識貝塞特氏症誤作感染性心內膜炎治療。

關鍵詞：貝塞特氏症、心臟MRI在貝塞特氏症、心內膜心肌纖維化

## Introduction

Endomyocardial fibrosis is rare in Behçet's disease (BD), we report 2 cases suffering from BD complicated by ventricular pseudo-tumor formation shown in the echocardiography. This deceptive appearance evoked the initial diagnosis of infective endocarditis with thrombosis.

## Case 1

Patient of 20-year-old with no particular medical history, admitted for an initial diagnosis of infective

endocarditis in the right heart. Physical examination disclosed murmur of tricuspid regurgitation, signs of right sided cardiac failure, and bilateral papillary oedema. Brain resonance magnetic imaging showed superior sagittal and right lateral sinus thrombosis. Electrocardiography showed right atrial hypertrophy and incomplete right bundle branch block.

Echocardiography disclosed an enlarged right atrium, severe narrowing of the in flow tract and the middle part of the right ventricle. Bright echoes were seen within the inferior and the middle parts of the right ventricular endocardium (Figure 1).

Nine blood cultures were negatives contrasting with disturbed inflammatory analysis and a fever, initially the patient was treated by intravenous antibiotics without any improvement, and it is with the onset of genital and oral ulceration, pseudo folliculitis in her skin that BD was established as a final diagnosis after an extensive work-up to exclude de infective endocarditis.

Cardiac magnetic imaging showed a mass of intermediate signal intensity on T1 weighted images with right ventricular dilation complicated by a cavitory

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thrombosis, late gadolinium enhancement was observed, with endocardial fibrous tissue present only in the subendocardium, appearing as a continuous area, commonly extending from the subvalvar region to the apex of the ventricle (Figure 2).

The endomyocardial biopsy was not possible because of the cardiac thrombi the patient was treated with cyclophosphamide boluses, prednisone (10 mg/d), colchicine (1 mg/d), and anticoagulants with a good



**Figure 1.** *Parasternal small-axis showing the exuberant formations lining the entire wall of the right ventricle.*

evolution and partial regression of the masses in the right ventricle after 7 weeks.

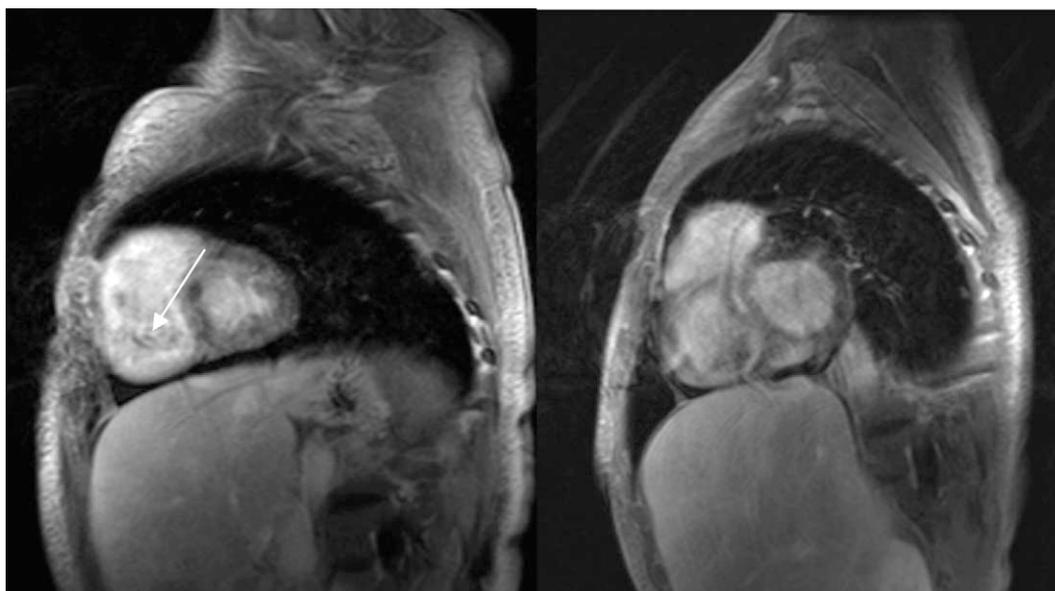
## Case 2

A young patient of 19-year-old, admitted in the cardiac emergencies for right heart failure with a fever lasting for three months, treated initially as an infective right endocarditis with 6 negatives blood cultures, without any improvement.

The patient reported having recurrent oral and genital aphthae, a posterior uveitis 2 months ago without healing; erythema nodosum in the abdomen and limbs. We completed by a pathergy test that was positive.

The echocardiography showed a dilated right ventricle with pseudotumoral formations lining the entire wall of the right ventricle and the septum inter atrial unlike the first case, with images of thrombi in the right ventricle, tricuspid valve was not affected by this process neither the others valves (Figure 3). Cardiac magnetic imaging for this patient was not available nearby.

The diagnosis of endomyocardial fibrosis in BD was established and a first dose of cyclophosphamide



**Figure 2.** *Cardiac Cine MRI, long-axis cine sequence, late gadolinium enhancement viewing the subendocardial thickening appearing as a continuous area in the right ventricle associated with the presence of several thrombi.*

combined with oral corticosteroid therapy were given to the patient, with a clinical improvement within 15 days.

## Discussion

BD is an inflammatory vasculitis, characterized by its frequency, in general with benign muco cutaneous, articular manifestations, but sometimes the severity of ocular, neurological, cardiac and vascular complications remains crucial.<sup>1</sup>

This disease mainly affects men (twice the woman) between 20 and 40 years. It is common in the Far East and the Mediterranean. The diagnosis is clinical and based on international criteria.<sup>2</sup> It is a disease that progresses in spurts sometimes spontaneously regressive and which treatment is largely symptomatic, of the fact many unknowns about its etiology,<sup>1</sup> but, as described in pathophysiology, the interplay between infectious-agent exposure and genetic factors may have a role. An environmentally triggered hyperactive primed state of autoimmunity ensues, resulting in two types of vascular damage. The first is vasculitic lesions that may be widespread. Sequelae depend on the various organ systems affected.

Some of the pathologic changes are due to thrombosis and / or clot formation caused by the

development of a hypercoagulable state. The mechanism is still undetermined; however, studies have demonstrated excessive thrombin formation and the potential role of impaired fibrinolytic kinetics in the generation of the hypercoagulable/prothrombotic state. Pathologic activation of the procoagulant cascade via endothelial injury has also been demonstrated in patients with Behçet disease.<sup>3</sup>

The frequency of cardiac involvement varies from less than 1% to 6% in clinical series and 16.5% in an autopsy series.<sup>3</sup> The three cardiac layers may be affected with pericarditis, myocardial injury, valvular and coronary tissue conduction. Intracardiac thrombosis is very rare, endomyocardial fibrosis still a part of the differential diagnosis of restrictive heart disease, and which can be presented as an intracardiac tumor also.<sup>4</sup>

Transthoracic echocardiography is the first-line examination and allows accurate systolic and diastolic functional assessment. It is however limited for tissue characterization and differential diagnosis of restrictive heart disease. Cardiac magnetic imaging has a key role in the diagnosis and prognosis of this disease, although few data have been reported in the literature.<sup>5,6</sup> It allows precise morphological evaluation of the endomyocardial fibrosis most often characterized by a diffuse thickening under endocardial right ventricle with the presence of several associated thrombus. Auricular areas are often increased in size due to severe diastolic dysfunction with restrictive disorder.<sup>2</sup>

The sequences of delayed enhancement affirm the diagnosis by showing a typical late enhancement, limited to subendocardium, and extended from the valve to the apex regions under the two ventricles where it usually dominates. Key element, a raise is not distributed in a vascular territory and is not accompanied by myocardial thinning in most cases. The presence of a thrombus is common at the apex of the LV and / or RV and. The prognostic role of MRI has also been recently suggested as the treatment of choice is the surgical resection of the endomyocardial fibrosis areas, in patients with Stage 3 or 4 NYHA.<sup>7,8</sup> MRI may help in the future planning of surgery and monitor its effectiveness. For our 2 patients, surgical treatment was deferred with the improvement under medical treatment with corticosteroids and anticoagulants.



**Figure 3.** Four chamber bidimensional end diastolic echocardiogram: bright echoes in the right ventricular endocardium.

## Conclusion

The discovery of endocardial masses in a patient suspected having an infective endocarditis, negative blood cultures with criteria of Behçet diseases, should arouse suspicion of the diagnosis of endomyocardial fibrosis. The cardiac MRI allows precise characterization of particular tissue fibrosis with the delayed enhancement sequences, and could help prognostic stratification and planning for therapeutic intervention in those patients.

## Disclosures

The authors declare that there is no conflict of interest.

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# 19th Annual Scientific Meeting of the Institute of Cardiovascular Science and Medicine and the 10th Across the Strait Scientific Conference on Cardiovascular Science

21 November 2015  
Hong Kong Convention and Exhibition Centre  
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## SCIENTIFIC PROGRAMME

### 21 NOVEMBER 2015 (SATURDAY)

08:30-09:00 **Registration**

09:00-10:40 **Invited Lectures**

*Chairpersons: Prof. Yu Huang, Chinese University of Hong Kong, Hong Kong  
Prof. Ying-Tung Lau, Chang Gung University of Science and Technology, Taiwan*

SIRT3 Mediates the Anti-oxidant effect of Hydrogen Sulfide in Endothelial Cells  
*Prof. Yong Ji, Nanjing Medical University, China*

MicroRNA Mediation of Endothelial Inflammatory Response to Smooth Muscle Cells and its Inhibition by Atheroprotective Shear Stress  
*Prof. Jeng-Jiann Chiu, National Health Research Institutes, Taiwan*

Salvianolic Acid A Ameliorates Vascular Endothelial Dysfunctions in Diabetic Rats through Anti-oxidants and Anti-hyperglycemia Stress  
*Prof. Guan-Hua Du, Chinese Academy of Medical Sciences and Peking Union Medical College, China*

Astragaloside IV Protects Against Cerebral Contusion, Neuronal Apoptosis, and Neurologic Deficits in Traumatic Brain Injured Rats  
*Prof. Cheng-Kuei Chang, Taipei Medical University, Taiwan*

09:00-10:40 **Oral Presentations for Young Investigator Award**

***Sponsored by Sun Chieh Yeh Heart Foundation***

*Chairpersons: Prof. Huang-Tian Yang, Shanghai Institutes for Biological Sciences, China  
Dr. Qin Yang, Chinese University of Hong Kong, Hong Kong*

10:40-11:00 Coffee break, poster viewing and booth visit

11:00-11:50 **Free Communications**

*Chairpersons: Prof. Cheng-Kuei Chang, Taipei Medical University, Taiwan  
Prof. Yong Ji, Nanjing Medical University, China*

11:00-11:50 **Poster Presentations for Young Investigator Award**

***Sponsored by Sun Chieh Yeh Heart Foundation***

*Chairpersons: Prof. Chao-Yu Miao, Second Military Medical University, China  
Dr. Susan Leung, The University of Hong Kong, Hong Kong*

11:50-13:10 **Plenary Lectures I**

*Chairpersons: Prof. Mao-Tsun Lin, Chi Mei Medical Center, Taiwan  
Dr. Heather Ballard, The University of Hong Kong, Hong Kong*

Sudden Cardiac Death in the Young  
*Dr. Anna Maria Lang-Choy, University of Dundee, UK*

Aquaporin-1 Translocation and Degradation Partially Mediates the Water Transportation Mechanism of Acetazolamide  
*Prof. Xue-Jun Li, Peking University, China*

Inflammation and Biomarkers in Congestive Heart Failure  
*Prof. Wei-Hsien Yin, National Yang-Ming University, Taiwan*

- 13:10-14:10 **Lunch**
- 14:10-14:40 **Opening Ceremony**
- 14:40-16:00 **Plenary Lectures II**  
*Chairpersons: Prof. G Liu, Peking University Health Science Center, China*  
*Prof. Bernard Cheung, The University of Hong Kong, Hong Kong*
- Hyperhomocysteinemia-promoted Atherosclerosis via T Cell Priming Activation  
*Prof. Xian Wang, Peking University, China*
- Tackling Atrial Fibrillation at the Population Level  
*Dr. David Siu, The University of Hong Kong, Hong Kong*
- Vascular Aging  
*Prof. Alex F. Chen, Central South University, China*
- 16:00-16:30 Coffee break, poster viewing and booth visit
- 16:30-17:45 **Invited Lectures**  
*Chairpersons: Prof. You-Yi Zhang, Peking University, China*  
*Dr. ML Fung, The University of Hong Kong, Hong Kong*
- The Janus Face of Angiotensin Like-2 in Heart and Vessels  
*Prof. Eric Thorin, Institut de Cardiologie de Montréal, Canada*
- Cardiac Differentiation of Pluripotent Stem Cells and Myocardial Repair  
*Prof. Huang-Tian Yang, Shanghai Institutes for Biological Sciences, China*
- Whole Body Cooling during Resuscitation Improves Neurotrauma Outcome in a Rat Model of Hemorrhagic Shock  
*Prof. Hung-Jung Lin, Taiwan Joint Commission on Hospital Accreditation, Taiwan*
- 16:30-17:45 **Invited Lectures**  
*Chairpersons: Prof. Alex F. Chen, Central South University, China*  
*Dr. Susan Leung, The University of Hong Kong, Hong Kong*
- NAMPT Metabolic Signaling in Vascular Repair  
*Prof. Chao-Yu Miao, Second Military Medical University, China*
- Teaching of Gender Difference in Cardiovascular Physiology  
*Prof. Ying-Tung Lau, Chang Gung University of Science and Technology, Taiwan*
- Human-Like Genetic Engineered Hamsters: Creation of a New Era of Translational Cardiovascular Research  
*Prof. G Liu, Peking University Health Science Center, China*
- 17:45-18:00 **Closing Remarks and Young Investigator Award Ceremony**  
*Prof. Bernard Cheung, The University of Hong Kong, Hong Kong*
- 18:00 **Annual General Meeting**

## ABSTRACTS

### Abstracts for Invited Lectures:

#### IL1.

##### **SIRT3 MEDIATES THE ANTI-OXIDANT EFFECT OF HYDROGEN SULFIDE IN ENDOTHELIAL CELLS**

LP Xie,<sup>1</sup> GL Meng,<sup>1</sup> S Li,<sup>1</sup> X Tang,<sup>1</sup> Y Ma,<sup>1</sup> Y Hang,<sup>2</sup> Y Ji<sup>1</sup>

<sup>1</sup>Key Laboratory of Cardiovascular Disease and Molecular Intervention, Nanjing Medical University, China; <sup>2</sup>Department of Vascular Biology, The Chinese University of Hong Kong, Hong Kong

**Aim:** Oxidative stress is a key contributor to endothelial dysfunction and associated cardiovascular diseases. Hydrogen sulfide (H<sub>2</sub>S) is an anti-oxidant gasotransmitter that protects endothelial cells against oxidative stress. Silent information regulator 2 (SIR2) is a functionally important transcriptional factor family in endothelial cells under oxidative stress. H<sub>2</sub>S was able to regulate activity of several transcriptional factors. The aim of this study is to investigate the possible role of SIRT3 in the antioxidant effect of H<sub>2</sub>S in endothelial cells.

**Results:** Cultured EA.hy926 endothelial cells were exposed to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as a model of oxidative stress-induced cell injury. GYY4137, a slow releasing H<sub>2</sub>S donor, improved cell viability and reduced oxidative stress and apoptosis following H<sub>2</sub>O<sub>2</sub> treatment. H<sub>2</sub>S reversed the H<sub>2</sub>O<sub>2</sub>-mediated inhibition of MAPKs phosphorylation, down-regulated of sirtuin3 (SIRT3) mRNA and enhanced expression of superoxide dismutase 2 and isocitrate dehydrogenase 2. H<sub>2</sub>S increased activator protein 1 (AP-1) binding activity of SIRT3 promoter via enhancing its S-sulfhydration which effect was absent in the presence of the specific AP-1 inhibitor SR11302 or curcumin. Paraquat injection into mice induced defected endothelium-dependent aortic vasodilatation and increased oxidative stress in mice aorta and small mesenteric artery, which were improved by GYY4137 administration. This vasculoprotective effect of H<sub>2</sub>S was absent in SIRT3 knockout mice.

**Innovation:** These results highlight a novel role for SIRT3 in the protective effect of H<sub>2</sub>S on oxidant damage in endothelium both *in vitro* and *in vivo*.

**Conclusion:** H<sub>2</sub>S enhances AP-1 binding activity with the SIRT3 promoter, thereby up-regulating SIRT3 expression and ultimately reducing oxidant-provoked vascular endothelial dysfunction.

#### IL2.

##### **MICRORNA MEDIATION OF ENDOTHELIAL INFLAMMATORY RESPONSE TO SMOOTH MUSCLE CELLS AND ITS INHIBITION BY ATHEROPROTECTIVE SHEAR STRESS**

JJ Chiu

Institute of Cellular and System Medicine, National Health Research Institutes, Taiwan

In atherosclerotic lesions, synthetic smooth muscle cells (sSMCs) induce aberrant microRNA (miR) profiles in endothelial cells (ECs) under flow stagnation. Increase in shear stress induces favorable miR modulation to mitigate sSMC-induced inflammation. We addressed the role of miRs in sSMC-induced EC inflammation and its inhibition by shear stress. Coculturing ECs with sSMCs under static condition causes initial increases of 4 anti-inflammatory miRs (146a/708/451/98) in ECs followed by decreases below basal levels at 7 days; the increases for miR-146a/708 peaked at 24 hours and those for miR-451/98 lasted for only 6 to 12 hours. Shear stress (12 dynes/cm<sup>2</sup>) to cocultured ECs for 24 hours augments these 4 miR expressions. *In vivo*, these 4 miRs are highly expressed in neointimal ECs in injured arteries under physiological levels of flow, but not expressed under flow stagnation. MiR-146a, miR-708, miR-451, and miR-98 target interleukin-1 receptor-associated kinase, inhibitor of nuclear factor-κB kinase subunit-γ, interleukin-6 receptor, and conserved helix-loop-helix ubiquitous kinase, respectively, to inhibit nuclear factor-κB signaling, which exerts negative feedback control on the biogenesis of these miRs. Nuclear factor-E2-related factor (Nrf)-2 is critical for shear-induction of miR-146a in cocultured ECs. Silencing either Nrf-2 or miR-146a led to increased neointima

formation of injured rat carotid artery under physiological levels of flow. Overexpressing miR-146a inhibits neointima formation of rat or mouse carotid artery induced by injury or flow cessation. Our results indicate that Nrf-2-mediated miR-146a expression is augmented by atheroprotective shear stress in ECs adjacent to sSMCs to inhibit neointima formation of injured arteries.

## ABSTRACTS

### Abstracts for Invited Lectures:

#### IL3.

##### **SALVIANOLIC ACID AMELIATES VASCULAR ENDOTHELIAL DYSFUNCTIONS IN DIABETIC RATS THROUGH ANTI-OXIDANTS AND ANTI-HYPERGLYCEMIA STRESS**

GH Du, R Zhao, JK Song, XY Yang, P Wu, BY Hou, L Sun, Y Lv, L Zhang  
Beijing Key Laboratory of Drug Target and Screening Research, Institute of Materia Medica, Chinese Academy of Medical Science and Peking Union Medical College, China

**Aim:** As a risk factor for cardiovascular disease, diabetes mellitus (DM) contributes to vascular complications. The pathological basis of that is endothelial dysfunctions, according to experimental evidences and substantial clinical reports. Salvianolic acid A (SalA) is an active compound isolated from the root of traditional Chinese medicine *Salvia miltiorrhiza* Bunge, which is used treating cardiovascular diseases for thousands of years. It has been found representing anti-inflammatory, antioxidant, anticarcinogenic, antiplatelet and antifibrotic properties. In present experiments, we launched the project aiming to figure out the *in vivo/in vitro* antidiabetic effect of SalA and the underlying mechanisms by alleviating endothelial dysfunctions.

**Methods:** The models of Alloxan-induced type 1 diabetic mice, streptozotocin (STZ, 60 mg/kg, i.p.) induced type 2 diabetic Sprague-Dawley (SD) rats, high-fat diet (HFD) and low-dose streptozotocin (STZ)-induced type 2 diabetic wistar rats were employed for the evaluation of SalA effects (1 mg/kg, p.o. 90% purity). The cultured endothelial cells were used to explore the mechanism of SalA actions.

**Results:** In both type 1 and type 2 diabetic animals, SalA regulated their fasting blood glucose (FBG) and blood glucose. In type 2 diabetic models, SalA reduced the level of serum Von Willebrand factor (vWF), ameliorated

the vascular reactivity of aorta ring, increased plantar blood perfusion and vascular activities, alleviated the vascular pathological changes in diabetic rats. SalA ameliorates diabetes symptoms and alleviates vascular endothelial dysfunction to keep vascular on healthy biological conditions. The underlying cellular and molecular mechanisms can be summarized as follows: a) regulate the glucose metabolism. SalA ameliorated the abnormal blood glucose by regulates glucose metabolism, increasing ATP production, decreasing mitochondrial membrane potential (MMP), improving mitochondrial function via Ca<sup>2+</sup>/calmodulin-dependent; b) Anti-oxidant effects. Oxidative stress is augmented in diabetic complications, treatment of diabetic animals with antioxidant SalA decreased the diabetes-induced MDA and NOS upregulation, reduced production of AGEs and ROS; c) Anti-inflammatory effects. Inflammatory mediators associated with vascular complications of diabetes, SalA may ameliorated vessel lesion by blocking inflammatory cascades.

**Conclusions:** The data indicated that SalA exhibits the antidiabetic effects, it could also protect against vascular endothelial dysfunction in diabetes, which might be owing to its antioxidative and anti-inflammatory effects.

#### IL4.

##### **ASTRAGALOSIDE IV PROTECTS AGAINST CEREBRAL CONTUSION, NEURONAL APOPTOSIS, AND NEUROLOGIC DEFICITS IN TRAUMATIC BRAIN INJURED RATS**

CK Chang,<sup>1</sup> CP Chang,<sup>2,3</sup> MT Lin<sup>4</sup>

<sup>1</sup>Graduate Institute of Injury Prevention and Control, Taipei Medical University; Neurosurgical Department, Taipei Medical University Shuang-Ho Hospital; <sup>2</sup>Department of Biotechnology, Southern Taiwan University of Science and Technology; <sup>3</sup>The Ph.D. Program for Neural Regenerative Medicine, Taipei Medical University; <sup>4</sup>Department of Medical Research, Chi Mei Medical Center, Taiwan

**Objectives:** Astragaloides (AST) is the main component of astragalus with the function of antioxidation, immune regulation, and promotion of intelligence. It was traditionally prescribed in the prevention and treatment of vascular and cerebrovascular diseases, aging, immune function disorders and other diseases. A mixture of astragalosides can improve memory in aged mice, and astragaloside IV can reduce brain infarction in mice or in rats after focal cerebral ischemia. However, the therapeutic effects of astragalosides in traumatic brain injury (TBI) models and how it affects the proposed microglial overexpression of TNF- $\alpha$  has not been determined.

**Materials:** Anesthetized rats, after the onset of traumatic brain injury, were divided into two groups and given the vehicle solution (1 mL/kg of body weight) or AST (80 mg kg<sup>-1</sup>). Vehicle or AST solutions were administered intraperitoneally and one hour after traumatic brain injury. AST or saline was injected immediately 1 h post-TBI, and the effect on the maximal angle of an inclined plane that the rats could cling to, neurological severity score (NSS), cylinder test, foot placing test, and ladder climbing test were assessed

1 day before surgery and 3 days post-TBI. The effect of rats' cerebral contusion zone was assessed 3 days after TBI. The effect on immunofluorescence staining for neuronal nuclei (NeuN) and TUNEL, GFAP, and Iba1, and Iba1 and TNF- $\alpha$ , in the rats' damage brain areas was assessed 3 days after TBI.

**Results:** Astragaloside causes attenuation of TBI-induced cerebral contusion, neuronal apoptosis, and neurological motor dysfunction. Traumatic brain injury-induced neuronal apoptosis, gliosis, and activated microglia (evidenced by changed their morphology into an amoeboid shape as well as microglia overexpression of tumor necrosis factor- $\alpha$ ) were all AST therapy-reduced.

**Conclusions:** Our results indicate that AST therapy may protect against traumatic brain outcomes after traumatic brain injury through mechanisms attenuating neuronal apoptosis, gliosis, and activated microglia in the injured brain tissues.

## ABSTRACTS

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### Abstracts for Invited Lectures:

#### IL5.

##### SUDDEN CARDIAC DEATH IN THE YOUNG

AM Choy

University of Dundee, UK

Sudden cardiac death (SCD) in a young person is a tragic experience and loss for families and communities, as victims are usually at the peak of their physical health, and socio economic potential. The causes of young SCD can be classified in to distinct entities that are often asymptomatic during life, with SCD being the first manifestation, and the challenge for clinicians is how these young deaths can be prevented. It is recognised that inherited cardiac conditions including the cardiomyopathies and channelopathies often underlie SCD, and it is important that the correct diagnosis is made by post-mortem, including genetic post-mortem, so that immediate relatives can be assessed for the condition and offered treatment, thus preventing further deaths. However, achieving this outcome is challenging as it requires professional awareness of these conditions; effective communication between the forensic pathologist, coroner, and general practitioner, as well as defined referral pathways and access to specialist cardiologists and clinical genetics. In my presentation, I will discuss the genetic causes of young SCD, screening programs and a multidisciplinary approach required for the effective management of families affected by ICC.

#### IL6.

##### AQUAPORIN-1 TRANSLOCATION AND DEGRADATION PARTIALLY MEDIATES THE WATER TRANSPORTATION MECHANISM OF ACETAZOLAMIDE

JZ Zhang, Y An, XJ Li

Department of Pharmacology, Peking University, China

It has been proposed that AQPs might have involved in certain clinical diseases such as brain and pulmonary edema, glaucoma and tumor etc. Our previous study has proved two carbonic anhydrase inhibitors (acetazolamide and topiramate) could significantly inhibit the expression of AQP1 protein both *in vivo* and *in vitro* and could dramatically inhibit the function of water transportation across the cell membrane. We also observed that the both drugs could depress the tumor metastasis and provided evidences that these compounds might dramatically produce the anti-angiogenesis effects in the tumor tissues. AQP1 being located in renal proximal tubules is required for urine concentration. In this present study, we testify whether regulating to AQP1 will mediate the diuretic effect of AZA. We utilized Sprague Dawley rats, as well as AQP1 knock-out (AQP1<sup>-/-</sup>) mice to examine urine volume, and human kidney-2 (HK-2) cell line was used for *in vitro* mechanism study. To detect AQP1-binding proteins, we established a Surface Plasmon Resonance (SPR)-based method, which is usually utilized to discover molecules interaction. We found that AZA decreased CAs activity initially but the activity gradually recovered. Contrarily, diuretic effect was consistently significant. AQP1 protein expression was significantly decreased on day 7 and 14. By utilizing AQP1<sup>-/-</sup> mice, we found diuretic effect of AZA was cancelled on day 14, while urine volume continuously increased in wild-type mice. SPR results indicated AQP1 was physiologically bound by myosin heavy chain (MHC). *In vitro* study results proved AZA facilitated AQP1 translocation onto cell membrane by promoting interaction with MHC, dependent on ERK/ myosin light chain kinase (MLCK) pathway activation. MHC inhibitor BDM and ERK inhibitor U0126 both abolished above effect of AZA. Eventually AZA induced AQP1 ubiquitination, while proteasome inhibitor MG132 reversed AZA's down-regulating effect upon AQP1. Our results identified AZA exerted diuretic effect through an innovative mechanism by regulating AQP1 and verified its inhibitory mechanism was via promoting MHC-dependent translocation onto cell membrane and then ubiquitin mediated degradation, implicating a novel mechanism and target for diuretic agent discovering.

#### IL7.

##### INFLAMMATION AND BIOMARKERS IN CONGESTIVE HEART FAILURE

WH Yin

Division of Cardiology, Heart Center, Cheng Hsin General Hospital and Faculty of Medicine, National Yang Ming University, Taiwan

Heart failure (HF) remains one of the most important problems in cardiology despite the progress in its treatment. HF is a complex syndrome with a host of pathophysiological mechanisms in action. Inflammation, an integral component of homeostasis, is a complex tissue response to stressors that attempts to mitigate their effect and initiate healing. Inflammation plays a critical role in the development, course, severity and outcomes of HF. The delicate balance of pro- and anti-inflammatory processes can lead to beneficial or detrimental effects to the failing heart. Early detection of changes developing in the heart is the key in improving the treatment's effectiveness. It appears that determining specific, sensitive biomarkers reflecting the complex pathophysiology of HF and using them to detect asymptomatic cardiac alterations may become a crucial screening tool, assisting in the identification of patients requiring further diagnostic examinations. In this presentation, the evidence on inflammatory biomarkers and their potential role in prognosis and therapeutic decisions for patients with HF will be reviewed.

## ABSTRACTS

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Abstracts for Invited Lectures:

### IL8.

#### **HYPERTHOMOCYSTEINEMIA - PROMOTED ATHEROSCLEROSIS VIA T CELL PRIMING ACTIVATION**

X Wang, J Feng, SL Lv

Department of Physiology and Pathophysiology, Basic Medical College, Peking University, China

Homocysteine (Hcy) is a sulfur-containing non-constitutive amino acid derived from the essential amino acid methionine. Numerous clinical studies have established hyperhomocysteinemia (HHcy) as an independent risk factor for cardiovascular diseases in human. We and others have demonstrated that HHcy accelerates atherosclerosis by affecting the immuno-inflammatory response by stimulating chemokine/cytokine secretion from monocytes and T lymphocytes. In this process, reactive oxygen species (ROS) act as a mediator in the T lymphocyte priming activation by Hcy. However, the exact mechanism of that has not been known yet. In this study, we postulated that by inducing the metabolic reprogramming, Hcy promoted T-cell proliferation and cytokine IFN- $\beta$  secretion with upregulated ER-mitochondrial coupling. Our results showed that Hcy (30  $\mu$ gM) increased T cell proliferation which was accompanied by elevated oxidative phosphorylation (OXPHOS), membrane potential, ATP production, mitochondrial mass, and endoplasmic reticulum (ER) stress. While stimulation of ER stress by ER stress inducer dithiothreitol (DTT) mimicked Hcy-mediated T cell proliferation, inhibition of ER stress by 4-phenylbutyric acid (PBA) or mitochondrial respiration by rotenone blocked Hcy-induced T cell proliferation and IFN- $\beta$  secretion. Mechanistically, we found that Hcy increased ER-mitochondria coupling as evidenced by SIM and tethering protein expression. The increased ER-mitochondria interaction contributed to the enhanced ER stress, increased

Ca<sup>2+</sup> transfer from ER to mitochondria, elevated OXPHOS and ATP production. Uncoupling ER-mitochondria by microtubule inhibitor nocodazole attenuated Hcy-stimulated mitochondrial membrane potential and IFN- $\beta$  secretion, suggesting that juxtaposition of mitochondria with the ER is required for the Hcy-promoted mitochondrial dysfunction and T cell activation as well as vascular Inflammation. In conclusion, we have found that Hcy promoted T-cell proliferation and IFN- $\beta$  secretion by inducing the metabolic reprogramming through regulation of ER-mitochondrial coupling. Our study highlights the importance of metabolic regulation in T cell priming activation, thus shed new light in understanding HHcy-accelerated atherosclerosis pathogenesis.

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### IL11.

#### **THE JANUS FACE OF ANGIOPOIETIN LIKE-2 IN HEART AND VESSELS**

E Thorin

Montreal Heart Institute, Research Center and Department of Surgery, Faculty of Medicine, Université de Montréal, Canada

Angiotensin-like 2 (angptl2) is a pro-inflammatory protein that induces vascular inflammation and endothelial dysfunction. The impact of angptl2 on cardiac function is, however, unknown. We hypothesized that angptl2 contributes to pressure overload-induced cardiac dysfunction. Cardiac and vascular endothelial functions were investigated in angptl2 knockdown mice (KD) versus wild-type (WT) littermates, in response to a 6-week pressure overload induced by transverse aortic constriction (TAC). The increase in systolic pressure in the aortic arch induced by TAC was lower in KD than in WT mice. In addition, while cerebral arteries displayed vascular remodeling and endothelial dysfunction in WT-TAC mice, arterial structure and function were preserved in KD-TAC mice. In contrast, KD-TAC mice displayed amplified cardiac hypertrophy (increased heart weight / tibia length ratio) and worsening of cardiac dysfunction measured by echocardiography (reduced ejection fraction and stroke volume) and Millar pressure catheter (increased minimal and end diastolic pressures, reduced relaxation rate) compared to WT-TAC mice. Cardiac mRNA and protein expression of NADPH oxidase NOX4, a major source of oxidative stress in the heart, was increased only in KD-TAC mice. Cardiac-specific reduction of NOX4 expression by single i.v. injection of adeno-associated virus AAV9 shRNA in KD-TAC mice significantly limited cardiac hypertrophy and remarkably prevented the cardiac over-dysfunction caused by the absence of angptl2 in response to TAC. In

conclusion, angptl2 knockdown paradoxically worsens cardiac hypertrophy and contractile dysfunction induced by pressure overload. Cardiac up-regulation of NOX4 expression and/or activity could contribute to this aggravated cardiac dysfunction.

## ABSTRACTS

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### Abstracts for Invited Lectures:

#### IL12.

##### **CARDIAC DIFFERENTIATION OF PLURIPOTENT STEM CELLS AND MYOCARDIAL REPAIR**

N Cao, H Liang, M Liu, JX Huang, Q Liu, L Qiang, W Qiang, P Zhang, HT Yang

Key Laboratory of Stem Cell Biology and Laboratory of Molecular Cardiology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences & Shanghai Jiao Tong University School of Medicine, China

Pluripotent stem cells (PSCs)-, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), derived cardiovascular progenitor cells (CVPCs) and cardiomyocytes hold great promise for the study of early cardiovascular development, drug screening, disease model, and myocardial regeneration. However, the efficient generation of these cells is a prerequisite for their applications. Moreover, the developmental logic and molecular regulation underlying their self-renewal versus the cardiac lineage decisions have not yet been fully understood. To address these issues, we investigated the regulatory mechanisms underlying the cardiac lineage fate decision, screened key signals controlling the generation and maintenance of CVPCs derived from PSCs (hPSC-CVPCs), and developed novel methods to efficiently induce human (h) CVPCs positive in MESP1/2, SSEA1, ISL1, MEF2C and GATA4 from hPSCs under feeder- and serum-free culture conditions. They possess the ability to generate three major cell lineages of the heart, yet lack tumorigenic ability. To determine their therapeutic values to myocardial infarction (MI), we then transplanted these cells to mice with acute MI. The post-MI cardiac performance in the hPSC-CVPCs group is significantly improved compared with the control one. Consistently, the infarct area in the hPSC-CVPC group is smaller than the one in the control. The

findings proved new insight into the regulatory mechanisms of cardiogenesis. The established systems permit one to efficiently obtain hPSC-derived CVPCs, which represent a powerful tool to study early embryonic cardiovascular development and offer a potentially safe source for developing approaches for myocardial repair (*Grants: Research Programs of CAS and CSIRO (XDA01020204, GJHZ1225); NSFC (31030050, 81470422), NBRPC (2011CB965300), NSTPC (2012ZX09501-001-001)*).

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#### IL14.

##### **NAMPT METABOLIC SIGNALING IN VASCULAR REPAIR**

CY Miao

Department of Pharmacology, Second Military Medical University, China

Vascular repair is an endogenous defense mechanism after ischemic disease such as stroke and myocardial infarction. Nicotinamide adenine dinucleotide (NAD), a well-known coenzyme involved in electron transport chain for generation of adenosine triphosphate, has emerged as a dictator regulating various biological signaling pathways. Nicotinamide phosphoribosyl transferase (NAMPT), also an adipokine known as visfatin, is the rate-limiting enzyme for NAD biosynthesis in mammals. NAMPT may also act in a non-enzymatic manner, presumably mediated by unknown receptor(s). Recently, we and others show that NAMPT and NAMPT-controlled NAD metabolism regulate fundamental biological functions in endothelial cells, vascular smooth muscle cells and endothelial progenitor cells. Sirtuins (SIRT; the most studied is SIRT1) are NAD sensors and may mediate the regulatory effects of NAMPT-NAD axis in these cells and vascular repair. Here, we discuss the current data regarding NAMPT and NAMPT-controlled NAD metabolism in vascular repair and the potential translational application of NAMPT-related products in treatment of cardio-cerebro-vascular disease.

#### IL15.

##### **TEACHING OF GENDER DIFFERENCE IN CARDIOVASCULAR PHYSIOLOGY**

YT Lau

Chang Gung University of Science and Technology, Taiwan

The differences in men and women's normal function and in their related diseases are often neglected in teaching of basic medicine. However, women differ significantly from men in most physiological systems. We thus have added a 20-minute unit on gender difference in the teaching of cardiovascular (CV) physiology for medical students in Chang Gung University. The content of this unit includes: (1) Gender differences in average lifespan; (2) Gender differences in cardiovascular diseases (CVD); (3) Gender differences in CV properties/functions; (4) Implications of gender difference in CV medicine. Several topics were listed (e.g. atherosclerosis, exercise and CV function) for the students to choose for this 20-minute period, and more than 80% students voted for gender difference in CVD in the past 3 years. It is important to address the impact of sex/gender on normal functions and CVD, an early exposure to this topic should be helpful for the medical students in this regard.

**ABSTRACTS**

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Abstracts for Invited Lectures:

**IL16.**

**HUMAN-LIKE GENETIC ENGINEERED HAMSTERS:  
CREATION OF A NEW ERA OF TRANSLATIONAL  
CARDIOVASCULAR RESEARCH**

G Liu

Peking University Health Science Center, China

Cardiac- and cerebral vascular diseases are the number one killer of human beings. Among these diseases, atherosclerosis represents the majority of underlying pathological alterations which cause the development and progression of the diseases. It was well established that high blood cholesterol alone is sufficient to result in atherosclerosis in coronary, cerebral and peripheral atherosclerosis. One of common causes of high blood cholesterol is familial hypercholesterolemia (FH), an autosomal dominant-inherited genetic disorder that leads to elevated blood cholesterol levels. FH may present as severely elevated total cholesterol and low density lipoprotein (LDL) cholesterol levels or as premature coronary heart disease (CHD). After the seminal discovery by Brown and Goldstein that mutations in the low-density lipoprotein receptor (LDLR) was the cause of monogenetic FH, over 1,500 mutations of this gene have been detected and these account for more than 80% of cases of FH. Heterozygous FH (plasma cholesterol around 300 mg/dl) is rather common among general population with an estimated prevalence of 1 in 300 to 500 in the world, while the incidence of homozygotes (Ch over 600 mg/dl) occurs about one per million. Though LDLR deleted mice had been generated 20 years ago and widely used until today, the lack of CETP, different lipoprotein profile from humans and absence of phenotypes in heterozygosity in mice limited the utility of LDLR KO mice as ideal human-like model in cardiovascular studies. In contrary, the hamsters as small rodents,

posses many features resembling that of human lipid metabolism, such as high CETP level, intestinal ApoB editing, endothelium-anchored hepatic lipase, low hepatic LDL receptor and hence enhanced susceptibility to atherosclerosis. To capitalize the full usefulness of hamsters we first optimized the methodology to manipulate the hamster's fertilized eggs and generated the first GFP transgenic hamsters. We then applied Crisp-Cas9 technology and created LDLR deleted hamsters. The heterozygous LDLR targeted hamsters developed hypercholesterolemia at age of 6 weeks on chow diet (plasma Ch 280 vs 180 mg/dl of wildtype littermates). Moreover, the homozygous males and females had plasma cholesterol levels near 1000 mg/dl, 4-5 times higher than wild type littermates. These LDLR targeted hamsters are under characterization of their lipoprotein profile, apolipoprotein distribution, response to various nutritional and pharmaceutical manipulations. Ultimately, the development of atherosclerosis in coronary and cerebral arteries will be evaluated in these LDLR deleted hamsters which will perhaps be the ideal small rodent models for human CAD and stroke.

## ABSTRACTS

### Abstracts for Oral Presentation:

#### OPI.

##### STABILIZATION OF G-QUADRUPLEX DOWN-REGULATE MICRORNA-24 EXPRESSION

J Gao,<sup>1</sup> YC Qi,<sup>1</sup> W Tan,<sup>2</sup> Y Gu,<sup>2</sup> M Xu<sup>#1</sup>

<sup>1</sup>Department of Cardiology, Institute of Vascular Medicine, Peking University Third Hospital, Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education and Key Laboratory of Cardiovascular Molecular Biology and Regulatory Peptides, Ministry of Health; <sup>2</sup>Beijing National Laboratory for Molecular Sciences, Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, Department of Chemical Biology, College of Chemistry and Molecular Engineering, Peking University, China

**Objectives:** G-quadruplexes (G4) are non-canonical nucleotide secondary structures formed by stacks of guanine tetrads. The function of G4 in gene coding regions has been extensively studied, however, less is known about G4 motifs in non-coding gene regions. Herein, this study focuses on whether the G-rich sequence upstream of miR-24-1 could fold into a G4 structure and regulate the expression of miR-24. Our research provides a new mechanism of microRNA regulation by stabilization of G4.

**Methods and Results:** (1) The G4 displayed parallel conformation characteristics by Circular dichroism spectroscopy (CD), Electrospray ionization mass spectrometry (ESI-MS) and Nuclear magnetic resonance spectroscopy (NMR) indicated the G4 has 3 layers of G-quartet planes. And the DMS-foot printing assays clarified the location of key guanines participated in the formation of G4. (2) Two types of adenoviral expression vectors were constructed to overexpress miR-24. The G4 type virus carried wild type G-rich sequence and the NG4 type virus carried mutation G-rich sequence, which lost the ability of folding into a G4. These adenovirus were transfected into HEK-293A cells, and small molecule tetrandrine (TET), G4 ligand, was added into HEK-293A cells for 12 hours, qRT-PCR was performed

to detect the miR-24 expression. The expression level of miR-24 was down-regulated by TET dose dependently in G4 type virus transfected cells, while was unchanged in the cells transfected NG4-AEV. (3) CRISPR/Cas9 system was used to construct G-rich sequence Knock-Out Sprague Dawley Rats (KO SD Rats). Compared to wild type SD rats (WT SD Rats), expression of miR-24 is much higher in Knock-Out SD Rats (WT:n=7, KO:n=7, P<0.001). Consequently, Junctophilin2 (JP2) and P27, the down-stream signaling of miR-24, were significantly down-regulated in KO SD Rats (WT:n=7, KO:n=7, P<0.05).

**Conclusion:** The stabilization of G4 down regulates the expression of miR-24. This study shed new light on microRNA regulation.

*National Natural Science Foundation of China (No. 91339105), Natural Science Foundation of Beijing (No. 7142165), Beijing Municipal Commission of Science and Technology (NO.Z141100000214006).*

#### OP2.

##### miR-17-3P IS REQUIRED FOR EXERCISE-INDUCED CARDIAC GROWTH AND PROTECTS AGAINST MYOCARDIAL ISCHEMIA-REPERFUSION INJURY

JJ Xiao,<sup>1,2\*</sup> J Shi,<sup>2</sup> H Wang,<sup>2</sup> YH Bei,<sup>1</sup> QK Xuan,<sup>2</sup> W Sun,<sup>2</sup> H Liu,<sup>2</sup> XL Li,<sup>2</sup> XQ Kong<sup>2\*</sup>

<sup>1</sup>Regeneration and Ageing Lab, Experimental Center of Life Sciences, School of Life Science, Shanghai University; <sup>2</sup>Department of Cardiology, The First Affiliated Hospital of Nanjing Medical University, China

**Aims:** We previously have reported that miR-222 was necessary for exercise-induced cardiac growth and protected against pathological cardiac remodeling. However, the role of other miRNAs in this process is unclear. This study aims at investigating the role of members of the miR-17-92 cluster and their star miRNAs in exercise-induced cardiac growth.

**Methods and Results:** miRNAs including miR-17-3p, -17-5p, -18a-3p, -18a-5p, -19a-3p, -19a-5p, -19b-1-5p, -19b-3p, -20a-3p, -20a-5p, -92a-3p and -92a-5p were determined by quantitative reverse transcription polymerase chain reactions (RT-PCRs) in heart samples from mice undergoing 3-week swimming training. miR-17-3p and miR-18a-3p were found to be increased in swimming mice models while only miR-17-3p was further found to be increased in voluntary cage wheel exercise mice models. Interestingly, miR-17-3p was decreased in ventricular tissues of DCM patients and TAC mice. In isolated cardiomyocytes, forced-expression of miR-17-3p induced cardiomyocytes hypertrophy and proliferation as determined by  $\alpha$ -actinin and EdU or Ki-67 staining. miR-17-3p was found to indirectly increase the AKT/PI3K pathway, contributing to cardiomyocytes hypertrophy. TIMP-3 was identified as a target gene responsible for cardiomyocyte proliferation by Western blotting, luciferase assays and rescue experiments. *In vivo*, using

heart weight-to-body weight (HW/BW (mg/gm)) ratio, heart weight-to-tibia length (HW/TL (mg/mm)) ratio and EdU/ $\alpha$ -actinin staining, mice with miR-17-3p inhibition (n=9) showed milder cardiac growth and less cardiomyocyte proliferation compared with control group (n=8) after the 3-week swimming exercise model. Moreover, subjected to ischemia-reperfusion injury, mice with forced-expression of miR-17-3p (n=10) showed significantly less impairment of cardiac function measured by echocardiography than negative control group (n=11). Also, cardiomyocyte proliferation was significantly enhanced in the miR-17-3p agomiR treated ischemia-reperfusion mice as assessed by EdU/ $\alpha$ -actinin staining.

**Conclusions:** miR-17-3p is required for exercise-induced cardiac growth and protects against myocardial ischemia-reperfusion injury. miR-17-3p represents a novel therapeutic target for cardiac repair and regeneration.

## ABSTRACTS

Abstracts for Oral Presentation:

### OP3.

#### MEDIATION OF ER STRESS IN HOMOCYSTEINE-INDUCED BK<sub>Ca</sub> CHANNEL DYSFUNCTION IN CORONARY ARTERIES: ROLE OF PERK-FOXO-3a ACTIVATION

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**Objectives:** Being abundantly expressed in vascular smooth muscle cells, large-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> (BK<sub>Ca</sub>) channels significantly contribute to the control of vascular tone. Although homocysteine is known as an endoplasmic reticulum (ER) stress inducer and previous studies suggest the inhibitory effect of homocysteine on BK<sub>Ca</sub> channels, whether ER stress is involved in homocysteine-induced channel inhibition remains unknown. Furthermore, by what mechanisms ER stress may mediate homocysteine-induced BK<sub>Ca</sub> channel dysfunction remains completely unexplored.

**Methods:** Porcine small coronary arteries denuded of endothelium were studied for the relaxant response to the BK<sub>Ca</sub> channel opener NS1619. Primary cultured porcine coronary arterial smooth muscle cells (PCASMCs) were used for mRNA and protein analysis of BK<sub>Ca</sub> channels, as well as patch-clamp recording of BK<sub>Ca</sub> channel current.

**Results:** The NS1619-induced relaxation was significantly attenuated in homocysteine-exposed arteries whereas co-incubation of the arteries with ER stress inhibitors, either TUDCA or 4-PBA restored the response. The selective PERK inhibitor GSK2606414 showed potent protective effect against homocysteine on the NS1619-induced relaxation and the whole-cell BK<sub>Ca</sub> current, suggesting the mediation of PERK-ER stress signaling in

homocysteine-induced channel dysfunction. Homocysteine lowered the protein level of  $\beta$ 1 but not  $\alpha$  subunit of BK<sub>Ca</sub> without downregulating mRNA expression of both subunits. Downregulation of BK<sub>Ca</sub>  $\beta$ 1 was attenuated by TUDCA, 4-PBA, and GSK2606414. Inhibition of PERK with GSK2606414 suppressed the nucleic translocation of forkhead box O transcription factor-3a (FOXO-3a) in homocysteine-exposed PCASMCs, associated with an increase of BK<sub>Ca</sub>  $\beta$ 1 protein level. In PCASMCs transfected with FOXO-3a siRNA, restoration of BK<sub>Ca</sub>  $\beta$ 1 protein level was observed after homocysteine exposure.

**Conclusions:** ER stress-mediated downregulation of BK<sub>Ca</sub>  $\beta$ 1 subunit is involved in homocysteine-induced smooth muscle BK<sub>Ca</sub> channel dysfunction in coronary arteries. Activation of FOXO-3a by PERK signaling plays a key role in the ER stress-mediated BK<sub>Ca</sub> channel inhibition.

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### OP4.

#### BONE MORPHOGENIC PROTEIN 4-SMAD INDUCED UPREGULATION OF PLATELET-DERIVED GROWTH FACTOR AA IMPAIRS ENDOTHELIAL FUNCTION

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**Objectives:** Bone morphogenic protein 4 (BMP4) is an important mediator of endothelial dysfunction in cardio-metabolic diseases while platelet-derived growth factors (PDGFs) are major angiogenic and pro-inflammatory mediator although the functional link between these two factors is unknown. The present study investigated whether PDGF mediates BMP4-induced endothelial dysfunction in diabetes.

**Methods and Results:** We generated Ad-BMP4 to overexpress BMP4 and Ad-Pdgfa-shRNA to knockdown Pdgfa in mice through tail intravenous injection. SMAD4-shRNA lentivirus, SMAD1-shRNA and SMAD5 shRNA adenovirus were used for knockdown in human endothelial cells. We found that PDGFAA impaired endothelium-dependent vasodilation in aorta and mesenteric resistance arteries. BMP4 upregulated PDGFAA in human and mouse endothelial cells, which was abolished by BMP4 antagonist noggin or knockdown of SMAD1/5 or SMAD4. BMP4-impaired relaxation in mouse aorta was also ameliorated by PDGF-AA neutralizing antibody. Tail injection of Ad-Pdgfa-shRNA ameliorates endothelial dysfunction induced by BMP4 overexpression (Ad-BMP4) *in vivo*. Serum PDGF-AA was elevated in the both diabetic patients and diabetic *db/db* mice compared with non-diabetic controls. Pdgfa-shRNA or Bmp4-shRNA adenovirus reduced serum PDGF-AA concentration in *db/db* mice. PDGF-AA neutralizing antibody or tail

injection with Pdgfa-shRNA adenovirus improved endothelial function in aorta and mesenteric resistance arteries from *db/db* mice.

**Conclusions:** The present study provides novel evidences showing PDGF-AA impairs endothelium-dependent vasodilation, and PDGF-AA mediates BMP4-induced adverse effect on endothelial cell function through SMAD1/5 and SMAD4 -dependent mechanisms. Inhibition of PDGF-AA ameliorates vascular dysfunction in diabetic mice.

## ABSTRACTS

Abstracts for Oral Presentation:

### OP5.

#### HOMOCYSTEINE ACTIVATES B CELLS VIA REGULATING PKM2-DEPENDENT METABOLIC REPROGRAMMING

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**Objectives:** Homocysteine (Hcy) is a sulfur-containing amino acid formed during the metabolism of methionine. Hyperhomocysteinemia (HHcy) has been implicated as an independent risk factor for atherosclerosis. In this study, we investigated the role of metabolic reprogramming in Hcy-induced B cell activation and the underlying mechanisms involved.

**Methods:** Splenic B cells isolated from C57BL/6J mice were stimulated by 100  $\mu$ M Hcy for different times. For certain experiments, B cells were pre-incubated with inhibitors for 30 min before Hcy stimulation. For *in vivo* experiments, ApoE<sup>-/-</sup> mice were intraperitoneal injected with a selective Pyruvate Kinase M2 (PKM2) inhibitor shikonin (SKN, 1.2 mg/kg) or solvent control every three days. Three days after first injection, ApoE<sup>-/-</sup> mice were fed normal mouse chow diet supplemented with or without 1.8 g/L Hcy in drinking water for 2 weeks. Aortic root atherosclerotic lesions were stained with oil red O. Immunoglobulin levels were determined by ELISA. The expression and activity of PKM2 were detected by Western blotting and a continuous assay coupled to lactate dehydrogenase, respectively. Oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) were measured with an XF24 extracellular flux analyser.

**Results:** Hcy induced B cell proliferation and antibody production both *in vitro* and *in vivo*. Following Hcy stimulation, B cells upregulated both lactate production and oxygen consumption, suggesting a relative balanced metabolic reprogramming. Glucose uptake was also increased in Hcy-induced B cells. B cell activation was abolished by glycolytic inhibitor 2-deoxyglucose,

indicating that glycolytic pathway is critical for this process. Further investigations indicate that PKM2, one of the key enzymes involved in glycolytic pathway, plays an important role in this process. While both the protein expression and enzyme activity of PKM2 were increased by Hcy in B cells, PKM2 inhibition with SKN sharply suppressed Hcy-induced metabolic reprogramming and B cell activation. Similarly, PKM2 small interfering RNA silencing diminished Hcy-induced B cell activation. Further studies suggest that reactive oxygen species and mTOR signaling pathway participate in Hcy-induced B cell activation and function through regulating PKM2. Consistent with our *in vitro* findings, we showed that SKN treatment *in vivo* ameliorated HHcy-accelerated atherosclerotic lesion formation and diminished plasma immunoglobulin levels as well.

**Conclusions:** Our data demonstrate that Hcy-activated B cells require PKM2 to support metabolic reprogramming, which leads to B cell proliferation and antibody production. Targeting metabolic regulator PKM2 may be a therapeutic approach to ameliorate HHcy-accelerated atherosclerosis.

### OP6.

#### OVER EXPRESSION OF DRAM1 PREVENT AGAINST MYOCARDIAL ISCHEMIA INJURY BY IMPROVING AUTOPHAGY FLOW

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**Objectives:** Our previous studies showed that autophagy was induced sharply in the early phase after acute myocardial infarction, but autophagy substrate P62 accumulated with the extension of ischemia. Our results indicated that autophagy flow was impaired after sustained myocardial ischemia. DRAM1 (Damage-regulated autophagy modulator 1) was reported as one of the most important lysosome membrane protein to mediate the interaction of autophagosome and the lysosome. In this study, we investigated whether DRAM1 may improve the autophagy flow and prevent against myocardial ischemia injury.

**Methods and Results:** Adenoviral vector expressing DRAM1 or null adenoviral vector was injected into rat peri-infarct myocardium after left anterior descending (LAD) coronary artery ligation. DRAM1 expression was confirmed by Western blotting and immunofluorescence. Echocardiographic analysis demonstrated relatively preserved cardiac function in the adeno-DRAM1 animals 21 days after LAD ligation. The left ventricular function of adeno-DRAM1 animals was improved compared to the adeno-null rats. Myocardial remodeling was attenuated after overexpression of DRAM1, while was exacerbated in the adeno-null rats respectively with hematoxylin-eosin staining and Masson's trichrome staining. Overexpression DRAM1 diminished myocardial apoptosis compared with the adeno-null rats with Tunel staining. Overexpression DRAM1 decreased infarct size and increased new blood

vessels density. Increased autophagy and autophagy-lysosome were found in the infarct border of hearts overexpressed with DRAM1. Overexpression DRAM1 attenuated the accumulation of autophagy LC3-II and substrate protein P62/SQSTM1 obviously, which caused by sustained ischemia with Western blotting.

**Conclusions:** Overexpression DRAM1 improved the impaired autophagy flow caused by sustained myocardial ischemia, and prevented against myocardial ischemia injury.

## ABSTRACTS

Abstracts for Oral Presentation:

### OP7.

#### THE ROLE OF miR-27a-REGULATED G-PROTEIN COUPLED RECEPTOR KINASE 6 IN ABNORMAL PROLIFERATION OF ENDOTHELIAL CELL DURING HYPERTENSION

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**Objectives:** In hypertension, elevated blood pressure results in high cyclic stretch, which causes abnormal paracrine of vascular smooth muscle cells (VSMCs) and induced abnormal proliferation of endothelial cells (ECs). It has been reported that G protein-coupled receptor kinases (GRKs) are involved in the EC dysfunction of hypertension. GRKs regulate a large amount of proteins and are also regulated by many molecules, including microRNA (miR). Recent studies revealed that miRs can be secreted extracellularly and participate in the communication between ECs and VSMCs during many cardiovascular disorders. Here we hypothesized that cyclic stretch may modulate the VSMC secretion of miRs via microparticles (MPs), which subsequently induces EC dysfunction during hypertension.

**Methods:** Using hypertension rat model, the expression of GRK6 in thoracic aorta ECs was detected by immunofluorescence, the proliferation of ECs was detected by *in situ* BrdU immunofluorescence kit, and the VSMC-derived MPs were observed by transmission electron microscope (TEM). Using FX-4000T Strain Unit, 5% and 15% cyclic stretch at 1.25 Hz were applied to VSMCs *in vitro* for 24 h, and then, ECs were treated with the MPs isolated from the VSMC media. In addition, miR-27a mimics and inhibitor, and GRK6 siRNA were transfected into ECs by Lipofectamine 2000. The expression of miR-27a was detected by real-time PCR, the expression of GRK6 was detected by western blotting and the proliferation of ECs was detected by BrdU ELISA assay.

**Results:** The *in vivo* experiments showed that the expression of GRK6 in ECs at thoracic aorta of hypertensive rat was lower than that in normotensive control, but EC proliferation was higher. Then, we found the existence of VSMC-MPs *in vivo* and *in vitro*, and revealed the expression of miR-27a in VSMC-MPs. The expression of miR-27a in MPs that isolated from the media of VSMCs subjected to 15% cyclic stretch (15%-VSMC-MPs) was higher than that in 5%-VSMC-MPs. Dual luciferase reporter assays proved the target between miR-27a and GRK6. The expression of GRK6 in ECs treated with 15%-VSMC-MPs was lower than that treated with 5%-VSMC-MPs, while the EC proliferation was higher. Further more, biotinylated miR-27a, which were previously transfected into VSMCs, was detected in the VSMC-MP-treated ECs, indicating the transfer of miR-27a from VSMCs to ECs via MPs. In addition, the up-regulation of miR-27a in ECs by mimics decreased the expression of GRK6 and increased the proliferation; while the down-regulation of miR-27a in ECs by inhibitor had the opposite effect. Suppressing the expression of GRK6 in ECs by GRK6 siRNA increased cell proliferation. **Conclusions:** MiR-27a and the target GRK6 played significant roles in the proliferation of ECs during hypertension. High cyclic stretch increased the secretion of miR-27a from VSMCs via MPs, which could subsequently be transferred into ECs, decreased the expression of GRK6, and finally induced the proliferation of ECs. (*This work was supported by grants from the NSFC No. 11232010, 11172178, 11222223*)

### OP8.

#### CARTILAGE OLIGOMERIC MATRIX PROTEIN DEFICIENCY PROMOTES ATHEROSCLEROTIC CALCIFICATION VIA MODULATING MACROPHAGE PHENOTYPES

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Vascular calcification significantly increases cardiovascular disease morbidity and mortality. We recently reported that cartilage oligomeric matrix protein (COMP), a normal vascular extracellular matrix, is an endogenous inhibitor of vascular smooth muscle cell (VSMC) calcification. However, whether COMP affects atherosclerotic calcification is unknown. We firstly evaluated the atherosclerotic plaques and calcification in COMP<sup>-/-</sup> mice. Compared with ApoE<sup>-/-</sup> mice, COMP<sup>-/-</sup>ApoE<sup>-/-</sup> mice fed with chow diet for 12 months displayed more extensive atherosclerotic lesions and calcification in the aortic roots, arches and the innominate arteries. Next, COMP was mainly expressed in vascular smooth muscle cells (VSMCs) and macrophages during atherosclerosis. To investigate which COMP contributed to atherosclerotic calcification, crossed bone marrow transplantation (BMT) was applied between ApoE<sup>-/-</sup> and COMP<sup>-/-</sup>ApoE<sup>-/-</sup> mice. Enhanced calcification was observed in both ApoE<sup>-/-</sup> and COMP<sup>-/-</sup>ApoE<sup>-/-</sup> mice transplanted with COMP<sup>-/-</sup>ApoE<sup>-/-</sup> bone marrow compared to the respective recipients transplanted with ApoE<sup>-/-</sup> bone marrow. These data indicated that macrophages lacking COMP played a critical role in atherosclerotic calcification. Furthermore, through comparable microarray analysis on WT and COMP<sup>-/-</sup> macrophages, we

explored that COMP deficient macrophages showed an atherogenic and osteogenic phenotype based on the upregulated genes related to inflammation, ROS production, lipid uptake and osteogenesis. These results reveal that COMP deficiency drove macrophages as the atherogenic and osteogenic phenotype to aggravate atherosclerotic lesion and calcification.

## ABSTRACTS

### Abstracts for Oral Presentation:

#### OP9.

##### CONCENTRATION-DEPENDENT VASCULAR EFFECTS OF DIVALENT MANGANESE

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**Objectives:** Divalent manganese is a cofactor for soluble guanylyl cyclase (sGC), the enzyme producing cyclic GMP in vascular smooth muscle cells that causes relaxation. Manganese competes with magnesium to activate sGC. These divalent cations can bias the activity of the enzyme to produce cyclic nucleotides other than cyclic GMP, in particular cyclic AMP and cyclic IMP. Cyclic IMP, preferably produced in the presence of magnesium, can cause contraction in precontracted arteries of the pig. The objective of the present study was to identify the mechanisms by which manganese, compared to magnesium [used in standard physiological solutions at millimolar concentrations for functional studies], affects endothelium-dependent and -independent relaxations by influencing intracellular levels of cyclic nucleotides.

**Methods:** *Ex vivo* experiments were designed using isolated rat aortae of Sprague-Dawley rats of 12-14 weeks of age and porcine coronary arteries (collected at the local abattoir). The arteries were cut into rings and used for measurement of vascular reactivity in conventional organ chambers using pharmacological inhibitors of endothelial nitric oxide (NO) synthase (L-NAME) and of cyclooxygenases (indomethacin). In some rings, the endothelium was removed through insertion of a wooden toothpick. After obtaining an optimal resting tension, the rings were treated with millimolar concentrations of manganese or magnesium for fortyfive minutes and precontracted with phenylephrine (rat aortae) or U46619 (porcine coronary

arteries). Subsequently, concentration-dependent responses were obtained with acetylcholine (rat arteries), bradykinin (porcine coronary arteries) and the NO-donor SNP. In parallel, *in vitro* experiments were conducted using cultured porcine coronary artery smooth muscle cells (PCASMCs). The cells were exposed to millimolar concentrations of manganese or magnesium in the presence or absence of a sGC-activator (YC-1). Intracellular cyclic GMP levels were measured using a commercially available ELISA kit.

**Results:** *In vivo*, millimolar ranges of manganese significantly decreased endothelium-dependent relaxations to acetylcholine in rat aortae and to bradykinin in porcine coronary arteries compared to physiological levels of magnesium. This effect was abolished by endothelium-removal. In addition, L-NAME partially reversed the effect of manganese, while indomethacin did not. Manganese also decreased, but to a smaller extent, endothelium-independent relaxations to SNP in both porcine coronary arteries and rat aortae. In parallel, the *in vitro* study showed that manganese alone or in combination with YC-1 blocks the production of cyclic GMP.

**Conclusions:** Taken into conjunction, these experiments show that manganese at millimolar levels decreases endothelium-dependent and -independent relaxations compared to physiological levels of magnesium. This is likely explained through a decrease in cyclic GMP production. The effect of manganese is endothelium-dependent. In the case of endothelium-dependent relaxations (to acetylcholine or bradykinin), the effect of manganese is mediated by endothelial NO synthase, but not by cyclooxygenases. These findings indicate that in the presence of manganese NO does not act as a vasodilator and must lead to production of cyclic nucleotides other than cyclic GMP to cause contraction, similar to what has been reported under hypoxic conditions.

#### OP10.

##### PROSTAGLANDIN E RECEPTOR SUBTYPE 4 (EP4) REGULATES LIPID DROPLET SIZE AND MITOCHONDRIAL ACTIVITY VIA Fsp27 IN WHITE ADIPOSE TISSUE

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**Objectives:** A salient feature of chronic  $\beta_3$ -adrenergic agonist activation is pronounced remodeling of white adipose tissue (WAT), which includes the transformation of the lipid vacuole from unilocular into multiocular appearance and mitochondrial biogenesis with resulting elevation in whole body metabolic rate. Although prostaglandin E receptor subtype 4 (EP4; one subtype of prostaglandin E2 receptor) stimulation affect metabolic functions, no available information focuses on its role in WAT remodeling. Hence, the aim of this study was to investigate whether or not genetic ablation of EP4 affects WAT remodeling mediated by  $\beta_3$ -adrenergic agonist.

**Methods:** EP4<sup>+/+</sup> and EP4<sup>-/-</sup> mice (12-15 weeks old male) received either saline or CL316243 (a highly selective  $\beta_3$ -adrenergic agonist, 1 mg/kg/day, *i.p.*) for 10 days. The effect of EP4 deficiency on energy expenditure (as determined by indirect calorimetry), fat tissues morphology (as determined by H&E staining), mitochondrial biogenesis (as determined with electron microscopy and with the MitoTracker dye through confocal microscopy) and mitochondrial activity (as reflected by mitochondria complex I/IV activity measurement) in mice with or without CL316243 treatment were compared. In addition, the expression of genes and proteins involved in lipid droplet formation in fat pads was also studied.

**Results:** The elevation metabolic rate caused by chronic CL316243 treatment was significantly greater in EP4<sup>-/-</sup> mice. CL316243 significantly fragmented the unilocular lipid droplet into multiocular lipid vacuoles. It also increased mitochondria biogenesis and mitochondria cytochrome c oxidase activity as well as coenzyme Q1 activity. These changes were significantly amplified in mice with EP4 deficiency and were selectively seen in subcutaneous WAT, but were absent in epididymal or brown adipose tissue. The mRNA and protein expression of fat specific protein 27 (Fsp27), a protein that promote fusion of triglycerides and formation of unilocular lipid droplet, were significantly diminished in subcutaneous WAT of EP4 deficient mice.

**Conclusions:** The current findings implies that EP4 regulates the extent of WAT remodeling and that in its absence, Fsp27 expression in subcutaneous WAT is repressed provoking greater CL316243-induced multiocular lipid droplets and mitochondrial activity-causing an overall increase in whole body metabolic rate in these animals.

*The work is supported by Research Grant Council Early Career Scheme (ECS) (Project code: 27101314).*

## ABSTRACTS

### Abstracts for Oral Presentation:

#### OP11.

##### THE ORPHAN RECEPTOR NOR1 PARTICIPATES IN CARDIAC HYPERTROPHY BY REGULATING PARP-1 AND SIRT1

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**Background and Purpose:** NOR1 belongs to NR4A subfamily of the nuclear hormone receptor superfamily and is involved in regulation of glucose and fat metabolism. However, its potential contribution to cardiovascular diseases remains to be elucidated. In the present study, the roles of NOR1 in cardiac hypertrophy induced by isoproterenol (ISO) and the underlying molecular mechanism were investigated.

**Experimental Approach:** The expression of NOR1 was detected in hypertrophic cardiomyocytes induced by ISO. After NOR1 overexpression by transfection or depletion by RNA interference in neonatal rat cardiomyocytes, cellular hypertrophy was monitored by measuring cell surface area and the mRNA levels of hypertrophic biomarkers. Further, the interaction between NOR1 and PARP-1 was investigated by co-immunoprecipitation. The expression of NOR1 and activity of PARP-1 were measured *in vivo*, using ISO model of cardiac hypertrophy in rats.

**Key Results:** ISO treatment significantly upregulated NOR1 expression and PARP-1 activity both *in vitro* and *in vivo*. Specific genetic silencing of NOR1 attenuated ISO-induced cardiomyocyte hypertrophy. Furthermore, we identified a physical interaction between NOR1 and PARP-1, whose enzyme activity could be enhanced by NOR1 transfection. Overexpression of NOR1 but not its mutant NOR1 (C293Y), resulted in increased cellular surface area and the mRNA levels of ANF and BNP, which could be inhibited by PARP-1 inhibitor 3AB or PARP-1 RNA interference.

**Conclusions and Implications:** Our findings provide the first evidence that NOR1 is involved in ISO-induced cardiac hypertrophy. The prohypertrophic effect of NOR1 can be at least partially attributed to its regulation of PARP-1 enzyme activity.

#### OP13.

##### LOSS OF OSTEOGLYCIN PROMOTES ANGIOGENESIS IN LIMB ISCHEMIA MOUSE MODELS VIA MODULATION OF VEGF-VEGFR2 SIGNALING PATHWAY

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**Objectives:** Osteoglycin (OGN) has been noted for implications in cardiovascular diseases in recent studies, particularly its correlation with cardiac remodeling. However, the relationship between OGN and angiogenesis remains unknown. Therefore, we aimed to investigate the effect of OGN on ischemia-induced angiogenesis and address the underlying mechanisms.

**Methods:** Immunofluorescence and western blot were used to detect OGN expression under ischemic condition. Limb ischemia model was established in OGN<sup>-/-</sup> (n=12) mice and wild type (WT, n=12). Perfusion recovery was estimated by Laser Doppler imaging. Histology, capillary formations and inflammation response in gastrocnemius muscle after ischemia were evaluated by H.E staining and immunohistochemistry. Besides, alkali induced corneal neovascularization model and *ex vivo* aortic ring sprouting model were established in both OGN<sup>-/-</sup> and WT mice and angiogenesis were assessed. *In vitro* studies, tube formation, proliferation and migration of human umbilical vein endothelial cells (HUVECs) were assessed after transfection of either OGN siRNA or overexpression plasmid. Western blot was used to detect expression and activation of VEGFR2 signaling. Protein-protein interaction was assessed by co-immunoprecipitation (co-IP) and detailed information was provided by molecular docking.

**Results:** We observed OGN down-regulation with angiogenesis in

#### OP12.

##### OBESITY EXACERBATES RAT ISCHEMIC STROKE THROUGH ENHANCING ADIPONECTIN-CONTAINING NEURONAL APOPTOSIS

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**Objectives:** A diet containing of high levels of saturated fat has been linked to a dramatic rise in obesity. Long term exposure to high fat, "Western diet" (WD), are detrimental to ischemic brain injury.

**Materials:** To explore the role of adiponectin (APN) and its adiponectin receptors (ADRs) in the development of acute cerebral injury, we subjected WD and control diet (CD) rats to 1 hour of middle cerebral artery occlusion following by 23 hours of reperfusion.

**Results:** Compared with CD rats, WD rats exhibited higher levels of brain infarct, neurologic deficits, brain edema and apoptosis of APN-containing neurons, upregulation of ADR-1 and p38 mitogen-activated protein kinase (p38 MAPK) and downregulation of ADR-2 in ischemic brain tissues including frontal cortex, striatum, and hippocampus. Increasing percentages of APN-containing neurons by baculovirus-mediated administration of APN, in addition to reducing apoptosis of APN-containing neurons in ischemic brain tissues, significantly attenuated brain infarct and edema, neurologic deficits, and altered expression of ADR-1, p38 MAPK, and ADR-2 in both WD and CD group rats.

**Conclusions:** These data suggest negative correlation between percentage of APN-containing neurons and ischemic stroke. Obesity could exacerbate rat stroke injury by enhancing apoptosis of APN-containing neurons in ischemic brain tissues, probably via modulating ADRs and p38 MAPK.

intermuscular endothelial cells of angiogenic tissues in patients with peripheral artery disease and mice of limb ischemia model. Although, histology change, inflammatory cell infiltration, and inflammatory cytokines expression after ischemia in OGN<sup>-/-</sup> mice did not differ from WT ones. Perfusion recovery rate was higher in OGN<sup>-/-</sup> mice. The percent of blood flow recovery of wild type mice was 24.8%, 44.6% and 63.5% respectively at day 4, day 7 and day 14 after limb ischemia, while OGN<sup>-/-</sup> mice was 46.5%, 71.5%, and 83.0% at the same time points. Capillary density was also higher in OGN<sup>-/-</sup> mice in gastrocnemius muscle of the ischemia limb. Moreover, enhanced of angiogenesis was observed in OGN<sup>-/-</sup> mice after alkali injury. Aortic rings isolated from OGN<sup>-/-</sup> mice had stronger sprouting than those from WT ones. Tube formation was increased in HUVECs with OGN knockdown, compared with the negative control. Proliferation and migration were also enhanced in HUVECs with OGN knockdown. Meanwhile OGN overexpression suppressed cell functions of tube formation, proliferation and migration. Further study revealed that OGN depletion significantly promoted the activation of vascular endothelial growth factor receptor 2 (VEGFR2) and its downstream signaling pathways without affecting the protein level of vascular endothelial growth factor (VEGF) or VEGFR2. co-IP assay revealed that OGN associated with VEGFR2 and negatively modulated the interaction between VEGF and VEGFR2. Molecular docking revealed that Tyr196, Glu176 and Glu108 of OGN form hydrogen bonds with residues Glu140, Tyr194 and Tyr190 of VEGF2, respectively, which is the main binding affinity between OGN and VEGF2.

**Conclusions:** OGN is an important negative regulator of VEGF-VEGFR2 signaling for endothelial cell tube formation, proliferation, migration, as well as ischemia induced angiogenesis. Thus, our findings provided a novel therapeutic target of ischemic vascular diseases.

## ABSTRACTS

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Abstracts for Oral Presentation:

### OP14.

#### THE MECHANISM AND PHARMACOLOGICAL RESCUE OF BERBERINE-INDUCED hERG CHANNEL DEFICIENCY

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**Aims:** The hERG (human ether-a-go-related gene) potassium channel which plays a crucial role in cardiac repolarization is the target of diverse therapeutic drugs. Berberine (BBR) was previously found that can simultaneously block hERG and inhibit its membrane expression. However, the regulatory mechanisms of BBR effects on hERG at cell membrane level remain unknown. The present study was designed to investigate in detail how BBR decreased hERG expression on cell surface and further explore its pharmacological rescue strategies.

**Methods and Results:** Transfection and western blotting were applied to investigate the role of caveolin-1 (Cav1) and the canonical drug binding sites of hERG (a tyrosine 652 and a phenylalanine 656) in S6 domain played in BBR-induced hERG expression defect on membrane. The whole-cell patch clamp technique was used to record hERG current and action potential duration (APD). After BBR treatment, the expression of caveolin-1 reduces and the use of caveolin-1 specific siRNA (Cav1-siRNA) diminishes BBR-caused hERG inhibition. In addition, BBR obviously decreases WT-hERG expression, whereas has no significant inhibition on hERG mutation Y652A-hERG and F656V-hERG. These results indicate that caveolin-1 and residues Tyr652, Phe656 account for BBR-induced hERG defects on membrane. The data of rescue experiments show that astemizole, fexofenadine and resveratrol can rescue surface expression of hERG channel, whereas only fexofenadine and resveratrol restore hERG current. Moreover, fexofenadine and resveratrol also shorten APD prolongation disrupted by BBR.

**Conclusion:** Our study demonstrates that (1) BBR promotes mature hERG degradation via accelerating caveolin-1 turnover, and (2) aromatic tyrosine (Tyr652) and phenylalanine (Phe656) in S6 domain mediate BBR-induced reduction of hERG on membrane, which suggest that BBR reduces hERG membrane stability with multiple mechanisms. Furthermore, fexofenadine and resveratrol, shorten APD prolonged by BBR, have a potential effect of alleviating the cardiotoxicity of BBR.

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### OP15.

#### RANDOMISED CONTROLLED TRIAL OF THE EFFECT OF PHYTOSTEROL-ENRICHED LOW-FAT MILK ON LIPID PROFILE IN CHINESE

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**Objective:** Phytosterols found naturally in plants are known to reduce cholesterol absorption in the gut. The Chinese diet typically contains many vegetables and not much meat; we therefore aimed to test if phytosterols are as effective in Chinese.

**Method:** There were 221 (41 men, 180 women; age 24-79) subjects who consented to participate in the study (ClinicalTrials.gov ID: NCT02541201), the protocol of which was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. Subjects were randomised to double-blind intake of a phytosterol-enriched low-fat milk or a conventional low-fat milk for three weeks. Before every breakfast and lunch, they had a 273 ml serving. Active treatment contained 1.5 g/day phytosterol. Fasting blood samples for lipid profile were taken before and at the end of study. Body weight, waist circumference and blood pressure were also measured.

**Results:** LDL-cholesterol decreased from 3.22±0.08 to 3.06±0.08 mmol/L in the phytosterol group and increased from 3.08±0.08 to 3.20±0.08 mmol/L in controls. Comparing treatment with control, the decrease in LDL-cholesterol was 9.5±2.0% (p<0.001). There were no significant changes in HDL-cholesterol, triglycerides, body weight or blood pressure. Five subjects (2.3%; 4 in treatment group) withdrew.

**Conclusion:** Consumption of a phytosterol-enriched low-fat milk led to a significant fall in LDL-cholesterol. This can be recommended as part of a healthy diet for people with mildly elevated cholesterol levels and not at high cardiovascular risk. Statins and ezetimibe remain the treatment of choice for those with high cholesterol levels or high cardiovascular risk.

## ABSTRACTS

### Abstracts for Chaired Posters:

#### CP1.

##### **NOREPINEPHRINE INCREASES THE MONOAMINE OXIDASE A AND OXIDATIVE STRESS IN SH-SY5Y CELLS**

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**Background:** Obstructive sleep apnea (OSA) is a major breathing disorder affecting 5-7% of adult population, which increases risks for stroke and cardiovascular morbidities and mortalities. Episodes of oxygen desaturation caused by obstruction of the airway of OSA patients manifested as intermittent hypoxia (IH) significantly contributes to the excessive production of reactive oxygen species, which cause oxidative stress and inflammation in the pathogenesis of cardiovascular disease. Also, OSA patients are reportedly with elevated levels of sympathetic and norepinephrine (NE) activities. Monoamine oxidase isoform-A (MAO-A) deaminates NE and produces H<sub>2</sub>O<sub>2</sub> as a byproduct during its catalytic reaction. Additionally, 2,3-dioxygenase (IDO) is closely associated with oxidative stress and inflammation [5]. We recently observed that inhibition of MAO-A decreases the IH-induced cell death in SH-SY5Y cells.

**Aims and Objectives:** We aim to examine the role of NE in the IH-induced cell death. In this study, we test the hypothesis that NE could increase the expression of MAO-A and IDO in SH-SY5Y cells.

**Methods:** Cultured SH-SY5Y cells were used in this study, which constitutively express MAO-A but not MAO-B. Cells were treated with NE at concentrations of 0.01  $\mu$ M and 0.1  $\mu$ M to mimic the NE overspill in OSA patients. Clorgyline (10  $\mu$ M) was used as a selective inhibitor of MAO-A;

Western blotting was used to assess the level of protein expression of MAO-A, IDO and antioxidant enzyme superoxide dismutase (SOD2). Also, GSSG/GSH ratio was obtained to evaluate the level of oxidative stress.

**Results:** We found significant increased levels of MAO-A and IDO expressions in the SH-SY5Y cells treated with NE (0.01  $\mu$ M and 0.1  $\mu$ M) for 48 hours. In addition, the ratio of GSSG/GSH was significantly increased and the level of SOD2 expression was decreased by the NE treatment, suggesting an elevated level of oxidative stress. Furthermore, the effects of NE on MAO-A and IDO expression and oxidative stress were partially antagonized by 10  $\mu$ M clorgyline.

**Conclusion:** The overspill of NE in the OSA patient could worsen the IH-induced oxidative stress and inflammation via increased levels of MAO-A and IDO expression, which may play a mechanistic role in the pathophysiological response to chronic intermittent hypoxia.

#### CP2.

##### **C1q/TUMOR NECROSIS FACTOR-RELATED PROTEIN 6 ATTENUATED POST-INFARCT CARDIAC FIBROSIS BY TARGETING RHOA/MRTF-A PATHWAY**

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**Objective:** C1q/tumor necrosis factor-related protein-6 (CTRP6) is a newly identified adiponectin paralog with modulation effects on metabolism and inflammation. However, the cardiovascular function of CTRP6 remains unknown. This study aimed to investigate the effect of CTRP6 on post-infarct cardiac fibrosis and its underlying mechanisms.

**Methods:** Experimental myocardial infarction (MI) was induced by left anterior descending coronary artery ligation in Sprague-Dawley rats. Picric acid Sirius red staining was used to determine cardiac fibrosis. Quantitative-PCR, western blotting and immunohistochemical staining were used to detect the expression of CTRP6 and pro-fibrotic molecules. Cardiac fibroblasts (CFs) isolated from adult rat heart were cultured to investigate transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1)-induced myofibroblast differentiation and its underlying mechanisms.

**Results:** Myocardial expression of CTRP6 was significantly decreased post-MI. CTRP6 supplement by intramyocardial injection of adenovirus alleviated cardiac fibrosis, and inhibited myofibroblast differentiation as well as the expression of collagen I, collagen III, and connective tissue growth factor post-MI. Treatment of CFs with recombinant CTRP6 significantly inhibited

transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1)-induced myofibroblast differentiation. CTRP6 increased the phosphorylation of AMP-activated protein kinase (AMPK) and Akt in cultured CFs and post-MI hearts. Pretreatment with adenine 9- $\beta$ -D-arabinofuranoside (AraA), an AMPK inhibitor, or LY294002, a phosphatidylinositol-3-kinase (PI3K) inhibitor, abolished the protective effect of CTRP6 on TGF- $\beta$ 1-induced profibrotic response. Furthermore, CTRP6 significantly decreased TGF- $\beta$ 1-induced RhoA activation and myocardin-related transcription factor-A (MRTF-A) nuclear translocation, whereas had no effect on TGF- $\beta$ 1-induced Smad3 phosphorylation and nuclear translocation, these effects of CTRP6 were blocked by AMPK or Akt inhibition.

**Conclusion:** CTRP6 attenuates cardiac fibrosis via inhibiting myofibroblasts differentiation post-MI. AMPK and Akt activation are responsible for the CTRP6-mediated anti-fibrotic effect by targeting RhoA/MRTF-A pathway.

**ABSTRACTS**

Abstracts for Chaired Posters:

**CP3.**

**MiR-33 MEDIATE VEIN GRAFT NEOINTIMAL HYPERPLASIA AND VENOUS SMCs PROLIFERATION UNDER CYCLIC STRETCH**

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**Objectives:** Autologous saphenous veins are often grafted to patients with multivessel-coronary-artery disease. After grafted to replace a diseased artery, the vein is exposed to substantially higher pressure and higher mechanical stretch inevitably, which contributes to neointimal hyperplasia. It has been proved that proliferation of venous smooth muscle cells (SMCs), induced by arterial cyclic stretch is crucial for neointimal hyperplasia and narrowing of the vessel lumen. MiR-33 has been shown to regulate cell proliferation, and cell cycle progression. However, the role of miR-33 in neointimal hyperplasia of grafted vein caused by arterial cyclic stretch remains unknown.

**Methods:** Vein grafts were generated by "cuff" technique, and the jugular vein on the right side was used as a control. Paraffin sections of grafted veins stained with Elastin-Van Gieson to detect neointimal hyperplasia; RT-PCR was carried out to disclose miR-33 expression levels; and western blotting was used to examine expressions of BMP-3, Smad2, p-Smad2, Smad5 and p-Smad5 in grafted veins. FX 4000 *in vitro* Strain Union was used to apply 10% cyclic stretch to cultured venous SMCs, which mimics the arterial environment. Web-accessible databases and dual luciferase reporter assay were used to detect the target of miR-33. The inhibitor and mimics of miR-33, and the specific siRNA and recombinant protein of BMP-3 were used respectively to demonstrate the effects of miR-33 and Bmp3 on proliferation of venous

SMCs. Furthermore, perivascular multi-point injection of agomiR-33 was performed to verify the function of miR-33 *in vivo*.

**Results:** Neointima of grafted veins was notably increased after 4-week grafts. The expression of miR-33 was decreased in 1-week, 2-week and 4-week grafted veins, and the remarkable decrease was detected in 2-week group. The expressions of BMP-3, p-Smad5 and p-Smad2 were all increased in grafted veins. The similar results were found in venous SMCs subjected to 10% (arterial) cyclic stretch *in vitro*. 10%-cyclic stretch significantly promoted proliferation of venous SMCs, declined expression of miR-33, and elevated expressions of BMP-3, p-Smad5 and p-Smad2. Accordingly, miR-33 mimics repressed, while inhibitor increased proliferation of venous SMCs. MiR-33 directly targeted to BMP-3 and regulated its expression. Recombinant BMP-3 increased proliferation of SMCs and expressions of p-Smad5, p-Smad2; while BMP-3 siRNA had the opposite effect. Furthermore, perivascular multi-point injection of agomiR-33 *in vivo* disclosed that agomiR-33 not only attenuated expressions of BMP-3, p-Smad2, and p-Smad5, but also decelerated neointimal formation of grafted veins.

**Conclusions:** MiR-33/BMP-3/Smad signaling pathway regulates proliferation of venous SMCs, which is induced by arterial cyclic stretch. MiR-33 is a target to attenuate neointimal hyperplasia of grafted vessel, may has potential clinical application. (This research was supported by grants from the National Natural Science Foundation of China, Nos. 11232010, 11222223, 11172178)

**CP4.**

**OBESITY IS RELATED TO IMMUNOGLOBULIN E**

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Immunoglobulin E (IgE), a measurement of allergy, plays an essential role in immunity and hypersensitivity. In order to know whether such an association can be also found in adults, we explored data on 1933 men and 1802 women in the National Health and Nutrition Examination Survey (NHANES) 2005-2006. We included participants aged above 20 years who had valid data on body mass index (BMI) and serum IgE level. Pregnant women were excluded. We found in adults that obesity (OR=2.63, 95%CI=1.62-4.27) was associated with higher odds of asthma episodes, compared with normal weight. Obesity, but not overweight, was also associated with allergic symptoms (OR=1.27, 95%CI=1.08-1.49). Elevated serum levels of liver enzymes (all  $P \leq 0.001$ ) and C-reactive protein ( $P < 0.001$ ) were associated with an increment in BMI. IgE level increased with BMI; it increased progressively from 33.70 kU/L in the bottom quartile to 45.62 kU/L in the top quartile of BMI. The results also showed an association between BMI and IgE level in the overall population ( $p=0.074$ ,  $P=0.002$ ) and among women ( $p=0.088$ ,  $P=0.006$ ). After adjustment for race/ethnicity, age, sex (except in sex-specific analysis), physical activity, alcohol consumption and smoking, the association between BMI and IgE level in the overall population ( $p=0.070$ ,  $P=0.005$ ) and women ( $p=0.074$ ,  $P=0.013$ ) remained significant. After further adjustment for C-reactive protein, serum levels of liver enzymes and correlations among serum levels of liver

enzymes, the association was attenuated but remained significant in the overall population ( $p=0.047$ ,  $P=0.044$ ) and women ( $p=0.059$ ,  $P=0.043$ ). The attenuation in the association between BMI and IgE after controlling for liver enzymes and C-reactive protein suggests that hepatic inflammation may account for part of the association. Although allergy may not be the most consequential health risk for adult obesity, it does provide additional motivation to undertake the difficult challenge of fighting obesity. If there is a causal link between obesity and elevated IgE level, efforts to control body weight may also be beneficial for the associated allergic diseases.

## ABSTRACTS

Abstracts for Chaired Posters:

### CP5.

#### CHRONIC INTERMITTENT HYPOXIA EXACERBATES DEPRESSIVE-LIKE BEHAVIORS IN HIGH FAT DIET-FED MICE

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**Background:** There is a high prevalence of obstructive sleep apnea (OSA) in the obese population. Depressive symptoms are commonly manifested in obese and OSA patients as well as in animal models, but the pathophysiological mechanism remains elusive. Neuroinflammation plays an important role in major depressive disorder (MDD) with an activation of brain indoleamine 2,3, dioxygenase (IDO-1), a catabolic enzyme of tryptophan and serotonin, which depletes serotonin availability. We have shown that chronic intermittent hypoxia (CIH) manifested as episodic oxygen desaturation in severe OSA condition induces oxidative stress and inflammation in the rat hippocampus. Significant neuroinflammation was also observed in obese mice.

**Aims and hypothesis:** We aim to examine whether CIH exacerbates high fat diet (HFD)-induced depressive-like behaviors. It is hypothesized that CIH aggravates HFD-induced depressive-like behaviors with an increased IDO-1 expression and activity mediated by oxidative stress and inflammation, leading to serotonin deficiency and apoptosis in the mice hippocampus.

**Methods:** Adult male C57BL/6N mice (4-week old after weaning) were either fed with HFD (45% fat) or regular diet for 13 weeks. The mice were treated with intermittent hypoxia (with inspired oxygen levels altering between

21-5% per min for 8 hours per day for a week, Apnea-Hyponea Index at 60) or in room air (normoxic control) starting at the 9th week for 4 weeks. Forced swimming test and sucrose preference test were employed to assess the behavioral despair and hedonic status of the mice. Hippocampi were harvested for the measurement of IDO-1 activity, oxidative stress, inflammatory and apoptotic markers assessed by Western blot and enzyme-linked immunosorbent assay.

**Results:** The immobility time was increased in the forced swimming test and the percentage of sucrose consumption was decreased in the sucrose preference test in the HFD or hypoxic group when compared to the normoxic control; these changes were doubled in the mice co-treated with HFD and hypoxia. In addition, the level of lipid peroxidation was increased and the protein expressions of antioxidant enzymes (SOD-2, GPx-1) were decreased in the HFD or hypoxic group and these changes were significantly augmented in the co-treated mice. Moreover, inflammatory mediators (TNF- $\alpha$ , IL-1 $\beta$  and IL-6) and apoptotic markers (cleaved caspase-3, cleaved PARP-1) were increased in the HFD or hypoxic group and were significantly increased in the co-treated group. Furthermore, levels of the IDO expression and activity (ratio of KYN/TRP) were increased in the HFD or hypoxic group and were significantly elevated in the co-treated mice.

**Conclusion:** CIH treatment aggravates depressive-like behaviors in HFD-fed mice with significant increased levels of oxidative stress, neuroinflammation and IDO-1 activity, which could augment the depletion of serotonin and apoptosis in the hippocampus.

### CP6.

#### IMMEDIATE EARLY GENE X1 (IEX-1) AMELIORATES VASCULAR CALCIFICATION THROUGH INHIBITING MITOCHONDRIAL REACTIVE OXYGEN SPECIES PRODUCTION

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**Introduction:** Vascular calcification (VC) is the major risk factor for cardiovascular mortality in chronic renal failure (CRF) patients, but the pathogenesis remains largely unknown and effective therapeutic targets are urgent to be explored. The Immediate Early Gene X-1 (IEX-1) plays a role in controlling cellular growth and differentiation. As IEX-1 expressed in the cartilage tissue and it can modulate protein phosphatase, we aimed to study whether IEX-1 affects VC.

**Methods and Results:** IEX-1 inhibited VC *in vitro* and *in vivo*. In cultured BASMCs, compared with cells overexpressing GFP then treated with Pi, the cells overexpressing IEX-1 then treated with Pi had decreased calcium content, BALP activity, Ca<sup>2+</sup> incorporation rate and osteogenic gene expression. Alizarin Red staining result confirmed the inhibitory effect of IEX-1 on VC. In contrast, BASMCs knock-down of IEX-1 expression had the opposite effect on VC. In adenine-induced CRF rat model, aortic calcium content also decreased in rats treated with adenovirus overexpressing IEX-1, when compared to CRF rats treated with pluronic gel as a control. We then demonstrated that IEX-1 expression was down-regulated in calcified aorta and BASMCs treated with Pi. Further, IEX-1 overexpression inhibited

Pi-induced osteogenic gene expression and reversed Pi-induced smooth muscle cell lineage markers in BASMCs. In our previous study, we demonstrated that mitochondrial reactive oxygen species (ROS)-activated nuclear factor- $\kappa$ B promotes Pi-induced calcification *in vitro* and *in vivo*. Thus we tested whether IEX-1 reduced VC via modulating mitochondrial ROS. ROS counting showed that IEX-1 overexpression significantly reduced H<sub>2</sub>O<sub>2</sub> activated mitochondrial ROS production, this result was further confirmed by Mito tracker staining, in which BASMCs overexpressing IEX-1 had significantly lower fluorescence intensity. Finally, western blotting showed that IEX-1 overexpression activated SOD2, but not SOD1 expression, in BASMCs.

**Conclusions:** The results demonstrated that IEX-1 blocked mitochondrial ROS production, causing ameliorated VC *in vitro* and *in vivo*.

## ABSTRACTS

### Abstracts for Chaired Posters:

#### CP7.

##### EFFECTS OF CARDIAC RESYNCHRONIZATION THERAPY ON DISPERSION OF REPOLARIZATION AND VENTRICULAR ARRHYTHMIA

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**Objective:** The prolongation of TpTe is the independent risk factor of chronic heart failure mortality. The purpose of this study was to determine the impact of cardiac resynchronization therapy (CRT) on dispersion of repolarization and ventricular arrhythmia in heart failure patients.

**Methods:** A total of 86 consecutive patients who underwent their first CRT implantation at our center were studied. The electrocardiogram and echocardiogram were collected at pre-operation, immediate post operation, and after 1 year follow-up respectively and were analyzed for QRS duration, QTc interval, TpTe. Ventricular arrhythmia data was obtained through the pacemaker records, including premature ventricular beats (PVCs) and PVC runs. Response was defined as absolute improvement in LVEF 10% from baseline. Changes of QRS duration, QTc interval, TpTe at different period and ventricular arrhythmias were compared between responders and non-responders.

**Results:** 43 patients as responders and 43 patients as non-responders, without any baseline and medication difference between groups. Although no statistical significance, subgroup analysis revealed obvious decreased TpTe in responders. After 1 year, QRS duration and TpTe were shorter than immediate post operation and pre-operation in responders (both  $P < 0.05$ ), while no significant differences were found in non-responders. After 1 year followup,

responders suffered less PVCs and PVC runs than nonresponders (both  $P < 0.01$ ). Multiple regression analysis demonstrated that postoperation TpTe as an independent predictor of Ig (PVCs) ( $B = 0.143$ ,  $OR: 1.154$ ,  $P = 0.001$ ) and Ig (PVC runs) ( $B = 0.122$ ,  $OR: 1.047$ ,  $P = 0.001$ ).

**Conclusions:** In heart failure patients who received CRT, response for this therapy can reduce QTc and TpTe, suggesting that CRT promotes clinically significant reverse electrical and mechanical remodelling. Increased TpTe is associated with increased incidence of ventricular arrhythmias in CRT.

#### CP8.

##### EXOGENOUS H<sub>2</sub>S INHIBITS HIGH-GLUCOSE-INDUCED APOPTOSIS VIA INDUCING PROTECTIVE AUTOPHAGY IN H9C2 CELLS

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**Objective:** Diabetic cardiomyopathy (DCM) has become a major cause of diabetes-related morbidity and mortality. Cardiac oxidative stress is associated with DCM, which is triggered by hyperglycemia. Increasing evidences have proved that hydrogen sulfide (H<sub>2</sub>S) fulfills a positive role in regulating diabetic myocardial injury. Autophagy is initiated by the formation of autophagosomes which nonselectively sequester long-lived proteins and cytoplasmic organelles such as mitochondria, endoplasmic reticulum and ribosomes. While overactivation of autophagy is incompatible with cell growth, this self-cannibalistic process may protect cells from various kinds of stress. In relation to its regulation, the AMP - dependent kinase (AMPK) and mammalian target of rapamycin (mTOR) pathways play important roles in the control of autophagy. In the present study, we demonstrated the protective autophagy by H<sub>2</sub>S in H9C2 cells with high glucose culture and the involvement of AMPK and mTOR signaling.

**Methods:** The present study was undertaken to investigate the effects of H<sub>2</sub>S on cell injury induced by high glucose in H9C2 cell line. H9C2 cells were incubated in normal glucose (5.5 mM), 25 mM, and 40 mM glucose for 24 h to mimic the hyperglycemia in DCM *in vitro*. Then we added 50, 100, and 200  $\mu$ M GYY4137, as donor of H<sub>2</sub>S, and measured the cell viability by MTT assay. 0.5 mM 5-amino-4-imidazole-carboxamide riboside (AICAR, an AMPK activator) and 1 mM Compound C (CC, an AMPK inhibitor) were used to identify whether the AMPK/mTOR signal pathway was involved in

H<sub>2</sub>S-mediated cardioprotection. We tested ultrastructural changes in the cells and the expression of autophagy-associated proteins in H9C2 cells. Treating the cells with H<sub>2</sub>S for 24 h or 48 h was used to observe the formation of autophagic vacuoles as determined by immunofluorescent staining for LC3-II. AMPK was lowered using target-specific siRNA molecules (Cell Signaling Technology, USA)

**Results:** We demonstrated that HG decreased cell viability. 25 mM HG treatment for 24 h was chosen as our model group for further study. 100  $\mu$ M GYY4137 treatments significantly attenuated HG-induced cell viability decrement; AICAR had similar effects to H<sub>2</sub>S treatment while CC attenuated H<sub>2</sub>S-mediated cardioprotection. Ultrastructural analysis by electron microscopy revealed the formation of autophagosome or secondary lysosomes with the residual digested material in H<sub>2</sub>S-treated H9C2. H<sub>2</sub>S also prevented high glucose-mediated changes in Bcl-2/Bax levels, mitochondrial membrane potential ( $\Delta\psi_m$ ) dissipation, cytochrome c release, caspase-3 activation. Moreover, exposure to H<sub>2</sub>S led to features characteristic of autophagy, including increased formation of LC3-II autophagic vacuoles and acidic vesicular organelles as determined by immunofluorescence and acridine orange staining, respectively. LC3-II levels were increased in high glucose group after treatment with H<sub>2</sub>S for 48 h. The expression of Beclin-1, another protein involved in macroautophagy, was also altered. Further mechanistic investigation revealed that H<sub>2</sub>S stimulated the phosphorylation of AMP-activated protein kinase (AMPK) and inhibited the phosphorylation of mammalian target of rapamycin (mTOR) compared with the HG model group while Compound C attenuated H<sub>2</sub>S-mediated AMPK phosphorylation increase and mTOR phosphorylation decrement.

**Conclusion:** Collectively, we demonstrate that H<sub>2</sub>S likely protects against HG-induced cytotoxicity by inducing protective autophagy via the AMPK pathway in H9C2 cells.

## ABSTRACTS

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### Abstracts for Chaired Posters:

#### CP9.

##### AUTOANTIBODIES AGAINST $\beta_1$ -ADRENOCEPTOR INDUCE ISLET INJURY THROUGH T LYMPHOCYTES

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**Objective:** To investigate the change of pancreas islet and blood glucose after the administration of  $\beta_1$ -AA and determine the role of T lymphocytes in this mechanism.

**Methods:**  $\beta_1$ -AA active immunization rats model was established, islet was collected at 24th, 28th, 32rd, 36th weeks and fixed in 10% formaldehyde and embedded in paraffin. And then the tissue sections were stained with hematoxylin and eosin (HE).  $\beta_1$ -AA passive immunization BALB/c mice/nude mice model were built up to detect the change of blood glucose by IPGTT. T lymphocytes were treated by  $\beta_1$ -AA alone or with metoprolol or  $\beta_1$ -AR-ECII for 6, 12, 24, 48 hours, then the supernatant of each group in each time point was added to NIT-1 $\beta$  cell line, after 48 hours incubation LDH and insulin level was detected.

**Results:** (1) Significant irregular structure change was witnessed at 28th week group compared with vehicle. The edge of islet is unclear, area of islet was decreased while T lymphocytes infiltration. (2) Blood glucose in  $\beta_1$ -AA group was significantly increased compared with vehicle (8th week:  $13.47 \pm 3.85$  v.s.  $8.3 \pm 1.39$  mmol/L, \*\* $P < 0.01$ ), but glucose level in passive immunization nude mice model had no apparent difference ( $P > 0.05$ ). (3) Insulin of NIT-1 cells was markedly decreased after the administration of T cells supernatant which treated by  $\beta_1$ -AA compared with vehicle (\*\* $P < 0.01$ ). And metoprolol can partially counteracted the effect of  $\beta_1$ -AA on the reduction of insulin

(\*\* $P < 0.01$ ). (4) LDH level in response to T cells supernatant was increased (\*\* $P < 0.01$ ) in the time-dependent form. While metoprolol and  $\beta_1$ -AR-ECII could suppress this impairment (6 hour: \* $P < 0.05$ , 48 hour: \*\*\* $P < 0.01$ ).

**Conclusions:**  $\beta_1$ -AA long term existence impair pancreas islet structure and increase blood glucose, and the change of insulin secretion and injury in  $\beta$  cell caused by  $\beta_1$ -AA may probably through T lymphocytes.

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#### CP10.

##### EXOGENOUS HYDROGEN SULFIDE AMELIORATES MITOCHONDRIAL FUNCTION BY UP-REGULATING Sirt3 IN EXPERIMENTAL DIABETES MELLITUS MODELS

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**Background:** Sirt3 is a member of the sirtuin family of NAD<sup>+</sup>-dependent protein deacetylases that is localized in mitochondria and regulates mitochondrial function. Mitochondrial dysfunction is a key contributing factor in type2 diabetes. The endogenous gasotransmitter hydrogen sulfide (H<sub>2</sub>S) can act in a cytoprotective manner.

**Methods:** We tested the mitochondrial respiratory rate, respiratory chain complex activity, the expression of Sirt3 and total acetylation level in *db/db* mice and treatment of NaHS in *db/db* mice. Neonatal cardiomyocytes were induced by hyperlipidemia and high glucose. We tested the effects of exogenous H<sub>2</sub>S on reactive oxygen species (ROS) production and mitochondrial membrane potential by MitoSOX Red and JC-1.

**Result:** In *db/db* mice, cardiac tissue developed lipid accumulation, mitochondrial dysfunction, the low expression of Sirt3 and hyperacetylation. The function of mitochondria was improved in *db/db* mice with treatment of exogenous H<sub>2</sub>S, the level of acetylation was decreased and the expression of Sirt3 was recovered after treatment of H<sub>2</sub>S.

**Conclusion:** We conclude that exogenous H<sub>2</sub>S plays a critical role in diabetes through up-regulating Sirt3 to improve cardiac mitochondrial function.

## ABSTRACTS

### Abstracts for Posters:

#### P01.

##### **CTRP3 ATTENUATES POST-INFARCT CARDIAC FIBROSIS BY TARGETING Smad3 ACTIVATION AND INHIBITING MYOFIBROBLAST DIFFERENTIATION**

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**Objectives:** C1q/tumor necrosis factor-related protein-3 (CTRP3) is a novel adipokine with modulation effects on metabolism, inflammation, and cardiovascular system. This study aimed to investigate the effect of CTRP3 on cardiac fibrosis and its underlying mechanism.

**Methods:** Rat myocardial infarction (MI) model was established by left anterior descending coronary artery ligation. Masson' trichrome staining was used to detect cardiac fibrosis. Adult rat cardiac fibroblasts (CFs) were cultured. Real-time PCR, western blotting and immunofluorescence were used to detect mRNA and protein expression of fibrosis-related markers.

**Results:** The myocardial expression of CTRP3 was significantly decreased post-MI. Adenovirus-delivered CTRP3 supplement attenuated myocardial hypertrophy, inhibited interstitial fibrosis, and decreased the number of myofibroblasts post-MI. In cultured CFs, CTRP3 inhibited whereas CTRP3 small interfering RNA (siRNA) facilitated the expression of  $\alpha$ -SMA, CTGF, collagen I, and collagen III induced by TGF- $\beta$ 1. CTRP3 also attenuated TGF- $\beta$ 1-induced Smad3 phosphorylation, nuclear translocation, and interaction with p300. CTRP3 increased the phosphorylation of AMP-activated protein kinase (AMPK) in both rat hearts and the cultured CFs. Adenine 9- $\beta$ -D-arabinofuranoside (AraA), an AMPK inhibitor, abolished the protective effect of CTRP3 against TGF- $\beta$ 1-induced profibrotic response and Smad3 activation.

**Conclusions:** CTRP3 attenuates cardiac fibrosis by inhibiting myofibroblast differentiation and the subsequent extracellular matrix production. AMPK is required for the anti-fibrotic effect of CTRP3 through targeting Smad3 activation and inhibiting myofibroblast differentiation.

#### P02.

##### **EXOGENOUS HYDROGEN SULFIDE RESTORES CARDIOPROTECTION OF ISCHEMIC POST-CONDITIONING VIA INHIBITION OF mPTP OPENING IN THE AGING CARDIOMYOCYTES**

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**Aims:** The physiological and pathological roles of hydrogen sulfide (H<sub>2</sub>S) in the regulation of cardiovascular functions have been recognized. H<sub>2</sub>S protects against the hypoxia/reoxygenation (H/R)-induced injury and apoptosis of cardiomyocytes, and ischemic post-conditioning (PC) plays an important role in cardioprotection from H/R injury in neonatal cardiomyocytes but not in aging cardiomyocytes. Whether H<sub>2</sub>S is involved in the recovery of PC-induced cardioprotection in aging cardiomyocytes is unclear.

**Methods:** The aging of cardiomyocytes was identified via detection of SA  $\beta$ -gal activity, cell division index and AGEs contents. The apoptosis was assessed by flow cytometry and Hoechst 33342 staining. The gene expression was analysed by western blotting and Real-Time PCR. The mitochondrial membrane potential and mitochondrial permeability transition pore (mPTP) opening were examined with JC-1, and incubation of calcein-AM and cobalt chloride, respectively, using laser con focal microscopy.

**Results:** We found that both H/R and PC decreased cystathionine- $\gamma$ -lyase (CSE) expression and the production rate of H<sub>2</sub>S. Supplementation of NaHS protected against H/R-induced apoptosis, the expression of cleaved caspase-3 and cleaved caspase-9, the release of cytochrome c (Cyt c), and mPTP opening. The addition of NaHS also counteracted the reduction of cell viability caused by H/R and increased the phosphorylation of ERK1/2, PI3K, Akt, GSK-3 $\beta$  and mitochondrial membrane potential. Additionally, NaHS increased Bcl-2 expression, promoted PKC- $\epsilon$  translocation to the cell membrane, and activated mitochondrial ATP-sensitive K channels (mitoK<sub>ATP</sub>). PC alone did not provide cardioprotection in H/R-treated aging cardiomyocytes, which was significantly restored by the supplementation of NaHS.

**Conclusions:** Our results suggest that exogenous H<sub>2</sub>S restores PC-induced cardioprotection via the inhibition of mPTP opening by the activation of the ERK1/2-GSK-3 $\beta$ , PI3K-Akt-GSK-3 $\beta$  and PKC- $\epsilon$ -mitoK<sub>ATP</sub> pathways in aging cardiomyocytes. These findings provide a novel target for the treatment of aging ischemic cardiomyopathy.

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## ABSTRACTS

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Abstracts for Posters:

### P03.

#### THE PROTECTIVE EFFECT OF N-ACETYLCYSTEINE ON ACUTE LUNG INJURY

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**Objectives:** To examine the pharmacological effects of N-Acetylcysteine (NAC) in a two-hit model of acid-aspiration induced inflammation followed by ventilator-induced lung injury (VILI).

**Methods:** Rats received intra-tracheal instillation of hydrochloric acid as a first hit to induce systemic inflammation. They were then randomized to receive mechanical ventilation (MV) as a second hit, with a high tidal volume (TV) of 15 mL/kg and zero positive end-expiratory (PEEP), or a protective strategy of low TV of 6 mL/kg with a PEEP of 5 cm H<sub>2</sub>O, with a fraction of inspired oxygen (FiO<sub>2</sub>) of 40% during the 4-hour experimental period. NAC (150 mg/kg) or placebo was administered intravenously before different MV strategies for 4 hours. The following data were collected: blood gases, mechanics (static compliance and respiratory elastance), lung edema, extended lung destruction (lung injury scores and lung histology), neutrophil recruitment in the lung and cytokine production.

**Results:** The hemodynamics including blood pressure and heart rates were similar at baseline and were not different in all animals during the study. Compared with placebo treated rats, NAC administration attenuated lung injury, with improved oxygenation, preserved lung mechanics and destruction and diminished inflammation.

**Conclusions:** Based on the two-hit model, NAC administration improved the physiologic and biologic profiles of this experimental model of VILI. Clinician may consider about the rescue effect of NAC to apply in further clinical practice.

### P04.

#### ACTIVATED EFFECT OF $\beta$ -ESTROGEN ON BK<sub>Ca</sub> IN MESENTERIC ARTERY SMOOTH MUSCLE CELLS OF PRE-MENOPAUSE AND POST-MENOPAUSE WOMEN

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**Objective:** Epidemiologic studies indicate that gender differences exist in essential hypertension. Premenopausal women have a much reduced incidence of hypertension compared with age-matched men. However, post-menopausal women develop increased incidence from hypertension. Laboratory researches suggest that estrogen has beneficial cardiovascular effects through their ability to modulate their function; however, these mechanisms remain incompletely understood.

**Methods:** To apply acute enzyme method to isolate women mesentery artery smooth muscle cells and record large-conductance Ca<sup>2+</sup>-activated potassium channel currents using perforate whole cell patch technique, and to examine the effects of  $\beta$ -E<sub>2</sub> on BK<sub>Ca</sub> of women mesenteric artery vascular smooth muscle cells (VSMCs) of pre-menopause women non-hypertension group (PNH), post-menopause women non-hypertension group (NH) and post-menopause women essential hypertension group (EH), and to explore the relation among  $\beta$ -E<sub>2</sub>, BK<sub>Ca</sub> and women essential hypertension, and to identify that the mechanisms of effect of  $\beta$ -E<sub>2</sub> on pre-menopause and post-menopause women essential hypertension.

**Results:** (1) Comparisons of effects of estrogen on BK<sub>Ca</sub> macroscopic currents between PNH, NH and EH groups: (i) At +60 mV, the current densities of BK<sub>Ca</sub> of PNH group increased 0.97±0.40 times after adding 100  $\mu$ M $\beta$ -E<sub>2</sub>. (ii) At +60 mV, the current densities of BK<sub>Ca</sub> of NH group increased 0.75±0.47 times after adding 100  $\mu$ M $\beta$ -E<sub>2</sub>. (iii) At +60 mV, the current densities of BK<sub>Ca</sub> of EH group increased 0.60±0.33 times after adding 100  $\mu$ M $\beta$ -E<sub>2</sub>. (2) Effects of ICI 182780 on BK<sub>Ca</sub> of women mesenteric artery smooth muscle cells. At +60 mV, the current densities of BK<sub>Ca</sub> of women mesenteric artery smooth muscle cells could increase from 15.89±4.87 pA/pF to 27.88±6.75 pA/pF ( $P$ <0.01, n=23). There was inhibitory effect on BK<sub>Ca</sub> after adding ICI 182780 subsequently. The current densities of BK<sub>Ca</sub> of women mesenteric artery smooth muscle cells could decrease to 20.15±6.21 pA/pF ( $P$ <0.05, n=23).

**Conclusions:** (1)  $\beta$ -E<sub>2</sub> could activate BK<sub>Ca</sub> macroscopic currents on PNH group, NH group, EH group. Compared with PNH group, the effect of estrogen on BK<sub>Ca</sub> in NH was lower. That suggest that  $\beta$ -E<sub>2</sub> play an important role in pre-menopause women' heart protection. Compared with NH group, the effect of estrogen on BK<sub>Ca</sub> in EH was lower. These data suggest that the responsiveness of effect of estrogen was lower on blood vessel after hypertension and menopause was a factor of happening of hypertension; (2)  $\beta$ -E<sub>2</sub> could make women mesenteric artery relax, and the effect could partly be inhibited by ICI 182780, so BK<sub>Ca</sub> and ER were involved mainly in the mechanisms of E-induced relaxation in mesenteric artery.

## ABSTRACTS

### Abstracts for Posters:

#### P05.

##### CIC-3 DEFICIENCY PREVENTS ATHEROSCLEROTIC LESION DEVELOPMENT IN ApoE<sup>-/-</sup> MICE

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**Objectives:** Recent evidence suggested that CIC-3, encoding Cl<sup>-</sup> channel or Cl<sup>-</sup>/H<sup>+</sup> 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.

**Methods and Results:** After a 16-week western-type high-fat diet, the CIC-3<sup>-/-</sup> ApoE<sup>-/-</sup> mice developed widespread atherosclerotic lesions in aorta. However, the lesion size was significantly reduced in aorta of CIC-3<sup>-/-</sup> ApoE<sup>-/-</sup> mice. Compared with the CIC-3<sup>+/+</sup> controls, there was significantly decreased ox-LDL binding and uptake in isolated peritoneal macrophages from CIC-3<sup>-/-</sup> mice. Moreover, the expression of scavenger receptor SR-A, but not CD36, was significantly decreased in both CIC-3<sup>-/-</sup> peritoneal macrophages and aortic lesions from CIC-3<sup>-/-</sup> ApoE<sup>-/-</sup> mice. These findings were further confirmed in ox-LDL-treated RAW264.7 macrophages, which showed that silencing of CIC-3 inhibited SR-A expression, ox-LDL accumulation and foam cell formation, whereas overexpression of CIC-3 produced the opposite effects. In addition, CIC-3 siRNA significantly inhibited, whereas CIC-3 overexpression 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<sup>-</sup>]<sub>i</sub> by low Cl<sup>-</sup> solution.

**Conclusions:** 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.

#### P07.

##### THE ROLE OF TRPV4 IN IMPAIRED FLOW-INDUCED DILATION OF MESENTERIC ARTERIES WITH ADVANCING AGE

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Shear stress induced by blood flow acts through the endothelial cells (ECs) to regulate vascular tone and diameter. The flow-stimulated intracellular Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>) rise in ECs serves as an important early event in flow-induced vessel dilation. Several studies have demonstrated an involvement of the transient receptor potential vanilloid subtype 4 (TRPV4), a Ca<sup>2+</sup> permeable cation channel, in facilitating this Ca<sup>2+</sup> increase. In the present study, pressure myography was utilized to show that both flow- and TRPV4 activator 4αPDD-induced vessel dilation of mesenteric arteries significantly decreased in aged rats compared to young rats. Flow- and 4αPDD-induced [Ca<sup>2+</sup>]<sub>i</sub> rise also declined in primary cultured mesenteric artery endothelial cells (MAECs) from aged rats. Western blotting and immunostaining studies showed a decrease in TRPV4 expression levels in MAECs of aged rats relative to young rats. We employed a lentiviral construct carrying *TRPV4* to enhance TRPV4 expression level. It is very interesting that flow- and 4αPDD-induced dilation was significantly restored in mesenteric arteries isolated from lenti-TRPV4-treated rats than in those from empty lentivector-treated rats. These results suggest that the TRPV4-mediated [Ca<sup>2+</sup>]<sub>i</sub> rise in ECs is critical to the cell-dependent flow response. The impairment of TRPV4-mediated Ca<sup>2+</sup> signal contributes to endothelial dysfunction with advancing age in rat mesenteric artery.

#### P06.

##### THE EFFECTS OF HEDAN TABLET ON OXIDATIVE STRESS AND ATHEROSCLEROSIS OF ApoE<sup>-/-</sup> MICE

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**Aim:** To observe the effects of Hedan tablet on ox-LDL, NO, IL-6, AMCP-1, AICAM-1 and expression of ICAM-1 of ApoE<sup>-/-</sup> mice.

**Methods:** Fifty ApoE<sup>-/-</sup> mice were randomly divided into normal control group, model group, two Hedan tablet treated groups and simvastatin treated group. Mice in control group were given ordinary chow, and mice in other groups were given high cholesterol diet. After 12 weeks, mice were sacrificed to observe ox-LDL, NO, AIL-6, MCP-1, ICAM-1 concentration. Expression of ICAM-1 in aorta were observed by immunohistochemistry.

**Results:** Compared with control group, ox-LDL, IL-6, MCP-1, ICAM-1 were significantly increased, NO was significantly decreased, expression of ICAM-1 in aorta was up-regulated in model group ( $P < 0.01$ ). Compared with model group, ox-LDL, IL-6, MCP-1, ICAM-1 were significantly decreased, NO was significantly decreased, expression of ICAM-1 in aorta was down-regulated in Hedan tablet treated groups and simvastatin treated group ( $P < 0.01$ ,  $P < 0.05$ ).

**Conclusion:** Hedan tablet can reduce ox-LDL, IL-6, MCP-1, ICAM-1, elevate NO and down-regulate expression of ICAM-1 in aorta of ApoE<sup>-/-</sup> mice, and then inhibit the formation of atherosclerosis of ApoE<sup>-/-</sup> mice.

#### P08.

##### CARDIOVASCULAR HEALTH IN CHILDREN WITH DEVELOPMENTAL COORDINATION DISORDER

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**Objectives:** Children with developmental coordination disorder participate less frequently in physical activities and have higher weight status than their typically-developing peers. These factors might compromise their cardiovascular health. This study aimed to compare the peripheral arterial resistance, arterial elasticity and resting heart rate and blood pressure between children with and without DCD.

**Methods:** Thirty-seven children with DCD (mean age±SD=7.7±1.4 years) and 50 age- and sex-matched children with typical development (mean age±SD=7.4±1.4 years) participated in the study. Peripheral (radial artery) arterial resistance and arterial elasticity were measured by a Doppler ultrasound machine and an arterial hardness and blood pressure monitor, respectively. Resting heart rate and blood pressure were also assessed using the same blood pressure monitor.

**Results:** Independent t test results revealed that peripheral arterial resistance (ultrasonic resistance index), arterial elasticity (H-value) and resting heart rate (in bpm) and blood pressure (in mmHg) were comparable between children with DCD and those with typical development (all  $p > 0.05$ ).

**Conclusions:** It seems that cardiovascular health of our DCD participants was as good as the typically-developing children. Further study may explore the changes in cardiovascular indices during exercise testing (i.e., cardiovascular fitness) and the functional aerobic capacity of children with DCD.

## ABSTRACTS

### Abstracts for Posters:

#### P09.

##### **CARTILAGE INTERMEDIATE LAYER PROTEIN-1 ATTENUATES PRESSURE OVERLOAD-INDUCED CARDIAC FIBROSIS BY TARGETING TGF- $\beta$ 1**

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**Objective:** Cartilage intermediate layer protein-1 (CILP-1), a monomeric extracellular matrix glycoprotein expressed mainly in the middle zones of articular cartilage, interacts directly with transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1). Recent studies showed that CILP-1 was upregulated in the heart tissue following cardiac ischemia reperfusion injury. This study explored the effect of CILP-1 on myocardial interstitial fibrosis and revealed the possible molecular mechanism.

**Methods:** Male C57BL/6 mice were subjected to transverse aortic constriction (TAC) for 2, 4, and 8 weeks. Neonatal rat cardiac fibroblasts (CFs) were obtained through enzymatic digestion of the hearts. Gene and protein expression of molecules was detected by using quantitative-PCR and Western blot analysis, respectively. Immunofluorescent staining was used to detect the distribution of CILP-1.

**Results:** We found that CILP-1 was expressed in both cardiac myocytes and fibroblasts in adult mice heart. Myocardial expression of CILP-1 was upregulated in mice subjected to TAC for 2, 4, and 8 weeks. AAV-9-mediated delivery of CILP-1 into mice increased the binding of CILP-1 with TGF- $\beta$ 1, attenuated interstitial fibrosis, and improved cardiac function. In cultured CFs, CILP-1 overexpression inhibited myofibroblast differentiation and expression of profibrotic molecules induced by TGF- $\beta$ 1. Furthermore, CILP-1 attenuated TGF- $\beta$ 1-induced Smad 3 phosphorylation and nuclear translocation.

**Conclusion:** CILP-1 alleviates pressure overload-induced cardiac fibrosis and dysfunction. CILP-1 exerts its anti-fibrotic effect through targeting TGF- $\beta$ 1 signaling. This study will offer a new therapeutic strategy for preventing and treating myocardial interstitial remodeling.

#### P11.

##### **FUNCTIONAL INHIBITION OF UREA TRANSPORTER UT-B INDUCES ENDOTHELIUM-DEPENDENT VASODILATION AND LOWERS BLOOD PRESSURE VIA L-ARGININE-ENOS-NOPATHWAY**

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**Objectives:** Urea transporter B (UT-B), a channel protein facilitating transmembrane urea transporting, is expressed in renal and multiple extrarenal tissues, especially in vascular endothelial cells. The purpose of this study was to examine the role of UT-B in regulating blood pressure and to investigate the underlying mechanism.

**Methods:** Two animal models, UT-B null mice and spontaneous hypertensive rats (SHRs), were employed in this study. PU-14, a UT-B inhibitor, is used as a tool drug for UT-B functional inhibition. Blood pressure was measured by the tail-cuff method. SHRs and Wistar-Kyoto rats (WKYs) were treated with PU-14 (50 mg/kg, subcutaneous injection, every 6 hours) for a week to measure the blood pressure and urine output. The vasodilation capacity to acetylcholine or PU-14 was determined with isolated vascular perfusion assay. Additionally, we detected some vasoactive factors in the plasma in wild-type and UT-B null mice with ELISA kits. The expression levels of eNOS, COX-1, COX-2, arginase I and arginase II were measured in the thoracic aortas of mice and SHRs with Western blot. Bovine aortic endothelial cells (BAECs) were studied as an *in vitro* model to further clarify the mechanism.

#### P10.

##### **INHIBITORY EFFECT OF QUERCETIN ON APOPTOSIS OF RAW264.7 CELLS AND THE EXPRESSION OF CHOP**

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**Aim:** To observe the effects and mechanisms of quercetin on the ER stress-induced apoptosis of RAW264.7 cells.

**Methods:** The ER stress-induced apoptosis of RAW264.7 cells was caused by thapsingargin. Quercetin was added into cells. Cell viability detected by MTT, the apoptotic rate was determined by flow cytometry assay, the morphological changes of the cells were observed under laser scanning confocal microscopy, the protein levels of CHOP were determined by Western blotting.

**Results:** In the RAW264.7 cells treated with thapsingargin (1  $\mu$ mol/L) 24h, the cell viability was decreased, and the apoptotic rate was increased, the expression of CHOP was up-regulated. In the cells treated with thapsingargin + quercetin, the cell viability was increased, and the apoptotic rate was decreased, the expression of CHOP was down-regulated as compared to those thapsingargin group.

**Conclusion:** Pretreatment of quercetin inhibits the development of apoptosis in RAW264.7 cells induced by thapsingargin, one of the mechanisms may be correlated with down-regulating the expression of CHOP.

**Results:** The deletion of UT-B caused lower blood pressure in mice. After administration of PU-14 for one week, diastolic blood pressure, systolic blood pressure and mean arterial pressure all dropped in both SHRs and WKYs. Acetylcholine-induced endothelium-dependent relaxation is augmented in UT-B null mouse aortas. PU-14 caused endothelium-dependent relaxations in mouse aortas and mesenteric arteries and these relaxations were abolished by the presence of N $\omega$ -Nitro-L-arginine methyl ester hydrochloride (L-NAME, NOS inhibitor). At the same time, PU-14 caused endothelium-dependent vasorelaxation in both WKYs and SHRs aortas, which can be blocked by L-NAME and/or indomethacin (COX inhibitor). PU-14 also caused similar endothelium-dependent vasodilation in rat aortas with injured or normal endothelium, suggesting that inhibition of UT-B has a protective role in impaired endothelium. Western blot analysis of thoracic aortas showed the up-regulated expression of eNOS, COX-1 and COX-2, and decreased expression of arginase I in UT-B null mice, with the same results showed in SHR model. In addition, plasma prostacyclin was increased and angiotensin II was decreased in UT-B null mice. In *in vitro* experiments, PU-14 and/or 25 mmol/L urea treatment increased NO level as well as the expression and eNOS, and COX-1 and COX-2, decreased arginase I in bovine aortic endothelial cells. And with increasing urea or PU-14 treatment, expression of arginase I was gradually decreased, while eNOS, p-eNOS, COX-1 and COX-2 were up-regulated in a dosage-dependent manner, with no changes of arginase II, meaning a mutual regulation between L-arginine-arginase-urea pathway and L-arginine-eNOS-NO pathway.

**Conclusion:** The present study suggests that UT-B may be a novel drug target of anti-hypertension medication, and UT-B inhibitor may be a potential diuretic for antihypertensive medication.

## ABSTRACTS

### Abstracts for Posters:

#### P12.

##### EFFECTS OF HYDROGEN SULFIDE ON INFLAMMATORY FACTORS IN ACUTE MYOCARDIAL ISCHEMIA INJURY IN RATS

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**Objective:** To observe study effects of H<sub>2</sub>S on the activity of NF-κB in myocardial tissue and inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and intercellular adhesion molecule-1 (ICAM-1) in acute myocardial ischemia in rats, and explore the possible mechanism.

**Methods:** Forty-eight male SD rats were randomly divided into sham operation group, ischemia group, ischemia + NaHS low, middle and high dose groups. The acute myocardial ischemia model was established by ligating the left anterior descending coronary artery (LAD) of the rats. Saline was intraperitoneally administrated in ischemia group. LADs were not ligated but only threaded in sham operation group in rats. In ischemia + NaHS low, middle and high dose groups NaHS (0.78, 1.56, 3.12 mg/kg) was intraperitoneally injected respectively at 3 hours after ischemia. All the rats were killed at 6 hours after the operation. The contents of IL-1β, IL-6 and TNF-α in serum and myocardial tissue were respectively measured by enzyme-linked immunosorbent assay (ELISA). The expression of ICAM-1, mRNA and NF-κB in myocardial tissue were respectively detected by semi-quantitative PCR and Western- blotting.

**Results:** (1) Compared with those of the sham operation group, the contents of IL-1β, IL-6 and TNF-α in serum were significantly increased in ischemia group in rats ( $P<0.01$ ). Compared with those of the ischemia group, the

contents of IL-1β and IL-6 in serum were significantly decreased in ischemia + NaHS low, middle and high dose groups in rats; and the content of TNF-α in serum was significantly decreased in ischemia + NaHS middle and high dose groups in rats. (2) Compared with those of the sham operation group, the contents of IL-1β, IL-6 and TNF-α in myocardial tissue were significantly increased in ischemia group in rats ( $P<0.01$ ). Compared with those of the ischemia group, the contents of IL-1β and TNF-α in myocardial tissue were significantly decreased in ischemia + NaHS low, middle and high dose groups in rats; and the content of IL-6 in myocardial tissue was significantly decreased in ischemia + NaHS middle and high dose groups in rats. (3) The expression of ICAM-1 mRNA in myocardial tissue was significantly increased in ischemia group compared with that of the sham operation group in rats ( $P<0.01$ ). Compared with that of the ischemia group, the expression of ICAM-1 mRNA in myocardial tissue was significantly decreased in ischemia + NaHS low, middle and high dose groups in rats ( $P<0.05$  or  $P<0.01$ ). (4) There was only trace of expression of NF-κB in myocardial tissue in the sham operation group in rats. Compared with that of the sham operation group, the expression of NF-κB was significantly increased in ischemia group in rats ( $P<0.01$ ). Compared with that of the ischemia group, the expression of NF-κB in myocardial tissue was significantly decreased in ischemia + NaHS middle and high dose groups in rats.

**Conclusion:** The administration of NaHS after acute myocardial ischemia could inhibit the activation of NF-κB, reduce the expression of IL-1β, TNF-α, IL-6 and ICAM-1, thus ameliorate the myocardial injury.

#### P13.

##### SYNERGISTIC EFFECT OF PAEONIFLORIN AND LIGUSTRAZINE PHOSPHATE ON THE ACTIN FILAMENT - INDUCED PLATELET ACTIVATION LEVEL AND PLATELET GELSOLIN *IN VITRO*

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**Objective:** To investigate the effect of Paeoniflorin and Ligustrazine phosphate on the actin filament (F-actin)-induced platelet activation level and platelet gelsolin *in vitro*.

**Methods:** Taken washing platelets of 15 healthy volunteers as the investigated subjects, and F-actin of different concentration as the platelet aggregation/activation inducers while Arachidonic Acid (AA), Adenosine diphosphate (ADP) and Thrombin as the positive control inducers. Meanwhile, we take combination of Paeoniflorin and Ligustrazine phosphate as the intervention drugs while aspirin as the positive control drug. The platelet CD62p expression is detected by FCM (flow cytometry), platelet aggregation rate is detected by platelet aggregation analyzer, and the platelet gelsolin is detected by ELISA.

**Results:** (1) Compare with control group, F-actin of different concentrations, AA, ADP and Thrombin groups all can induce platelet aggregation and activation *in vitro* markedly ( $P<0.05$  or  $P<0.01$ ), and concentration-dependent

of F-actin. F-actin of 10 μmol/L has the similar induced platelet aggregation/activation with AA, ADP and Thrombin at the same concentration ( $P>0.05$ ); (2) Compare with the control group, F-actin of 2.5 μmol/L, ADP and Thrombin have no marked difference on platelet gelsolin level ( $P>0.05$ ), while F-actin of 5 μmol/L and 10 μmol/L and AA can increase platelet gelsolin significantly ( $P<0.05$ ), and F-actin of 10 μmol/L has the similar effect on the platelet gelsolin level with AA at the same concentration ( $P>0.05$ ); (3) Paeoniflorin (1 mg·mL<sup>-1</sup>) and ligustrazine phosphate (1 mg·mL<sup>-1</sup>) can inhibit F-actin (10 μM) and AA (10 μM) induced platelet aggregation/activation *in vitro* ( $P<0.05$ ), which have the similar effect with aspirin ( $P>0.05$ ). Pretreatment of paeoniflorin and ligustrazine phosphate on the washing platelet can reduce the platelet gelsolin level of after F-actin and AA-induced platelet aggregation/activation *in vitro* ( $P<0.05$ ), but pretreatment of aspirin has no similar effect ( $P>0.05$ ).

**Conclusion:** F-actin can induce platelet aggregation/activation *in vitro*, and high concentration of F-actin can increase the platelet gelsolin level of activated platelet, which has the similar effect with AA. Paeoniflorin and ligustrazine phosphate can inhibit platelet aggregation/activation *in vitro* and also reduce the platelet gelsolin level of activated platelet. But aspirin has no such effect *in vitro*.

## ABSTRACTS

### Abstracts for Posters:

#### P14.

##### VAGAL ACTIVATION IMPROVES MITOCHONDRIAL QUALITY CONTROL IN CARDIOVASCULAR DISEASES

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**Background:** Mitochondrial dysfunction is considered to be a causative factor in the pathophysiology of diverse cardiovascular diseases, thus mitochondria represent a promising target for future therapeutic interventions. It is well-known that vagal nerve stimulation (VNS) and vagal neurotransmitter acetylcholine (ACh) exert favorable effects in cardiovascular diseases. However, little information is available on the effects of vagal activation on mitochondrial quality control and the underlying mechanisms are still unknown.

**Objectives:** The present studies were designed to determine the effects of VNS on mitochondrial dynamics in response to myocardial injury, and evaluate the influence of ACh on mitophagy, mitochondrial unfolded protein response (UPR<sup>m</sup>) and mitochondrial ROS production during hypoxia/reoxygenation (H/R) in cardiomyocytes or vascular endothelial cells.

**Methods:** Using models of isoproterenol-induced myocardial infarction rats and hypoxia/reoxygenation-injured cardiomyocytes and endothelial cells, we detected the protein expression of mitochondrial dynamics (fission and fusion), mitophagy as well as UPR<sup>m</sup> by Western blot. The mitochondrial morphology was observed by transmission electron microscopy. Mitochondrial ROS production and the release of proapoptotic signals from mitochondria were measured by confocal microscopy and immunofluorescence. The siRNA transfection was performed for genetic

downregulation of type-3 muscarinic ACh receptor (M3AChR) and M2AChR. **Results:** Our results demonstrate that vagal activation play important roles in mitochondrial quality control. (1) VNS balanced mitochondrial fission and fusion which was disrupted in isoproterenol-induced myocardial infarct rats. (2) ACh promoted mitophagy to eliminate damaged mitochondria and maintained normal mitochondrial morphology and function in H/R-injured cardiomyocytes. (3) ACh markedly attenuated H/R-induced mitochondrial ROS overproduction in cardiomyocytes, and M2AChR siRNA blocked the antioxidant effects of ACh. (4) In endothelial cells, H/R stimulation increased the index of UPR<sup>m</sup> (heat shock protein 60, lon protease) and the release of proapoptotic signals, which were prevented by ACh. Additionally, these protective effects of ACh were abrogated by 4-diphenylacetoxymethylpiperidine methiodide (a M3 AChR inhibitor) or M3AChR siRNA. (5) ACh depressed endoplasmic reticulum-mitochondria interaction at reperfusion, thereby improving mitochondrial morphology and function and protecting the vascular endothelial cells.

**Conclusion:** Vagal activation plays a salutary role in cardiovascular protection via improving mitochondrial quality control. These results increase our understanding of vagal activation-afforded beneficial effects on mitochondrial regulation, indicating that vagal modulation is a novel and promising strategy for prevention and management of cardiovascular diseases.

**Supported by:** Grant from National Natural Science Foundation of China (Major International Joint Research Project, No. 81120108002; General Project, No. 81473203), Specialized Research Fund for the Doctoral Program of Higher Education (No. 20130201130008).

#### P15.

##### GLUCAGON-LIKE PEPTIDE-1 IMPROVES ENDOTHELIAL FUNCTION BY UNCOUPLING PROTEIN 2-MEDIATED AUTOPHAGY IN ANGIOTENSIN II-INDUCED HYPERTENSIVE MICE

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**Objective:** Glucagon-like peptide-1 (GLP-1) has protective effects on vascular endothelial function. Autophagy plays an important role in the homeostasis of cells and tissues. However, the regulatory role of GLP-1 in autophagy of vascular endothelium in hypertension remains largely unknown.

**Methods:** Angiotensin II (Ang II)- or PBS-loaded osmotic pumps were implanted under ketamine/xylazine anesthesia (75/6 mg/kg body weight) in C57BL/6 and received dipeptidyl peptidase-IV inhibitor MK-626 administration (3 mg/kg/day) or vehicle for 2 weeks. Aortae were isolated and suspended in the myograph for force measurement. Protein expression or phosphorylation was evaluated by Western blot.

**Results:** Ang II induced the increase of blood pressure in C57BL/6J mice and impaired endothelium-dependent relaxation in mouse aortae. Moreover, Ang II resulted in the reduced phosphorylations of AMPK and eNOS, the decreased expressions of UCP2 and Atg7, the increased ratio of LC3B-I/LC3B-II, and the elevated levels of p62 and mTOR. However, MK-626 inhibited the increase of blood pressure induced by Ang II, increased active GLP-1 level in plasma, improved endothelium-dependent relaxations, elevated phosphorylations of AMPK and eNOS, up-regulated expressions of UCP2 and Atg7, and reduced ratio of LC3B-I/LC3B-II and levels of p62 and mTOR. Furthermore, UCP2 silencing virus inhibited the protective effects of GLP-1 on endothelial function.

**Conclusion:** GLP-1 induced the activation of AMPK/UCP2 and then enhanced autophagy, thus ameliorating endothelial dysfunction in Ang II-induced hypertensive mice. The present study elucidates the new mechanisms underlying the improvement of vascular endothelial function by GLP-1 in hypertensive mice (supported by National Natural Science Foundation of China, No. 81200183 and 81570445).

#### P16.

##### GLUCOSE METABOLISM IN VASCULAR REMODELING

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The pentose phosphate pathway (PPP) plays an essential role in cell proliferation via production of NADPH. Smooth muscle (SM) 22 $\alpha$  protein is involved in the regulation of vascular smooth muscle cell (VSMC) phenotypes. To identify the relationship between NADPH production and SM22 $\alpha$  activity in the development and progression of vascular diseases, glucose-6-phosphate dehydrogenase (G6PD) activity was measured using a specific kit, and apoptosis was determined by TUNEL assay. We showed that the expression and activity of G6PD is promoted in PDGF-BB-induced proliferative VSMCs. PDGF-BB induced G6PD membrane translocation and activation in a SM22 $\alpha$  K21 ubiquitination-dependent manner. The ubiquitinated SM22 $\alpha$  interacted with G6PD, and mediated G6PD membrane translocation. Furthermore, we found that TNF receptor associated factor (TRAF) 6 mediated SM22 $\alpha$  K21 ubiquitination in a K63-linked manner upon PDGF-BB stimulation. Knockdown of TRAF6 decreased the membrane translocation and activity of G6PD, parallel with reduced SM22 $\alpha$  K21 ubiquitination. Increased NADPH generation enhanced VSMC viability, and reduced apoptosis *in vivo* and *in vitro* via glutathione (GSH) homeostasis. We provide evidence that TRAF6-induced SM22 $\alpha$  ubiquitination maintains VSMC survival through increase in G6PD activity and NADPH production. TRAF6-SM22 $\alpha$ -G6PD pathway is a novel mechanism by which SM22 $\alpha$  provides a link between glucose metabolism and VSMC survival, and plays an important role for vascular impairment after injury and plaque stability during development of atherosclerosis.

## ABSTRACTS

### Abstracts for Posters:

#### P17.

##### **ROLE OF HYDROGEN SULFIDE IN ENDOTHELIUM-DERIVED RELAXING FACTORS-MEDIATED RELAXATION AND HYPERPOLARIZATION IN RAT CEREBRAL ARTERIES**

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Endothelium-derived hyperpolarizing factor (EDHF), nitric oxide (NO) and prostaglandin I<sub>2</sub> (PGI<sub>2</sub>) are major endothelium-derived relaxing factors (EDRFs). Role of H<sub>2</sub>S in EDRFs-mediated responses was investigated in rat cerebral arteries. By using siRNA technique, rat cystathionine- $\gamma$ -lyase was knocked down as indicated by decreases of cystathionine- $\gamma$ -lyase expression in cerebral arteries and serum H<sub>2</sub>S. Acetylcholine induced endothelium-dependent hyperpolarizations of vascular smooth muscle cells and dilations in rat cerebral middle artery and basilar artery. These responses were attenuated in the presence of NO synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME, 30  $\mu$ mol/L) and cyclooxygenase inhibitor indomethacin (10  $\mu$ mol/L). Acetylcholine-induced non-NO/PGI<sub>2</sub>-mediated responses were abolished by K<sup>+</sup> channel inhibitors charybdotoxin + apamin. In cerebral arteries from cystathionine- $\gamma$ -lyase knockdown rat, acetylcholine-induced responses were attenuated in the absence or presence of L-NAME and indomethacin, and the remaining non-NO/PGI<sub>2</sub>-mediated responses were abolished by charybdotoxin + apamin or cystathionine- $\gamma$ -lyase inhibitor propargylglycine. L-NAME but not indomethacin attenuated acetylcholine-induced responses in normal rat basilar artery and cystathionine- $\gamma$ -lyase knockdown did not affect NO-mediated responses. In rat cerebral arteries, bradykinin-induced dilations were attenuated by propargylglycine or co-application of L-NAME and indomethacin. NaHS significantly augmented Ca<sup>2+</sup>-activated K<sup>+</sup> current in rat basilar artery vascular smooth muscle cells, and this effect was eliminated by tetraethylammonium.

**Conclusion:** EDHF and NO are involved in the responses of rat cerebral arteries; The EDHF-mediated responses were due to H<sub>2</sub>S activating Ca<sup>2+</sup>-activated K<sup>+</sup> channel.

#### P19.

##### **LncRNA Hand2-AS1 REGULATES VSMC PHENOTYPIC SWITCH**

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Vascular smooth muscle cell (VSMC) phenotypic switch is a common pathological feature of vascular remodeling diseases. Long non-coding RNAs (lncRNAs) have many important regulatory functions, but the functions in VSMC phenotypic switch are largely unknown. Here, we identified that Hand2 (heart and neural crest derivatives expressed 2) gene and lncRNA Hand2 antisense RNA 1 (Hand2-AS1) are co-expressed, and their expression levels are significant decreased in dedifferentiated VSMC by RNA-seq and qRT-PCR analysis. By using both gain-of-function and loss-of-function approaches, we found Hand2 promote VSMC phenotypic switch by regulating SM22 $\alpha$ , a differentiated VSMC marker gene. Furthermore, we demonstrated that lncRNA Hand2-AS1 binds to the Hand2 gene promoter, and increases Hand2 expression at transcriptional level. MiR-138-5p inhibits Hand2 expression by targeting its 3'-untranslated region. LncRNA Hand2-AS1 is a competitive endogenous RNA, blocks miR-138-5p to targeting Hand2, and increases Hand2 expression at post-transcriptional level. In summary, these findings provide a novel mechanism that one lncRNA can regulate one target gene from both transcriptional and post-transcriptional level, our results indicate lncRNA Hand2-AS1, Hand2, and miR-138-5p can form a regulation loop to participate in VSMC phenotypic switch.

#### P18.

##### **TRAF6-MEDIATED SM22A K21 UBIQUITINATION PROMOTES G6PD ACTIVATION AND NADPH PRODUCTION, CONTRIBUTING TO GSH HOMEOSTASIS AND VSMC SURVIVAL IN VITRO AND IN VIVO**

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**Objective:** To identify the relationship between dihydronicotinamide adenine dinucleotide phosphate (NADPH) production and SM22 $\alpha$  activity in the development and progression of vascular diseases.

**Methods:** Vascular smooth muscle cells (VSMCs) were transduced for 24 hours with the respective adenovirus constructs, using a multiplicity of infection of 100. VSMC apoptosis was determined by TUNEL assay. For *in vivo* analyses using a carotid artery ligation model, 25% pluronic F-127 gel containing the adenovirus at a concentration of 10<sup>10</sup> pfu/mL was spread evenly around the outside of the left carotid arteries of the subject mice. Carotid arteries were harvested 14 days after ligation.

**Results:** We showed that the expression and activity of glucose-6-phosphate dehydrogenase (G6PD) are promoted in platelet-derived growth factor (PDGF)-BB-induced proliferative VSMCs. Platelet-derived growth factor-BB induced G6PD membrane translocation and activation in an SM22 $\alpha$  K21 ubiquitination-dependent manner. Specifically, the ubiquitinated SM22 $\alpha$  interacted with G6PD and mediated G6PD membrane translocation. Furthermore, we found that tumor necrosis factor receptor-associated factor (TRAF) 6 mediated SM22 $\alpha$  K21 ubiquitination in a K63-linked manner on PDGF-BB stimulation. Knockdown of TRAF6 decreased the membrane translocation and activity of G6PD, in parallel with reduced SM22 $\alpha$  K21 ubiquitination. Elevated levels of activated G6PD consequent to PDGF-BB induction led to increased NADPH generation through stimulation of the pentose phosphate pathway, which enhanced VSMC viability and reduced apoptosis *in vivo* and *in vitro* via glutathione homeostasis.

**Conclusions:** We provide evidence that TRAF6-induced SM22 $\alpha$  ubiquitination maintains VSMC survival through increased G6PD activity and NADPH production. The TRAF6-SM22 $\alpha$ -G6PD pathway is a novel mechanism underlying the association between glucose metabolism and VSMC survival, which is beneficial for vascular repair after injury but facilitates atherosclerotic plaque stability.

## ABSTRACTS

### Abstracts for Posters:

#### P20.

##### EFFECTS AND MECHANISM OF DIOSCIN ON RAT CARDIAC CONTRACTILITY

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**Objective:** To investigate the effects of Dioscin (Dio) on sodium current ( $I_{Na}$ ), L-type calcium current ( $I_{Ca,L}$ ) and intracellular free calcium concentration in isolated rat ventricular myocytes and to study its effects on rat myocardial contractility as well as explore its mechanism preliminarily.

**Methods:** (1) Single rat ventricular myocyte was isolated from adult rat heart by enzymatic dissociation. Effects of dioscin on sodium current ( $I_{Na}$ ), L-type calcium current ( $I_{Ca,L}$ ) were observed and recorded with whole-cell patch clamp. (2) Left ventricle contractile function was measured using the Langendorff non-recirculating mode of isolated rat heart perfusion. Effects of dioscin and dioscin in presence of SEA0400, sodium-calcium exchanger (NCX) inhibitor, were investigated by measuring left ventricular systolic pressure (LVSP) and left ventricular end diastolic pressure (LVEDP). Also, heart rate (HR), peak rate of rise/fall of left ventricular pressure ( $\pm dp/dt_{max}$ ) of isolated rat heart were calculated. After perfusion, isolated rat hearts were collected to analyze the content of superoxide dismutase (SOD) using SOD kit. (3) Effects of dioscin and SEA0400 on intracellular free calcium concentration in rat H9c2 cells were measured by labeling intracellular free calcium ion with Fluo3-AM and then detected the fluorescence intensity with confocal microscopy. Mitochondrial membrane potential was detected with multifunctional microplate reader in order to determine whether dioscin may cause cellular injuries.

**Results:** (1) Dioscin shifted downward the I-V curve with increased current density of sodium current compared with those in the control group. With the 0.1, 1, 10  $\mu\text{mol}\cdot\text{L}^{-1}$  dioscin, the peak current density were dose-dependently changed from  $-52.91\pm 1.58 \text{ pA}\cdot\text{pF}^{-1}$  to  $-57.93\pm 2.28$  ( $n=8$ ,  $p<0.05$ ),  $-76.14\pm 2.76$  and  $-82.40\pm 4.71 \text{ pA}\cdot\text{pF}^{-1}$  ( $n=8$ ,  $p<0.01$ ) respectively. Dioscin facilitated the

activation and recovery process of sodium current, but not the inactivation process. (2) Compared with the control group, dioscin shifted upward the I-V curve with decreased current density of calcium current. With the 0.1, 1, 10  $\mu\text{mol}\cdot\text{L}^{-1}$  dioscin, the peak current density were dose-dependently changed from  $-3.46\pm 0.15 \text{ pA}\cdot\text{pF}^{-1}$  to  $-3.31\pm 0.12$  ( $n=8$ ,  $p<0.05$ ),  $-3.03\pm 0.13$  and  $-2.69\pm 0.18 \text{ pA}\cdot\text{pF}^{-1}$  ( $n=8$ ,  $p<0.01$ ). Dioscin retarded the activation and recovery process of calcium current, but had no significant effects on the inactivation process. (3) With 0.1, 1, 10  $\mu\text{mol}\cdot\text{L}^{-1}$  dioscin, the relative fluorescence intensity of intracellular free calcium concentrations were increased significantly from  $16.62\pm 0.89$  to  $21.48\pm 0.80$ ,  $25.68\pm 0.69$  and  $19.84\pm 0.66$  ( $n=8$ ,  $p<0.01$ ) respectively. While in presence of SEA0400, the relative fluorescence intensity was changed by 0.1, 1, 10  $\mu\text{mol}\cdot\text{L}^{-1}$  dioscin to  $11.20\pm 0.82$ ,  $17.09\pm 0.63$  and  $14.80\pm 0.47$  ( $n=8$ ,  $p<0.01$ ). With 1  $\mu\text{mol}\cdot\text{L}^{-1}$  dioscin, the relative fluorescence intensity was changed to  $8.17\pm 0.50$  and  $12.73\pm 0.39$  ( $n=8$ ,  $p<0.01$ ) without calcium or sodium in the extracellular fluid. (4) According to the results from multifunctional microplate reader, 0.1, 1  $\mu\text{mol}\cdot\text{L}^{-1}$  dioscin, SEA0400 as well as 1  $\mu\text{mol}\cdot\text{L}^{-1}$  dioscin with SEA0400 all showed no significant effects on the mitochondrial membrane potential of rat H9c2 cells, while with effects of 10  $\mu\text{mol}\cdot\text{L}^{-1}$  dioscin, the ratio of JC-1 monomer and J-aggregates was changed from  $1.14\pm 0.03$  to  $1.35\pm 0.06$  ( $n=6$ ,  $p<0.01$ ), causing a decrease in the mitochondrial membrane potential. (5) With 0.1, 1  $\mu\text{mol}\cdot\text{L}^{-1}$  dioscin, LVSP and  $dp/dt_{max}$  were significantly enhanced by 13%, 18% and 11%, 9% respectively. With the 10  $\mu\text{mol}\cdot\text{L}^{-1}$  dioscin, LVSP and  $dp/dt_{max}$  were both decreased. (6) 1  $\mu\text{mol}\cdot\text{L}^{-1}$  SEA0400 alone showed no significant effect but can partly inhibited the effects of dioscin on cardiac performances in isolated Langendorff-perfused rat hearts. (7) With 0.1, 1, 10  $\mu\text{mol}\cdot\text{L}^{-1}$  dioscin, the content of SOD in isolated Langendorff-perfused rat hearts was changed from  $2.67\pm 0.23 \mu\text{g}\cdot\text{mL}^{-1}$  to  $3.16\pm 0.10$ ,  $3.32\pm 0.24$  ( $n=6$ ,  $p<0.01$ ) and  $2.95\pm 0.16 \mu\text{g}\cdot\text{mL}^{-1}$  ( $n=6$ ,  $p<0.05$ ) respectively.

**Conclusions:** Dioscin shows positive inotropic effect on isolated rat heart, enhancing the LVSP and  $dp/dt_{max}$  by increasing  $\text{Na}^+$  influx and facilitating the reverse mode of the sodium-calcium exchanger.

#### P21.

##### THE PROTECTIVE EFFECT OF DIOSCIN-CONTAINING SERUM AGAINST HYDROGEN PEROXIDE-MEDIATED OXIDATIVE STRESS AND APOPTOSIS IN NEONATAL RAT CARDIOMYOCYTES

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**Objectives:** To investigate the effect of dioscin on hydrogen peroxide ( $\text{H}_2\text{O}_2$ )-induced oxidative stress and apoptosis in cardiomyocytes in vitro and its underlying mechanisms with the method of Serum Pharmacology.

**Methods:** Primary cultures of neonatal rat cardiomyocytes pretreated with dioscin-containing serum or blank serum were exposed to 100  $\mu\text{mol}/\text{L}$   $\text{H}_2\text{O}_2$  for 2h. Cell viability, malondialdehyde (MDA) contents, lactate dehydrogenase (LDH) release, total superoxide dismutase (T-SOD) and catalase activities were measured. Apoptosis and caspase-3 activity were determined by Hoechst staining or fluorometric assay kit. To explore the mechanisms, western blot analysis was performed to detect the expressions of Bax, Bcl-2 and activation of AKT.

**Results:** Dioscin-containing serum significantly protected the cardiomyocytes from  $\text{H}_2\text{O}_2$  injury, as evidenced by decreased MDA contents, LDH release and increased cell viability in a concentration-dependent manner. More importantly, dioscin decreased cardiomyocyte apoptosis while increased the activities of T-SOD and catalase. Furthermore, dioscin-containing serum pretreatment up-regulated AKT phosphorylation of AKT, reduced the ratio of Bax/Bcl-2, and decreased caspase-3 activity.

**Conclusions:** Dioscin protects  $\text{H}_2\text{O}_2$ -induced cardiomyocytes injury by reducing oxidative damage and apoptosis. The underlying mechanism might be activation of PI3K/AKT signaling pathway and regulation of proapoptotic signaling cascades.

#### P22.

##### PROTECTIVE EFFECTS OF SALVIANOLIC ACID A ON MYOCARDIAL ISCHEMIA INDUCED BY LIGATING CORONARY ARTERY IN RATS

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**Aim:** To investigate the protective effect of Salvianolic acid A (Sal A) on acute myocardial ischemia and its possible mechanism, and compare its potency with salvianolate.

**Methods:** Left anterior descending (LAD) coronary artery in rats was occluded for 1h and Sal A dissolved in saline was administered intraperitoneally. Coronary vasodilating action was evaluated in the isolated rat heart perfusion model. Electrocardiograph, infarction index and serum myocardial enzymes were determined to evaluate the effect of Sal A. Some other observations were carried out to explore whether inhibiting inflammation and relaxing coronary artery is involved in the mechanisms underlying SalA.

**Results:** In myocardial ischemia injured rats Sal A could dose-dependently improve ischemic ECG changes, reduce myocardial infarction index, myocardial enzyme leakage and myocardial inflammatory cytokine level such as IL-6 and TNF- $\alpha$ . Sal A (10  $\mu\text{mol}\cdot\text{L}^{-1}$ ) could also improve coronary flow significantly.

**Conclusions:** These results suggested that Sal A can protect rat against myocardial ischemia injury. Anti-inflammation and relaxing coronary artery may contribute to the protective effect of Sal A.

**Foundation item:** This study was supported by the national scientific & technological major special project "significant creation of new drugs" (2013ZX09103001-008, 2012ZX09103101-078, 2013ZX09508104) and the National Natural Science Foundation of China (No. 81573645).

## ABSTRACTS

### Abstracts for Posters:

#### P23.

##### GENETIC VARIATION REGULATES THE TSLP/TSLP RECEPTOR AXIS AND ASSOCIATES WITH CORONARY ARTERY DISEASE

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**Objectives:** It has been reported that thymic stromal lymphopoietin (TSLP) and the TSLP receptor axis play an important role in cardiovascular diseases, including coronary artery disease (CAD). However, the exact relationship between this pathway and CAD is not clear yet. Here, we investigate the genetic role of the TSLP/TSLP receptor axis in CAD in the Chinese Han population.

**Methods:** A three-stage case control association analysis was performed for 3628 CAD cases and 3776 controls using common variants in the genes *TSLP*, *interleukin 7 receptor (IL7R)* and *TSLPR*. In addition, reporter gene analysis and circulation-level study were carried out, in order to clarify the function of the reference variants. Finally, interaction analysis between the reference functional variants in association with CAD was conducted. Logistic regression analysis was used to perform the interaction analysis and adjustment of traditional risk factors for CAD.

**Results:** Each gene had a variant significantly associated with CAD (rs3806933<sup>T</sup> in *TSLP*,  $P_{adj}=4.34 \times 10^{-5}$ , OR=1.18, 95%CI: 1.09-1.27; rs6897932<sup>T</sup> in *IL7R*,  $P_{adj}=1.13 \times 10^{-7}$ , OR=1.31, 95%CI: 1.19-1.45; g.19646A>G<sup>A</sup> in *TSLPR*,  $P_{adj}=2.04 \times 10^{-6}$ , OR=1.20, 95%CI: 1.11-1.29). Luciferase activity analysis indicated that changes in the alleles of rs3806933 and rs6897932 could influence their gene expression with *P* values of less than 0.005. In addition, the reference "T" allele of rs3806933 might increase the TSLP protein expression level. Furthermore, interaction analysis showed that the subjects with both rs3806933-TT in *TSLP* and rs6897932-TT in *IL7R* were more likely to suffer from CAD, with an OR value of 4.65.

**Conclusions:** Genetic variants in the TSLP/TSLP receptor axis could regulate the expression of the genes involved and contribute to a risk of CAD, indicating a causal role of the axis in the development of CAD.

#### P25.

##### IL-21 PROMOTES MYOCARDIAL ISCHEMIA/ REPERFUSION INJURY THROUGH MODULATION OF NEUTROPHIL INFILTRATION

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**Background:** The immune system plays important roles in driving the acute inflammatory response following myocardial injury. Interleukin-21 (IL-21) is a cytokine with multiple immunomodulatory effects and promotes the development of autoimmune diseases and inflammatory disorders. A recent study indicated that T cell-derived IL-21 promoted brain injury following ischemic stroke in mice. However, its role in myocardial ischemia/reperfusion (I/R) injury remains unknown.

**Methods:** Effects of IL-21 were examined in a mouse model of myocardial I/R injury. Myocardial injury, neutrophils infiltration and expressions of chemokine KC and MIP-2 were studied *in vivo*. In cultured cardiomyocytes (CMs) and cardiac fibroblasts (CFs) isolated from neonatal mouse, we determined the effects of IL-21 on the expression of KC and MIP-2 by real-time PCR and ELISA. The mechanisms of signaling pathway underlying these effects were explored by western blot.

**Results:** IL-21 was elevated within the acute phase of murine I/R injury. Exogenous IL-21 aggravated myocardial injury as illustrated by increased infarct size, cardiac troponin T levels and reduced cardiac function, whereas anti-IL-21 neutralizing antibody treatment exerted the opposite effects. IL-21 increased cardiac infiltrating neutrophils as detected by flow cytometry, and increased myocardial mRNA expressions of KC, MIP-2 following I/R

#### P24.

##### EFFECTS OF SODIUM HYDROSULFIDE ON LARGE-CONDUCTANCE $Ca^{2+}$ ACTIVATED $K^{+}$ CHANNEL IN MIDDLE CEREBRAL ARTERY SMOOTH MUSCLE CELLS

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Previous studies suggest that hydrogen sulfide ( $H_2S$ ) mediate vasorelaxation and hyperpolarization of the vascular smooth muscle cells (VSMCs) of cerebral arteries. However, the ionic mechanisms underlying this action at the cellular level remain unclear. Large-conductance  $Ca^{2+}$ -activated  $K^{+}$  channels ( $BK_{Ca}$ ) in VSMCs are critical regulators of membrane potential and vascular tone. In this study, the effects of sodium hydrosulfide (NaHS), the donor of  $H_2S$ , on large-conductance  $Ca^{2+}$ -activated  $K^{+}$  channel were investigated in VSMCs of rat middle cerebral artery (MCA). Single smooth muscle cells from MCA of rats were studied. Whole-cell patch-clamp recording was conducted to detect the  $BK_{Ca}$  current. Western blot was also used to explore the protein expression of  $BK_{Ca}$  in VSMCs. In rat middle cerebral VSMCs, NaHS (10-500 nmol/L) increased the  $BK_{Ca}$  current remarkably in a concentration-dependent manner. This response was significantly inhibited by iberiotoxin (IBTX, 100 nmol/L) which can particularly block the  $BK_{Ca}$  current. The protein expression of  $BK_{Ca}$  in middle cerebral VSMCs treated with NaHS or IBTX has no significant difference when compared with the control. Our data suggest that NaHS increase the  $BK_{Ca}$  current in middle cerebral VSMCs and without changing the protein expression of  $BK_{Ca}$  channel, which may contribute to its regulatory action in the vasorelaxation and hyperpolarization in cerebral vessel.

*in vivo*. *In vitro* study demonstrated IL-21 receptor expressions on isolated murine neutrophils, CMs and CFs. IL-21 treatment induced neutrophil migration as revealed by chemotaxis assay. Meanwhile, IL-21 induced mRNA and protein expression of KC and MIP-2 in CMs and CFs. The mechanisms underlying the induction of IL-21 on chemokine expression were associated with the activation of AKT and NF- $\kappa$ B in CMs, and p38 MAPK and NF- $\kappa$ B in CFs. Moreover, inhibition of AKT or NF- $\kappa$ B pathways in CMs, and inhibition of p38 MAPK or NF- $\kappa$ B pathways in CFs reversed these effects.

**Conclusions:** IL-21 plays a pathogenic role in myocardial I/R, most likely by promoting cardiac neutrophil infiltration.

ABSTRACTS

Abstracts for Posters:

P26.

**EFFECTS OF SIMVASTATIN ON THE EXPRESSION OF eNOS IN RENAL ISCHEMIA REPERFUSION INJURY**

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**Objective:** To investigate the effects of Simvastatin on the expression of eNOS in renal ischemia-reperfusion injury (RI/RI).

**Methods:** Sixty male Sprague-Dawley rats were divided into five groups randomly: (1) Sham group; (2) ischemia-reperfusion group (I/R); (3) low-dose Simvastatin group (Sim-L, 5 mg/kg/d); (4) middle-dose Simvastatin group (Sim-M, 20 mg/kg/d); (5) high-dose simvastatin group (Sim-H, 40 mg/kg/d). Sim-L, M and H group rats were given oral Simvastatin 5, 20 and 40 mg/kg/d treatment respectively for 2 weeks. The model of RI/RI was induced by bilateral clamping the renal artery and vein for 45 minutes followed by reperfusion. After 6 and 24 hours of reperfusion, the blood samples were taken for detecting contents of serum creatinine (Scr), serum urea nitrogen (BUN). After blood was taken, both side of kidney were excised for observing renal histological examination, content of Nitric Oxide (NO), activity of superoxide dismutase (SOD), the content of malondialdehyde (MDA) and the expression of endothelial nitric oxide synthase (eNOS).

**Results:** After RI/RI, the renal tubule epithelial cells showed signs of damage in I/R group rats. the contents of Scr, BUN and MDA were significantly increased in I/R group rats than that of sham group rats ( $P < 0.01$ ); Compared with the I/R group rats, contents of Scr, BUN and MDA were significantly lower in Sim-L, M and H groups ( $P < 0.05$ ). Contents of NO and activity of SOD were significantly increased ( $P < 0.01$ ) in Sim-M and Sim-H groups; Compared with the I/R group, the protein expression of eNOS were significantly increased in Sim-M and Sim-H groups, but not in Sim-L group rats.

**Conclusions:** These results suggest that Simvastatin could reduce renal tissue injury and improve renal function in RI/RI rats. The protective effects of Simvastatin to the RI/RI may be related to the anti-free radical damage and upregulates eNOS expression and NO production.

P27.

**Chlamydia pneumoniae INFECTION PROMOTES ANGIOGENESIS VIA THE IQGAP1 RELATED SIGNALING PATHWAY**

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**Objectives:** *Chlamydia pneumoniae* (*C. pneumoniae*) infection contributes to atherosclerotic plaque instability and rupture. Intraplaque neovascularization favors the progression of atherosclerotic plaque toward rupture. However, it is still an enigma how *C. pneumoniae* infection induces angiogenesis, although *C. pneumoniae* infection plays a possible role in this process. Therefore, we investigated the effect of *C. pneumoniae* infection on angiogenesis, and further explored the roles of IQ domain GTPase-activating protein 1 (IQGAP1) - Neural Wiskott-Aldrich syndrome protein (N-WASP) signaling in *C. pneumoniae* infection-induced angiogenesis.

**Methods and Results:** *C. pneumoniae* infection significantly enhanced angiogenesis as assessed by the tube formation assay possibly by inducing vascular endothelial cell (VEC) migration in the wound healing and Transwell migration assays. Subsequently, immunoprecipitation and western blot results showed that *C. pneumoniae* infection induced significant increases in the phosphorylations of IQGAP1 and N-WASP in VECs. Then, we found that the phosphorylations of IQGAP1 and N-WASP were both required for the angiogenesis induced by *C. pneumoniae* infection by using a Src tyrosine kinase inhibitor 4-Amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo(3,4-d)pyrimidine (PP2), and the PKC inhibitors (chelerythrine and GF 109203X) and an N-WASP inhibitor wiskostatin. Immunoprecipitation results revealed that IQGAP1 physically associated with N-WASP after *C. pneumoniae* infection of VECs. Actin polymerization assay further showed that in *C. pneumoniae*-infected VECs, IQGAP1 and N-WASP were both recruited to filamentous actin, and shared some common compartments in localization at the leading edge, which was impaired after the depletion of IQGAP1 by using siRNAs. Moreover, siRNA-mediated knockdown of IQGAP1 also significantly decreased N-WASP phosphorylation at Tyr256 induced by *C. pneumoniae* infection.

**Conclusions:** *C. pneumoniae* infection promotes angiogenesis by inducing VEC migration via IQGAP1-N-WASP signaling pathway.

P28.

**GRANULOCYTE COLONY-STIMULATING FACTOR PROMOTES THE PROLIFERATION OF CARDIAC SIDE POPULATION CELLS BY AKT-GATA4 PATHWAY**

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**Objectives:** Granulocyte colony-stimulating factor (G-CSF) was initially reported to induce myocardial regeneration by promoting mobilization of bone marrow stem cells or side population cells to the injured heart after myocardial infarction (MI). Cardiac side population cells (CSPs), one of candidates for cardiac stem cells, has been reported to be able to differentiate into cardiovascular cells *in vitro* and *in vivo*. Our previous study showed that G-CSF improved cardiac function against ischemic-reperfusion injury by Stat3/HSF1 pathway in animal study. However, whether the cardiac-protection of G-CSF is associated with the effect on CSPs is unclear. Here, we aim to study the effect and the potential mechanisms of G-CSF on CSPs.

**Methods:** MI model was established by ligation of left anterior descending artery in mice. Mice were treated with recombinant human G-CSF (100 ug/kg/day) or saline by subcutaneous injection for 7 days consecutively after MI. CSPs were isolated and counted from mice by fluorescence-activated cell sorting (FACS) analysis. *In vitro*, CSPs from neonatal rat were purified and cultured with or without G-CSF. The proliferation of cultured CSPs was analyzed by luminescent cell viability assay.

**Results:** After MI, the ratio of CSPs was increased 2-fold compared to sham mice, and G-CSF greatly promoted the effect. *In vitro*, G-CSF significantly enhanced the proliferation of CSPs in concentration-dependent manner. Further analysis showed that G-CSF increased phospho-AKT and expression of GATA4 in cultured CSPs. AKT inhibitor dramatically suppressed G-CSF-induced-proliferation of CSPs *in vitro* and *in vivo*. It also inhibited the

upregulation of GATA4 induced by G-CSF in cultured CSPs. Si-GATA4 not only effectively downregulated the expression of GATA4 by about 80%, but also partly abolished the phospho-AKT induced by G-CSF in cultured CSPs. In addition, G-CSF induced-proliferation of CSPs was greatly reversed by si-GATA4 *in vitro*. G-CSF-treated-CSPs ( $5 \times 10^6$ ) were transplanted into injured heart after MI. Immunofluorescence staining showed that transplanted CSPs could express  $\alpha$ -MHC, one of markers of cardiomyocytes at 2-week after MI. Cardiac function was significantly improved by CSPs transplantation at 4-week after MI.

**Conclusions:** G-CSF greatly activated AKT signaling, the phospho-AKT upregulated the expression of GATA4, which in turn, promoted the activation of AKT, resulting in the proliferation of CSPs. Thus, G-CSF may exert cardioprotective effect against MI by promoting the proliferation of CSPs and support myocardial regeneration in injured heart.

## ABSTRACTS

### Abstracts for Posters:

#### P29.

##### EFFECT OF ZINC IN MYOCARDIAL ISCHEMIA/ REPERFUSION INJURY

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While the role of endoplasmic reticulum stress (ERS) in myocardial ischemia/ reperfusion (I/R) injury has been extensively investigated, the precise mechanism by which inhibition of ERS induces cardioprotection remains unclear. We aimed to explore the mechanism of ERS inhibition-induced cardioprotection against I/R injury, focusing on the role of Zn<sup>2+</sup> and the mitochondrial permeability transition pore (mPTP). Exposure of H9c2 cells to 800 μM H<sub>2</sub>O<sub>2</sub> for 20 min increased GRP78 and GRP94 expressions, suggesting that H<sub>2</sub>O<sub>2</sub> can induce ERS. Cells treated with H<sub>2</sub>O<sub>2</sub> showed a significant decrease in TMRE fluorescence compared to the normal group, indicating that H<sub>2</sub>O<sub>2</sub> can induce the mPTP opening. In contrast, ERS inhibitor TUDCA prevented the loss of TMRE fluorescence, implying that inhibition of ERS can prevent the mPTP opening. This effect of TUDCA was blocked by zinc chelator TPEN, indicating a role of Zn<sup>2+</sup> in the action of TUDCA on the mPTP opening. In support, TUDCA increased intracellular free zinc, as indicated by a marked increase in Newport Green DCF fluorescence intensity. In isolated rat hearts, GRP78 expression was not altered at 10 min of reperfusion, but was markedly increased 30 and 60 min after the onset of reperfusion. Hearts treated with TUDCA showed a significant reduction of GRP78 expression, and effect that was reversed by TPEN. The immunofluorescence study also showed that the effect of TUDCA on GRP78

expression was reversed by TPEN. Experiments with transmission electron microscopy and hematoxylin-eosin staining revealed that TUDCA prevented endoplasmic reticulum and mitochondrial damages at reperfusion, which was blocked by TPEN. In conclusion, inhibition of ERS protects the heart from reperfusion injury through prevention of the mPTP opening. Increased intracellular free Zn<sup>2+</sup> accounts for the cardioprotective effect of ERS inhibition.

*This work was supported by the China Postdoctoral Science Foundation (CPSF 2014M560190), the Research Project of Education Department of Hebei Province (ZD 2014006, QN2014092), North China University of Science and Technology Foundation for Outstanding Young Scholars (JP 201302) and Project of Hebei administration of traditional Chinese Medicine (2014196).*

#### P30.

##### THE INHIBITORY EFFECT OF HYDROGEN SULFIDE ON PLATELET AGGREGATION AND THE UNDERLYING MECHANISMS

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**Objectives:** The present study explored the effects of H<sub>2</sub>S on human platelet in vitro and on mouse platelet *ex vivo*, which was also studied under dyslipidemia conditions.

**Methods:** Platelet aggregation was analysed using lumi-aggregometer. Thromboxane A<sub>2</sub> formation was measured via ELISA kit. Cytosolic Ca<sup>2+</sup> was detected using fura-2 AM. Protein phosphorylation was analyzed by Western blotting.

**Results:** NaHS at concentrations from 0.01 to 10 mM inhibited the aggregation of washed human platelets with an IC of 93.8 μM. The collagen-induced platelet aggregation, ATP release, and TXA<sub>2</sub> formation were also inhibited by NaHS incubation. Furthermore, NaHS significantly decreased intracellular calcium level in washed human platelets stimulated with collagen and inhibited collagen-induced c-PLA<sub>2</sub>, p38 MAPK, ERK, JNK, PLC-γ<sub>2</sub> and Akt phosphorylation. Injection of NaHS (1 μmol/g) into mice significantly inhibited the collagen-induced platelet aggregation. Finally, NaHS inhibited the aggregation of washed human platelets induced by ox-LDL plus collagen. The collagen-induced rapid platelet aggregation in apoE knockout mice was also significantly decreased by NaHS treatment.

**Conclusions:** Our study showed that H<sub>2</sub>S was able to inhibit platelet aggregation induced by collagen. The underlying mechanisms are related to H<sub>2</sub>S-induced changes in various signaling pathways as well as [Ca<sup>2+</sup>]<sub>i</sub> in the platelet. The interaction of H<sub>2</sub>S and platelets is also affected by lipid metabolism.

#### P31.

##### HYDROGEN SULFIDE INHIBITS AMPKA AND PREVENTS ROS-MEDIATED ENDOTHELIAL APOPTOSIS AND AUTOPHAGE IN DIABETES MELLITUS

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Vasculopathy is a major complication of diabetes mellitus and endothelial dysfunction contributes to the development of this complication, but the mechanisms of endothelial dysfunction in this setting are incompletely understood. We have previously reported that hydrogen sulfide (H<sub>2</sub>S) prevents diabetic vasculopathy by inhibiting reactive oxygen species (ROS) production, which resulted in an increased consumption of endogenous H<sub>2</sub>S. The goal of the present studies was to investigate the role of changes in H<sub>2</sub>S homeostasis in the pathogenesis of hyperglycemic endothelial apoptosis and autophagy. High glucose (40 mmol/L) and palmitate (100 nmol/L) increased intracellular ROS through a mechanism involving in regulation between cytosolic and mitochondrial ROS generation. NaHS (100 μmol/L) inhibited high glucose-induced ROS generation, mitochondrial membrane potential collapse, and endothelial cell apoptosis and autophagy. Additionally, the AMPK siRNA and the inhibitor compound C mimicked protective effects of H<sub>2</sub>S. H<sub>2</sub>S replacement therapy normalized hyperglycemia induced AMPKα phosphorylation, ROS generation, and autophagy in endothelial cells. These findings highlight a novel mechanism by which H<sub>2</sub>S inhibits AMPKα and protects against hyperglycemia-induced vasculopathy.

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ABSTRACTS

Abstracts for Posters:

P32.

**SALVIANOLIC ACID A INIBITS SMOOTH MUSCLE CELL PROLIFERATION THOUGH CREB-MEDIATED P21 INDUCTION AND PREVENTS INJURY-INDUCED NEOINTIMAL HYPERPLASIA**

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**Objectives:** Cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA)/cAMP response element (CRE) binding protein (CREB) signaling cascade negatively regulates platelet derived growth factor-BB (PDGF-BB) induced smooth muscle cell (SMC) proliferation, which is a critical event in the initiation and development of restenosis and atherosclerotic lesions. Salvianolic acid A (SAA) is one of the most abundant polyphenols extracted from salvia. The aim of this study is to investigate whether SAA exerts an action on PDGF-BB-induced proliferation via cAMP/PKA/CREB mechanism.

**Methods and Results:** SAA blunts PDGF-BB-induced human umbilical artery smooth muscle cell (hUASMC) proliferation via p21 induction, as evidenced by its increased mRNA and protein expression levels. The SAA-induced upregulation of p21 involves the cAMP/PKA signaling pathway; a cAMP analog mimicked the effects of SAA, and a specific cAMP/PKA inhibitor opposed these effects. SAA also activated CREB, including phosphorylation at Ser133, and induced its nuclear translocation. Deletion and mutational analysis of the p21 promoters, co-immunoprecipitation and western blot analysis showed that CRE is essential for SAA-induced p21 protein expression. Transfection of dominant-negative CREB (mutated Ser133) plasmids into hUASMCs attenuated SAA-stimulated p21 expression. SAA upregulated p21 expression and activated CREB in the neointima of balloon-injured arteries in vivo.

**Conclusions:** Our results indicate that SAA promotes p21 expression in SMCs through the cAMP/PKA/CREB signaling cascade in vitro and prevents injury-induced neointimal hyperplasia.

P34.

**MECHANISM OF ATRIAL FIBRILLATION IS RELATED TO CHANGES OF COLLAGEN TYPES AND ULTRASTRUCTURE IN RHEUMATIC HEART VALVULAR DISEASE**

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**Purpose:** Atrial fibrillation (AF) is a common complication in heart valvular disease and is associated with cellular structure abnormality. We aimed to study the collagen types and to investigate the changes of morphology and cell ultrastructure in the tissue of the right atrium from patients with mitral valvular disease and permanent AF.

**Methods:** The right atrial appendage obtained from patients with mitral valvular disease associated with either sinus rhythm (SR, n=4) or permanent AF (AF ≥6 months, n=4). One part of the sample was used for histological analysis and collagen fiber quantitation; the other part was analyzed by transmission electron microscope to observe cell ultrastructure.

**Results:** In both patients, Type II collagen was found under capillary endothelial and vascular basement membrane. Type II collagen was positive in cardiomyocytes and fibril cardiac muscle. Type III collagen was colored in mesothelial cells, vascular endothelial cells, and myocyte cytoplasm. Type IV collagen was located around cardiomyocytes, especially on vascular basement membrane and myocardial cell membrane. Atrial fibrosis was higher in AF patients than that of SR patients. In AF patients, there was a large number of immature fibrocytes among myocytes. The connection of myocytes was disrupted or disorientated. Electron microscope study revealed that the cell striations were cracked or disappeared and that "Glycogen Lake" existed in the myocardial cells. Further, mitochondrion was swelling.

P33.

**THE ROLE OF CATESTATIN IN CHRONIC INTERMITTENT HYPOXIA-INDUCED HYPERTENSION IN RATS**

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**Objective:** To address whether catestatin (CST) is involved in intermittent hypoxia (IH)-induced hypertension in rats.

**Methods:** Rats were randomly divided into control (Con), IH, and IH+CST group. Mean pressure (MP), systolic pressure (SP), and diastolic pressure (DP) were measured by left heart catheterization. Levels of CST, NE, E, and His in plasma were detected by ELISA. Expression of chromogranin A (CHGA) in the cerebrum, cerebellum, medulla oblongata, left ventricle, and lung tissue were detected by western blot.

**Results:** Compared with Con group, rats in IH group exhibited a significant increase of blood pressure after IH treatment for 10 days ( $p < 0.05$ ), which was markedly decreased with CST application ( $p < 0.05$ ). In addition, MP, SP, and DP in IH group were significantly increased, which were greatly decreased with CST application ( $p < 0.05$ ). IH treatment increased the ratio of RV/LV+S, dp/dt max and dp/dt min, while IH+CST decreased dp/dtmax, in the left ventricular. Mechanistically, IH treatment decreased CST and His while increased NE and E levels in plasma, which were reversed with CST treatment. CHGA levels in left ventricle and lung tissue as well as in central system, in IH group were decreased, which were restored with CST treatment.

**Conclusions:** Decrease of CST while increase of NE and E levels in plasma may be involved in IH-induced hypertension in rats. CST treatment ameliorates hypertension induced by IH in rats.

*This work was supported by the National Natural Science Foundation of China (81200039), the Natural Science Foundation of Zhejiang Province of China (LY12H01004), the Foundation of Zhejiang Educational Committee (Y201326833).*

**Conclusions:** Our study identified different types of collagen in the atrial appendage and revealed that there are atrial fibrosis and ultrastructural abnormalities related to AF in the mitral valvular disease. The present study provides evidence of structure changes that may lead to the abnormal conductance of the electrical signal and arrhythmia in the heart valvular disease.

## ABSTRACTS

### Abstracts for Posters:

#### P35.

##### **TERUTROBAN, A TP RECEPTOR ANTAGONIST, RESTORES RENAL ARTERY TONE, BUT NOT RENAL FUNCTION IN MOUSE WITH 5/6 NEPHRECTOMY**

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*\*Both authors contribute equally*

**Objectives:** Thromboxane A2 (TXA2) is assumed to contribute to the process of renal dysfunction. The present study was designed to investigate whether terutroban, a specific antagonist of thromboxane/prostaglandin (TP) receptor, protects against renal damage in 5/6 nephrectomy.

**Methods:** C57/BL6 mice were randomly grouped into sham-operated (2K), 5/6 nephrectomy groups (5/6K-off) and 5/6 nephrectomy treated with terutroban (10 mg/kg/d) groups (Terutroban). Renal artery and kidney were collected for vascular function study, Western blotting, immunohistochemistry (IHC) assay and enzyme-linked immunosorbent assay (ELISA), respectively.

**Results:** Four weeks after the surgery, arterial blood pressure was comparable among the three groups. However mice in terutroban group had higher levels of serum creatinine and lower survival. Compared with 2K groups, 5/6K-off mice had significantly higher levels of renal blood flow as well as a blunted relaxation to acetylcholine. Production of prostacyclin (PGI2) and thromboxane B2 (TXB2), but no prostaglandin E2 (PGE2), were significantly increased in the renal artery of 5/6K-off group. Terutroban restored the renal blood flow, but not the acetylcholine-induced relaxation in the renal artery. It is probably due to the blockade effect of terutroban on the smooth muscle since terutroban

treatment significantly reduced U46619-induced vasoconstriction in renal arteries. Interestingly, terutroban increased the production of TXB2, but not PGI2 or PGE2, in the renal artery. This probably is a compensatory effect on prostaglandins production. In kidney cortex, 5/6K-off group had significantly lower levels of PGE2 and TXB2 when compared with 2K group. Terutroban markedly increased all three prostaglandins levels.

**Conclusion:** Terutroban restores renal artery function, but not renal function in mouse with 5/6 nephrectomy. It suggests that kidney has more complicated regulations than renal artery. High levels of prostanoids in kidneys may contribute to renal damage in terutroban group. Further experiments will focus on examining the underlying mechanisms.

#### P36.

##### **SPERMINE INHIBITS HYPOXIA-INDUCED PULMONARY ARTERY SMOOTH MUSCLE CELL PROLIFERATION AND RELATED PATHWAYS**

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**Background:** Spermine, one composition of polyamine, plays a decisive role in the periodic cell proliferation and apoptosis. Pulmonary vascular remodeling is the significant pathological feature of hypoxia-induced pulmonary hypertension (HPH), while pulmonary artery smooth muscle cell (PASMCs) proliferation plays the leading role in pulmonary vascular remodeling. Therefore, the present study was performed to observe the relationship between hypoxia-induced PASMCs proliferation and polyamine metabolism and to explore the effects and related mechanism of exogenous spermine in the PASMCs proliferation progress.

**Methods:** PASMCs were cultured with Cobalt chloride (CoCl2) to establish hypoxia model, and spermine at different final concentration (0.1, 1, 10, 100  $\mu\text{mol/L}$ ) were added to the medium of PASMCs 40 min before hypoxia for pretreated. Cell proliferation was measured with MTT, cell counting kit-8 and BrdU. Cell cycle was measured by flow cytometry. The related protein expressions were measured using western blot.

**Results:** The results showed that PASMCs treated by CoCl2 at 50  $\mu\text{mol/L}$  for 24h were obviously proliferated. The expression of ODC, the key enzymes of polyamine biosynthesis, was decreased and the expression of SSAT, the key enzymes of terminal degradation of polyamine was increased in hypoxia condition. Exogenous spermine at 1 and 10  $\mu\text{mol/L}$  concentration significantly inhibited hypoxia-induced PASMCs proliferation, arrested the cells in G1/G0-phase, decreased cyclin D1 expressions, increased P27 expressions, and suppressed

phosphorylation of ERK1/2, PI3K and AKT, but the above-mentioned parameters were not affected obviously by spermine at 0.1 and 100  $\mu\text{mol/L}$  concentration.

**Conclusions:** These results suggested that hypoxia could cause polyamine metabolism disorder, and spermine at 1 and 10  $\mu\text{mol/L}$  concentration could inhibit chemical hypoxia-induced human PASMCs proliferation via suppressed ERK 1/2 and PI3K / AKT-associated pathways. These findings will provide new insight into the prevention and treatment of HPH.

**Acknowledgements:** This research is supported by the National Natural Science Foundation of China (No. 81070123, 81270311)

## ABSTRACTS

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### Abstracts for Posters:

#### P37.

##### ANTIINFLAMMATION OF APELIN IN AMELIORATION HYPOXIA PULMONARY ARTERY HYPERTENSION IS THROUGH AN INHIBITION OF NF- $\kappa$ B PATHWAY

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**Objective:** To investigate whether antiinflammation of apelin in amelioration of HPAH is through an inhibition of nuclear factor- $\kappa$ B (NF- $\kappa$ B) pathway.

**Methods:** Mice were exposed in a normobaric hypoxic chamber with a fraction of inspired oxygen (FiO<sub>2</sub>) of ~10%, or hypoxia combined with apelin application, 23 h/d, continued for 2 weeks. Mean right ventricular pressure (mRVP) was measured by pulmonary artery catheterization. Right ventricle hypertrophy (RVH) was assessed by the ratio of right ventricle/left ventricle plus septum (RV/LV+S). Pulmonary vascular remodeling, inflammatory response, and nucleus translocation of NF- $\kappa$ B were also examined.

**Results:** Hypoxia increased mRVP and RVH as well as TNF- $\alpha$  and IL-1 $\beta$  levels in lung tissue, which were blocked with apelin application. Hypoxia-increased NF- $\kappa$ B translocation into the nucleus of lung tissue was suppressed by apelin pretreatment, suggesting amelioration of apelin in hypoxic PAH in mice is through antiinflammation by a robust inhibition of the NF- $\kappa$ B pathway. Utilizing mice with NF- $\kappa$ B deletion (p50<sup>-/-</sup>), we found there was no obvious mRVP or RVH or inflammation response after two weeks hypoxia exposure, further confirming NF- $\kappa$ B activation is involved in the development HPAH and RVH.

**Conclusion:** Our data highlights the potential role of apelin/NF- $\kappa$ B axis as a novel regulatory pathway involved in antiinflammation and amelioration of HPAH and RVH.

*This work was supported by the Natural Science Foundation of Zhejiang Province of China (LY12H01004, Y2091033).*

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#### P38.

##### EFFECT OF EXOGENOUS CST ON BLOOD PRESSURE AND CARDIAC FUNCTION IN RENAL HYPERTENSION RATS

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**Objective:** To examine the role of catestatin (CST) in cardiac function and blood pressure regulation in renal hypertension induced by the method of '2-kidney 1-clip (2K1C)' in rats.

**Methods:** Forty male Sprague Dawley rats were randomly divided into four groups: Control, Control+CST, 2K1C, and 2K1C+CST group. Six weeks after 2K1C operation, CST (80  $\mu$ g/100g weight) was administrated. The blood pressure and cardiac function were measured by left ventricular and arteria cruralis catheter. Levels of histamine (His), epinephrine (E) and CST in plasma were measured by ELISA assay. Levels of nitro oxide (NO) in plasma and left ventricular tissue were measured by nitrate reduction method. Calcium receptor-like receptor (CRLR) gene expression levels were tested by real-time PCR.

**Results:** Compared with Control group, (1) the blood pressure of rats in 2K1C group was increased gradually from the 3rd week after 2K1C operation, and reached maximum in the 6th week after 2K1C operation. (2) Systolic function and diastolic function parameters of rats in 2K1C group were higher than those in Control group, 2K1C combined with CST application significantly lowered systolic function and diastolic function parameters, when compared with those in 2K1C group. (3) The pressure volume loop (PVL) in 2K1C group was to the right and its area was increasingly shifted under the effect of pressure load. However, the PVL in 2K1C+CST group was left and its area was decreasingly shifted, when compared with those in 2K1C group.

(4) Levels of CST in 2K1C group were lower than those in Control group. However, levels of His, E and NO in 2K1C group were higher than those in Control group. 2K1C combined with CST application significantly increased His and NO, when compared with those in 2K1C or Control group. (5) Results from real-time PCR showed that levels of CRLR gene expression in left ventricular of rats in 2K1C group were lower when compared with those in the Control group. 2K1C combined with CST application significantly decreased CRLR gene expression in left ventricular, when compared with those in 2K1C group.

**Conclusions:** These results indicate CST plays an important role in anti-hypertention and cardiac function inhibition, the role of CST may be through His, calcitonin gene-related peptide and NO pathway.

*This work was supported by the National Natural Science Foundation of China (81200039), the Natural Science Foundation of Zhejiang Province of China (LY12H01004), the Foundation of Zhejiang Educational Committee (Y201326833).*

ABSTRACTS

Abstracts for Posters:

P39.

**TETRAMETHYLPYRAZINE REGULATES SOLUBLE EPOXIDE EXPRESSION IN CORONARY ENDOTHELIUM: ANTI-ER STRESS AGAINST ANGIOTENSIN II**

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**Objectives:** Tetramethylpyrazine (TMP), an active ingredient of Chinese herb Chuanxiong, is known for cardioprotective benefits due to its anti-oxidant and anti-inflammatory properties. Endoplasmic reticulum (ER) stress-induced endothelial dysfunction is involved in cardiovascular disorders such as hypertension. Whether TMP possesses anti-ER stress effect on endothelium against angiotensin II (Ang-II) remains unexplored. Dysregulation of endothelial soluble epoxide hydrolase (sEH) occurs in hypertension. Whether Ang-II affects sEH through the induction of ER stress and whether TMP regulates sEH in ER-stressed endothelium are barely studied.

**Methods:** Endothelial cells were isolated by enzymatic digestion from porcine coronary arteries and primary cultured cells were used for western blot analysis.

**Results:** Endothelial cells exposed to Ang-II showed marked upregulation of ER stress molecules including p-PERK, p-IRE1a, and GRP78, along with a significant increase of sEH expression, which were suppressed by co-incubation of the cells with ER stress inhibitors, either 4-PBA or TUDCA. TMP was effective in inhibiting Ang-II-induced ER stress at concentrations

$\geq 10 \mu\text{M}$  with maximal effect achieved by  $100 \mu\text{M}$ . Downregulation of ER stress molecules by TMP in Ang-II-exposed cells is accompanied by a significant decrease in sEH expression.

**Conclusions:** ER stress mediates Ang-II-induced sEH upregulation in coronary endothelial cells. TMP downregulates Ang-II-induced sEH upregulation, which is related to its anti-ER stress capacity.

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P40.

**THE ROLE OF CaSR IN oxLDL-INDUCED PROLIFERATION AND MIGRATION IN A7r5 CELLS**

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**Objective:** To explore the role of Calcium-sensing receptor (CaSR) in oxidized low-density lipoprotein (oxLDL)-induced proliferation and migration in rat thoracic aorta smooth muscle cells (A7r5 cells) and signaling mechanisms.

**Method:** BrdU incorporation assay to detect cell proliferation; Wound healing assay and Transwell migration analysis to detect cell migration; The protein expression of CaSR, PCNA, ERK MAPK pathway and PI3K/AKT pathway was detected by Western blot analysis.

**Results:** (1) Low dose oxLDL ( $10 \mu\text{g}/\text{mL}$ ) for 24h induced proliferation and migration in A7r5 cells; (2) oxLDL increased CaSR expression in A7r5 cells; (3) CaSR antagonist (NPS2390) inhibited the effect of oxLDL, and the agonist Gdcl3 further enhanced the effect of oxLDL; (4) oxLDL stimulated the protein expression of p-AKT and p-ERK; (5) PI3K/AKT pathway inhibitor (LY294002) and ERK MAPK pathway inhibitor (PD98059) could inhibit oxLDL-induced proliferation and migration in A7r5 cells; (6) NPS2390 inhibited oxLDL-induced the expression of p-AKT and p-ERK, whereas GdCl3 has the opposite effect.

**Conclusion:** (1) OxLDL induced proliferation and migration in A7r5 cells; (2) Involvement of CaSR in oxLDL-induced proliferation and migration in A7r5 cells; (3) Involvement of CaSR in oxLDL-induced proliferation and migration in A7r5 cells via PI3K/AKT and ERK MAPK signal pathway.

**Acknowledgments:** This study was supported by the National Natural Science Foundation of China (no. 81300200)

P41.

**CONDITIONAL KNOCKOUT OF FGF13 IN MURINE HEARTS CAUSES FLECAINIDE-INDUCIBLE VENTRICULAR TACHYARRHYTHMIA**

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**Aims:** Cardiac  $\text{Na}^+$  channels play critical roles in impulse generation and conduction in ventricular tissue. Fibroblast growth factor homologous factors (FHF) are essential modulators of  $\text{Na}^+$  channels and *FGF12*, the dominant FHF in human heart, has recently been described as a locus for Brugada Syndrome. *FGF13*, the primary FHF in murine heart, has been shown to modulate cardiac  $\text{Na}^+$  channels and affect impulse conduction in cultured cardiomyocytes. However, the roles of FHF on cardiac electrical activities *in vivo* remain largely unknown.

**Methods and Results:** Here, we generated inducible, cardiomyocyte-restricted *FGF13* conditional knockout mice and studied their cardiac electrophysiological properties *in vivo* and *in vitro*. Compared to wildtype and *Cre* control mice, *FGF13* knockout mice had a prolonged QRS duration on electrocardiogram. Administration of flecainide, a class IC  $\text{Na}^+$  channel blocker, further prolonged QRS duration in *FGF13* knockout mice and triggered ventricular tachyarrhythmias. Action potentials in *FGF13* knockout mice exhibited slower upstrokes, reduced amplitude, and longer durations. Whole-cell patch clamp recordings from ventricular myocytes demonstrated a  $\sim 25\%$  reduction in peak  $\text{Na}^+$  channel current densities and a hyperpolarizing shift in steady-state inactivation, suggesting that observed arrhythmias in *FGF13* knockout mice resulted at least in part from a loss of functioning  $\text{Na}^+$  channels.

**Conclusions:** *FGF13* is a critical cardiac  $\text{Na}^+$  channel modulator and *FGF13* mediated sodium channel dysregulation is arrhythmogenic in mice.

## ABSTRACTS

Abstracts for Posters:

### P42.

#### MACROPHAGE MIGRATION INHIBITORY FACTOR PROMOTES EXPRESSION OF GLUT4 GLUCOSE TRANSPORTER THROUGH MEF2 AND Zac1 IN CARDIOMYOCYTES

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**Objective:** Evidence shows that both macrophage migration inhibitory factor (MIF) and GLUT4 glucose transporter are involved in diabetic cardiomyopathy (DCM), but it remains largely unknown whether and how MIF regulates GLUT4 expression in cardiomyocytes. The present study aims to investigate the mechanism underlying the modulation of GLUT4 by MIF in cardiomyocytes.

**Material and Methods:** Activations of AKT and AMPK signaling, and expressions of MIF, GLUT4 and the candidate GLUT4 regulation associated transcription factors in the diabetic mouse myocardium were determined. The screened transcription factors mediating MIF-promoted GLUT4 expression were verified by RNA interference (RNAi) and electrophoretic mobility shift assay (EMSA), respectively.

**Results:** MIF was increased, but GLUT4 was decreased in the diabetic mouse myocardium. MIF could enhance glucose uptake and up-regulate GLUT4 expression in NMVCs. Expressions of transcription factor MEF2A, -2C, -2D and Zac1 were significantly up-regulated in MIF-treated neonatal mouse ventricular cardiomyocytes (NMVCs), and markedly reduced in the diabetic myocardium. Knockdown of MEF2A, -2C, -2D and Zac1 could significantly inhibit glucose uptake and GLUT4 expression in cardiomyocytes. Moreover,

EMSA results revealed that transcriptional activities of MEF2 and Zac1 were significantly increased in MIF-treated NMVCs. Additionally, MIF effects were inhibited by an AMPK inhibitor compound C and siRNA targeting MIF receptor CD74, suggesting the involvement of CD74-dependent AMPK activation.

**Conclusions:** Transcription factor MEF2 and Zac1 mediate MIF-induced GLUT4 expression through CD74-dependent AMPK activation in cardiomyocytes.

### P43.

#### CHOLINERGIC STIMULATION WITH PYRIDOSTIGMINE EXERTS PROTECTIVE EFFECTS IN RAT MODELS OF CARDIAC REMODELING

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**Background:** Cardiac remodeling in cardiovascular diseases is always accompanied by enhanced sympathetic activity and withdrawal of parasympathetic control. Recent studies showed that vagal nerve stimulation reduced myocardial infarction areas and improved cardiac function. Pyridostigmine, a reversible cholinesterase inhibitor, may be potentially beneficial for cardiovascular protection through improving vagal activity. However, little information is available regarding the effects of pyridostigmine on cardiac remodeling.

**Methods:** The present study investigated the effect of pyridostigmine on two rat models of cardiac remodeling: i) myocardial infarction was induced by coronary artery ligation, and ii) cardiac hypertrophy was established by constriction of abdominal aorta. Vagal nerve activity was evaluated by the intensity and frequency of discharge and the serum level of acetylcholine. Hemodynamic parameters and cardiac function were measured by PowerLab and echocardiography. Cardiomyocyte hypertrophy and interstitial fibrosis was measured by hematoxylin-eosin staining, Masson's trichrome and Picrosirius red. The biomarkers and possible signaling pathways for cardiac remodeling were quantitated by western-blot analyses.

**Results:** Pyridostigmine restored vagal tone and markedly attenuated cardiac dysfunction in both models. i) In rats with myocardial infarction, treatment

with pyridostigmine significantly improved hemodynamics and reduced collagen deposition and the level of biomarkers for cardiac fibrosis (MMP-2 expression and activity). The protective effect of pyridostigmine on cardiac fibrosis may be related to inhibition of TGF- $\beta$ 1/TAK1 pathway. ii) In rats with abdominal aortic constriction, pyridostigmine administration enhanced discharge of vagal nerve and increased serum ACh level. Pyridostigmine attenuated cardiomyocyte hypertrophy evidenced by decreased cardiomyocyte cross-sectional diameter and left ventricular weight/body weight. Pyridostigmine reduced the expression of ANP, BNP and b-MHC. Our results suggested that pyridostigmine inhibited CaN/NFAT3/GATA4 signaling cascade to protect myocardium against myocardial hypertrophy.

**Conclusion:** Increased vagal tone induced by pyridostigmine is important to restore the balance of the autonomic nervous system and ameliorated cardiac dysfunction in two models of cardiac remodeling. Our study may provide evidences that pharmacological treatment involving reversible cholinesterase inhibitor exerted beneficial effects in cardiovascular diseases.

**Supported by:** Grant from National Natural Science Foundation of China (Major International Joint Research Project, No. 81120108002; General Project, No. 81473203), Specialized Research Fund for the Doctoral Program of Higher Education (No. 20130201130008).

## ABSTRACTS

### Abstracts for Posters:

#### P44.

##### IMPROVING VAGAL ACTIVITY AMELIORATES CARDIAC FIBROSIS INDUCED BY ANGIOTENSIN II: *IN VIVO* AND *IN VITRO*

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**Background:** Cardiac remodeling is characterized by overactivity of the renin-angiotensin system (RAS) and withdrawal of vagal activity. We hypothesized that improving vagal activity could attenuate cardiac fibrosis induced by angiotensin II (Ang II) *in vivo* and *in vitro*.

**Methods:** Rats were subjected to abdominal aorta constriction (AAC) with or without pyridostigmine (PYR) (31 mg/kg/d). After 8 weeks, PYR significantly decreased Ang II level, AT1 protein expression, and collagen deposition in cardiac tissue and improved heart rate variability and baroreflex sensitivity (BRS), which were abolished by atropine. *In vitro*, treatment of cardiac fibroblasts (CFs) with Ang II ( $10^{-7}$  M) for 24 h increased cell proliferation, migration, transformation, and secretory properties, which were significantly diminished by acetylcholine (ACh,  $10^{-6}$  M).

**Results:** Ang II significantly increased collagen type I expression as well as metalloproteinase (MMP)-2 expression and activity. Transforming growth factor (TGF)- $\beta$ 1 expression and Smad3 phosphorylation presented a similar trend. Notably, the knockdown of the acetylcholine M2 receptor (M2 AChR) by siRNA could abolish ACh anti-fibrotic action.

**Conclusion:** These data implicated cholinesterase inhibitor can increase vagal activity and reduce local Ang II level, and ACh can inhibit Ang II pro-fibrotic effects. Our findings suggested that the parasympathetic nervous system can serve as a promising target for cardiac remodeling treatment.

**Supported by:** Grant from National Natural Science Foundation of China (Major International Joint Research Project, No. 81120108002; General Project, No. 81473203, 81471471, 81000102), Specialized Research Fund for the Doctoral Program of Higher Education (No. 20130201130008).

#### P45.

##### TARGETED INHIBITION OF ATP-BINDING CASSETTE TRANSPORTER A1 PROTEIN DEGRADATION PROMOTES REVERSE CHOLESTEROL TRANSPORT AND REDUCES ATHEROSCLEROSIS IN MICE

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ABCA1 is a 2,261-amino-acid integral membrane protein that comprises two halves of similar structure. Each half has a transmembrane domain containing six helices and a nucleotide binding domain (NBD) containing two conserved peptide motifs known as Walker A and Walker B, which are present in many proteins that utilize ATP, and a Walker C signature unique to ABC transporters. ABCA1 is the important regulator of circulating HDL-C and cellular cholesterol homeostasis, which utilizes ATP to generate the energy needed to transport free cholesterol and phospholipids across membranes to lipid-free apoA-I. It participates in the plasma HDL-C biogenesis and initiates the reverse cholesterol efflux. Mutations in ABCA1 genes cause a variety of diseases, including Tangier disease, familial HDL deficiency and disturbances in lipid and lipoprotein metabolism. Overexpression of ABCA1 protein leads to increase foam macrophages cholesterol efflux and alleviate atherosclerotic plaque formation. Studies found that LXR agonists upregulate the expression of ABCA1 and ABCG1 to accelerate cholesterol efflux to reduce atherosclerotic plaque development. Unfortunately, current synthesized LXR agonists are nonspecific activate LXR $\alpha$  and  $\beta$  to induce fatty acid synthesis, which result in hypertriglycerides, fatty liver or hepatic steatosis. However, there were fewer studies focused on posttranscriptional regulating of ABCA1

protein. Thus, we hypothesized that inhibition of ABCA1 protein degradation could raise ABCA1 turnover and enhance cholesterol efflux, resulting in reduced atherosclerotic plaque formation. Pulse-chase analysis revealed that a small molecule (IMM-H007) significantly increased ABCA1 protein expression *in vitro* and *in vivo*, without obviously effects on ABCA1 mRNA levels, indicated that IMM-H007 may not regulate ABCA1 on transcriptional levels. Next, we observed that IMM-H007 inhibited ABCA1 degradation and facilitated its cell-surface localization in THP-1 macrophages. The mechanism maybe involved in suppressing calpain activity to inhibit calpain-mediated ABCA1 protein degradation. The increased ABCA1 distribution on plasma membrane would be predicted to the augment of HDL-C biogenesis, which has been proved in different animals fed with chow diet or western diet. In addition, enhanced fecal neutral cholesterol excretion was also found in parallel with the increased HDL-C levels. *In vitro* isotope and fluorescence staining tests demonstrated that IMM-H007-treated HDL was functional as facilitating cellular cholesterol efflux, reducing intracellular lipid accumulation. Further, we observed that IMM-H007 promoted cholesterol efflux via ABCA1-mediated pathway in the study of different cholesterol efflux pathways' contributions. *In vivo* RCT assay showed that IMM-H007 enhanced HDL-dependent cholesterol efflux function by promoting RCT to the plasma, liver, and feces. Finally, apoE<sup>-/-</sup> mice fed with western diet containing 1.25% cholesterol and indicated doses of IMM-H007 treatment showed reduction of plaque size and lipid content, in addition, the number of macrophages decreased and collagen content increased in aortic root cryosection, suggesting IMM-H007 increase markers of plaque stability. In conclusion, our findings reveal that raising HDL levels by inhibiting ABCA1 protein degradation via suppressing calpain activity, enhances RCT and decreases plaque formation, suggesting that it is a promising HDL therapeutic strategy to protect against atherosclerotic. IMM-H007 could be a potential compound for the development of drugs for raising HDL quantity and function.

ABSTRACTS

Abstracts for Posters:

P46.

**LIPOPROTEIN (A) IS A RISK FACTOR FOR CORONARY ARTERY DISEASE IN CHINESE HAN ETHNIC POPULATION MODIFIED BY SOME TRADITIONAL RISK FACTORS: A CROSS-SECTIONAL STUDY OF 3,462 CASES AND 6,125 CONTROLS**

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**Background:** Lipoprotein (a) (Lp (a)) is a well-established risk factor for coronary artery disease (CAD) in Caucasians. However, data regarding the association of Lp (a) with CAD are lacking in Chinese Han population.

**Methods:** Cross-sectional study of 3,462 cases and 6,125 controls was performed for identifying the association of Lp (a) with CAD and its possible interactions with risk factors on CAD in Chinese.

**Results:** The Lp (a) levels in Chinese Han population were on average much lower than those in Caucasians. The suggested Lp (a) cutoff for discriminating CAD from non-CAD was 78.9 mg/dl. On a continuous scale, after fully-multivariate adjustment, the odds ratios per higher 10 mg/dl Lp (a) levels were 1.079 for initial IHD, 1.129 for initial MI, and 1.139 for prior CAD. On a categorical scale, after fully-multivariate adjustment, the odds ratios for initial IHD were 1.093 for Q2 (2nd Lp (a) quintile), 1.184 for Q3, 1.236 for Q4, and 1.586 for Q5, in reference to Q1; the odds ratios for initial MI were 1.493 for Q2, 1.633 for Q3, 1.933 for Q4, and 2.821 for Q5, in reference to Q1; the odds ratios for prior CAD were 1.201 for Q2, 1.208 for Q3, 1.379 for Q4, and 2.096 for Q5, in reference to Q1. Significant interactions were found between Lp (a) and primary hypertension, body mass index, total cholesterol, and creatinin on CAD subdivisions.

**Conclusions:** The Lp (a) distribution in Chinese Han population differs from that in Caucasian populations. The higher Lp (a) level is a confirmative risk factor for CAD, which can be modified by some risk factors in Chinese Han population. Our study provides direct evidences for differential approach to higher Lp (a) levels in Chinese.

P48.

**MICRORNA-133B REGULATES PROLIFERATION OF VASCULAR SMOOTH MUSCLE CELLS INDUCED BY LOW SHEAR STRESS**

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**Objectives:** Shear stress, especially low shear stress (LowSS), plays a crucial role in vascular remodeling, which is a major component of many cardiovascular diseases. Our previous study revealed that shear stress induces the secretion of insulin-like growth factor1 (IGF1) from endothelial cells (ECs) and then modulates vascular smooth muscle cells (VSMCs) via paracrine control. The crosstalk between ECs and VSMCs has been proved to be very important to vascular remodeling. MicroRNAs (miRs) are crucial for diverse cellular processes, but the expressions and functions of miRs in VSMCs responding to IGF1 induced by mechanical stimuli remain unclear. In the current study, we sought to determine the effect of miRs on endothelial IGF1- induced VSMC proliferation under shear stress.

**Methods:** Using parallel-plate flow chamber system, normal shear stress (NSS, 15 dyn/cm<sup>2</sup>) and LowSS (5 dyn/cm<sup>2</sup>) were applied to ECs co-cultured with VSMCs. The prediction of miRs modulated by IGF1 were analyzed by Ingenuity Pathway Analysis software (IPA). By using the websites of miRWalk and TargetScan, we picked out five target genes of miRs. 100 ng/mL recombinant protein of IGF1 (rIGF1) was added to the culture medium to stimulate VSMCs for 12 h. The expression level of miRs and mRNA level of their target genes were analyzed by Real-time RT-PCR. The expression of certain target genes was detected by western blot. miRs mimics and inhibitor were used to confirm the interplay between miRs and their target genes. The proliferation of VSMCs was determined by EdU flow cytometry assay kits.

P47.

**UPCONVERSION NANOPARTICLE-MEDIATED PHOTODYNAMIC THERAPY INDUCES THP-1 MACROPHAGE APOPTOSIS THROUGH THE MITOCHONDRIAL CASPASE PATHWAY**

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**Objectives:** Atherosclerosis (AS) is the most vital cardiovascular disease, which poses a great threat to human health. Macrophages play an important role in the progression of AS. Photodynamic therapy (PDT) has emerged as a useful therapeutic modality not only in the treatment of cancer but also in the treatment of AS. The purpose of this study was to determine the molecular mechanisms underlying the activity of PDT, using mesoporous-silica-coated upconversion fluorescent nanoparticles encapsulating chlorin e6 (UCNPs-Ce6) in the induction of apoptosis in THP-1 macrophages.

**Methods:** We investigated the ability of UCNPs-Ce6-mediated PDT to induce THP-1 macrophage apoptosis by facilitating the induction of reactive oxygen species (ROS) and regulation of mitochondrial permeability transition pore (MPTP) to depolarize mitochondrial membrane potential (MMP). Both Bax translocation and the release of cytochrome C were examined using immunofluorescence and Western blotting.

**Results:** Our results indicated that the levels of ROS were significantly increased in the PDT group, resulting in both MPTP opening and MMP depolarization, which led to apoptosis. In addition, immunofluorescence and Western blotting revealed that PDT induced both Bax translocation and the release of cytochrome C, as well as upregulation of cleaved caspase-9, cleaved caspase-3, and cleaved poly (ADP-ribose) polymerase.

**Conclusions:** UCNPs-Ce6-mediated PDT induces apoptosis in THP-1 macrophages via ROS bursts. The proapoptotic factor Bax subsequently translocates from the cytosol to the mitochondria, resulting in the MPTP opening and cytochrome C release. This study demonstrated the great potential of UCNPs-Ce6-mediated PDT in the treatment of AS.

**Foundation:** This study was supported by the National Natural Science Foundation of China (81000688, 81271734, 81571833)

**Results:** IPA revealed that IGF1 might regulate 4 types of miRs, i.e. miR-1, miR-133b and miR-378a. In static, the expression of miR133b and miR-378a was significantly increased by rIGF1 stimulation. Compared to NSS, ECs subjected to LowSS induced miR-133b expression in co-cultured VSMCs but miR-378a did not change obviously. NdrG1, a putative target gene of miR133b, was suppressed under LowSS. I miR-133b expression was up-regulated after miR-133b mimic transfection and down-regulated after miR-133b inhibitor transfection. The predicted target genes of miR-133b, Ptbpl and NdrG1, were significantly decreased by miR-133b mimics treatment at mRNA and protein levels. NdrG1 was significantly increased by miR-133b inhibitor treatment at mRNA level. After miR-133b mimics transfection, the proliferation of VSMCs was promoted.

**Conclusions:** Our results suggest that LowSS induces the secretion of IGF-1 from ECs, which subsequently paracrine influences the expression of miR-133b in the neighboring VSMCs, which regulates its target gene NdrG1 and then results in the proliferation of VSMCs. (This research was supported by grant from the National Natural Science Foundation of China, Nos. 11002091, 11232010).

ABSTRACTS

Abstracts for Posters:

P49.

**THE ROLE OF Rab28 IN VASCULAR REMODELING IN HYPERTENSIVE RATS**

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**Objectives:** Rab28 is a mechanosensitive protein, which is regulated by shear stress and mechanical strain in vascular tissues and cells. The study was to investigate the role of Rab28 in vascular remodeling and related molecular mechanisms.

**Methods:** Hypertensive rat model was established by abdominal aortic constriction, and sham-operated animal was used as control. Vascular smooth muscle cells (VSMCs) were subjected to the strains *in vitro* of 0% (static control), 5% (normal tensile strain), or 15% elongation (high tensile strain) at 1.25 Hz for 12 or 24 h. The expression level of Rab28, FOXO4, p-FOXO4, FOXO1, p-FOXO1 as well as differentiated VSMC markers, including  $\alpha$ -actin, sm22 and calponin, were detected by western blot. Rab28 siRNA was transfected to VSMCs, and then the expression levels of Rab28,  $\alpha$ -actin, sm22 and calponin were detected by western blot.

**Results:** The expressions of Rab28, FOXO4, p-FOXO4, FOXO1, p-FOXO1 and differentiated VSMC markers, including  $\alpha$ -actin, sm22 and calponin, were all significantly increased in hypertension rat, in contrast to sham control. In contrast to 5% elongations (normal strain), 15% strain (high strain), *in vitro*, significantly increased expressions of Rab28, FOXO4, p-FOXO4, FOXO1, p-FOXO1. In contrast to 0% elongation,  $\alpha$ -actin, sm22 and calponin increased in 5% elongation, while the expressions of  $\alpha$ -actin, sm22 and calponin were significantly increased in 15% strain after applied for 12 and 24 hours. Specific siRNA of Rab28 also significantly decreased expressions of  $\alpha$ -actin, sm22 and calponin.

**Conclusions:** Cyclic strain modulates expression of Rab28, which induces expressions of FOXO4, p-FOXO4, FOXO1, p-FOXO1, and further regulates VSMC differentiation. These results indicate that Rab28 and FOXOs play key roles in vascular remodeling in hypertension. (This work was supported by grants from the National Natural Science Foundation of China, Nos. 31170892, 11232010)

P50.

**NEUROPEPTIDE Y PROMOTES VASCULAR REMODELING ON PATHOGENESIS RESEARCH IN PREGNANCY HYPERTENSIVE**

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**Objectives:** The increased proliferation of vascular smooth muscle cells (VSMCs) plays important roles in pathophysiological vascular remodeling during pregnancy in hypertension. However, the mechanisms involved in this process remain unclear. Recent researches revealed that peripheral Neuropeptide Y (NPY) may participate in vascular remodeling during hypertension. Here, we detected the role of NPY in proliferation and migration of VSMCs during gestational hypertension.

**Methods:** Using pregnant hypertensive rats, induced by intraperitoneal injection of L-nitro-arginine methylester (L-NAME), the plasma concentration of NPY was detected by radioimmunoassay. Opening angle and H&E staining were used to detect the vascular remodeling during pregnant in hypertension *in vivo*. Expressions of collagen I and collagen III were analyzed by western blotting. Further more, recombinant NPY and different NPY receptor antagonist were used to stimulate cultured VSMCs *in vitro*. Proliferation of VSMCs was detected by WST-1.

**Results:** Compared the saline groups, the NPY concentration, the thickness of smooth muscle layer and artery open angle were all significantly increased in rats with hypertension in pregnant. The ratio of collagen I to collagen III was significantly decreased in pregnant hypertension. In cultured VSMCs, NPY most effectively stimulated the proliferation of VSMCs at  $10^{-6}$  mol/L, similar to the plasma concentration in L-NAME hypertension in pregnant rats. NPY up-regulated the expressions of both Y1 and Y5 receptors, and increased the

phosphorylations of STAT3 on Tyr705 and Ser727 residues. The NPY-induced VSMC proliferation was reduced by Y5 receptor antagonist, and fully blocked by combinations with other antagonist, including Y2+Y5, Y1+Y5, and Y1+Y2+Y5.

**Conclusions:** These results suggest that the elevated plasma concentration of NPY during hypertension in pregnancy may induce VSMC proliferation mainly via Y5 receptor, which subsequently modulates STAT3 signaling pathway. NPY plays important roles in pathophysiological vascular remodeling during pregnancy in hypertension. (This work was supported by grants from the National Natural Science Foundation of China, Nos. 11102112, 11372190.)

ABSTRACTS

Abstracts for Posters:

**P51.**

**MINOCYCLINE-SUPPRESSED PERIPHERAL INFLAMMATION IS ASSOCIATED WITH HYPOXIA-INDUCED NEONATAL BRAIN INJURY**

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**Objective:** While extensive studies report that neonatal hypoxia-ischemia (HI) induces long-term cognitive impairment via inflammatory responses in the brain, little is known about the role of leukocyte-mediated peripheral inflammation response in HI injury.

**Methods:** Here we used a HI rodent model by subjecting postnatal day 0 (P0d) rat pups to systemic hypoxia (3.5h), a condition that is commonly seen in clinic neonates, to examine how leukocytes responded to hypoxia and whether this peripheral inflammation response was associated to cognitive deficits.

**Results:** We found that hypoxia significantly increased leukocytes not only in blood, but also increased the monocytes in CNS, indicated by expression level of C-C chemokine receptor type 2 (CCR2). The early onset of peripheral inflammation response was followed by a late onset of brain inflammation that was demonstrated by level of cytokine IL-1 $\beta$  and ionized calcium binding adapter molecule 1 (ba1; activated microglial cell marker). Interrupted blood-brain barrier (BBB), hypomyelination and learning and memory deficits were seen after hypoxia. Interestingly, the cognitive function was highly correlated with hypoxia-induced leukocyte response. Notably, administration of minocycline even after the onset of hypoxia significantly suppressed leukocyte-mediated inflammation as well as brain inflammation, demonstrating neuroprotection in systemic hypoxia-induced brain damage.

**Conclusion:** Our data provided new insights that systemic hypoxia induces long-term cognitive dysfunction, which involves the leukocyte-mediated peripheral inflammation response.

**P52.**

**ATORVASTATIN SUPPRESSES TRPM2 CHANNEL TO PROTECT ENDOTHELIAL CELLS FROM OXIDATIVE INJURY**

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**Objective:** Vascular oxidative injury is involved in numerous cardiovascular diseases. Statins - a family of lipid lowering drugs - appear to display non-lipid modifying abilities. The present study was designed to investigate the role of TRPM2, a ROS-sensitive Ca<sup>2+</sup> entry channel, in the vasoprotective effect of atorvastatin.

**Methods and results:** Atorvastatin (50 mg·kg<sup>-1</sup>·d<sup>-1</sup>) was intragastrically administrated to rats for 4 weeks. Segments of third-order branches of the mesenteric artery were isolated for isometric tension recording. After H<sub>2</sub>O<sub>2</sub> exposure, improvement of acetylcholine-induced relaxation was showed in arterial rings from atorvastatin-administrated rats, compared with these from control group. Immunoblots showed that atorvastatin led to a down-regulated expression of TRPM2 in isolated arterial endothelial cells. Compared with the endothelial cells from control group, the TRPM2-mediated [Ca<sup>2+</sup>]<sub>i</sub> rise in response to hypoxanthine-xanthine oxidase was reduced in the endothelial cells from atorvastatin group. H<sub>2</sub>O<sub>2</sub>-induced apoptosis of endothelial cells was examined using MTT assay, DNA ladder formation analysis, and DAPI-based nuclear condensation assay. Cells from atorvastatin group were more resistant to the H<sub>2</sub>O<sub>2</sub>-induced apoptosis. The difference in cell apoptosis between atorvastatin group and control group was abolished by the TRPM2-specific blocking antibody TM2E3.

**Conclusion:** Atorvastatin reduces oxidative stress-induced endothelial cell injury and improves arterial function by suppressing the expression/function of TRPM2 channels.

**P53.**

**THE CHANGE OF PLASMA PROTEIN C ACTIVITY IN MYOCARDIAL INFARCTION**

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**Objective:** To explore the change of plasma protein C activity and conceivable mechanisms in myocardial infarction.

**Methods:** 30 male SD rats were randomly divided into control group and acute myocardial infarction (AMI) group. Rats from AMI group needed for cardiac left anterior descending coronary artery ligation and the control group without any treatment. RM6240 biological signal collector was performed for ECG signal collection. Rats carotid artery blood were taken in 0.5h, 1h, 2h and 4h after surgery, dual channel blood coagulation instrument was used for routinely testing of fibrinogen (FIB), activation of blood coagulation time (APTT), prothrombin time (PT) and thrombin time (TT); chromogenic substrate assay was used to test the activity of plasma protein C; antigen-antibody reaction was applied to detect fibrin degradation products (FDP), D-Dimer (D-Dimer); ELISA assay was used to test Vascular endothelial cell protein C receptor (EPCR), thrombosis, regulatory proteins (TM) and the content of serum troponin I (cTNI). All rats were sacrificed for cardiac HE staining after above testing.

**Results:** Contrasting with control group, ECG of rats in AMI group displayed that S-T section pushed up of arch upward, QRS complex morphological changed and T wave rised; the activity of plasma protein C began to decrease in 1h after surgery ( $P<0.05$ ) and the content of EPCR and TM began to increase and more obviously in 2h ( $P<0.05$ ); APTT and PT were shorter and the level of FIB was increased in 2h after operation (more markedly in 4h,  $P<0.01$ ); both FDP and D-Dimer got to particle agglutination in 2 h and more significantly in 4h ( $P<0.01$ ).

**Conclusion:** In myocardial infarction, plasma protein C system was activated and gradually consumed, which is the key part of ischemic injury. Plasma protein C testing may be the predictive indicator in clinical myocardial infarction patients.

**ABSTRACTS**

Abstracts for Posters:

**P54.**

**GENOME-WIDE ASSOCIATION STUDY OF ESSENTIAL HYPERTENSION IN CHINESE TIBETAN POPULATION**

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Essential hypertension (EH) is a complex trait disease, resulting from the interaction of genetic and environmental factors. In China, there is a significant difference of EH incidence and prevalence among different ethnic groups and regions. As one of the major ethnic minority groups in China, the unique genetic background and unsuitable hypoxia environment make Tibetans an ideal ethnic group for research on the effects of genetic environmental factors and the interaction of both. In order to identify the susceptibility genes to EH, a two-stage genome-wide association study (GWAS) was conducted. The first, we performed genotyping based-pooling DNA in cases-controls by Affymetrix SNP 6.0 array, which comprised 300 patients (SBP >160 mmHg and DBP >95 mmHg) and 300 unrelated control individuals (SBP <130 mmHg and DBP <80 mmHg) within families with EH. After a series of quality control procedures, we retained 591912 autosomal SNPs for the estimation of relative allele frequency (RAF). Using the Z combination test, more than the 600 top-ranking loci ( $P < 5 \times 10^{-5}$ ) were screened and 23 loci reached statistical significance at the genome-wide scale (Bonferroni correction,  $P < 5.8 \times 10^{-8}$ ). In the stage 2 replication study, we performed the genotyping for 120 top-ranking loci by SpectroCHIP11-G384 array, in a large population ( $n > 1000$  individuals for each) which were recruited from the region at altitudes from 3700 meters to 5000 meters above sea level. The results showed that there was 3 possibilities based on the odd ratio (OR) and *P* values, among 120 loci, 12 loci genotype is associated with susceptibility of EH ( $OR \geq 1.20$ ,  $P < 0.05$ ); 11 loci genotype is associated with prevention of EH ( $OR < 0.8$ ,  $P < 0.05$ ), rest OR located 0.81-1.19 as mediate. Our results add evidence for synergistic effect of susceptibility gene and preventive genes on genetic predisposition for EH.

**P56.**

**ADPRC EXPRESSION IS REGULATED BY RasGRP1 AND DOWNSTREAM OF RAS/JNK PATHWAY IN AORTA OF ApoE<sup>-/-</sup> MICE**

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As a transmembrane glycoprotein, adenosine diphosphate ribosyl cyclase (ADPRC) is expressed in various cells including vascular smooth muscle cell (VSMC) and has a multitude of diverse functions, such as catalyzing cADPR and NAADP production which enhance intracellular Ca<sup>2+</sup> concentration to promote VSMC proliferation and migration in pathogenesis of atherosclerosis. By now, the signal pathway of ADPRC expression in aorta of AS mice is still unclear. The current study was designed to explore the mechanisms of ADPRC expression in VSMC of aorta in ApoE<sup>-/-</sup> mice. In this study, 20 Male ApoE<sup>-/-</sup> mice (6 weeks old, 18-22 g) on a C57BL/6J background were randomly divided into four groups. Group 1 (NC group, n=5) was fed with normal chow. Group 2 (HFD group, n=5) was fed with high-fat Western-type diet (0.25% cholesterol, 21% fat), Group 3 (HFD+SP group, n=5) was fed with high-fat Western-type diet and JNK specific inhibitor SP600125 (30 mg/kg body weight per day), Group 4 (HFD+Cur group, n=5) was fed with high-fat Western-type diet and AP-1 inhibitor of nordihydroguaiaretic acid (NDGA, 100 mg/kg body weight per day) for 8 weeks. Five male C57BL/6J mice served as control. After 8 weeks, mice were anesthetized with chloral hydrate; aorta was removed and immediately frozen in liquid nitrogen. Aortic atherosclerotic lesions, VSMC proliferation and migration were assessed by histomorphological observation. ADPRC expression and alterations of RasGRP1, Ras, JNK, phospho-JNK, c-Jun and phospho-c-Jun were determined by Western Blot. AP-1 DNA binding activity were determined

**P55.**

**SURFACEOME ANALYSIS OF HUMAN PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES**

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**Objectives:** Human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) are highly heterogeneous and show a variety of chamber types (ventricular, atrial and pacemaker) and maturation states. Immunophenotyping and isolation of homogeneous subpopulations using antibodies against surface proteins would be critical for the use of hPSC-CMs for drug screening and transplantation. Our aim is to characterize the surfaceome of hPSC-CMs.

**Methods and Results:** Using a chemoproteomic approach, we have developed a cell surface proteome repository containing >700 N-linked glycoproteins from hPSC-CMs cultured for 15, 30, 60 and 90 days. Of these, some candidates show differential abundance across different stages of differentiation, and/or ventricular/atrial prevalence, and may be used for the isolation of stage- and chamber-specific subpopulations.

**Conclusions:** Immunophenotyped subpopulations will permit more robust disease modelling and pharmacotoxicology studies; and in the long-term, may help identify suitable cells for therapeutic applications.

by Electrophoretic Mobility Shift Assay (EMSA). The results showed that an increased expression of ADPRC and VSMC proliferation and migration in aortic atherosclerotic lesions derived from HFD group were observed followed by increased expression of RasGRP1, Ras, JNK, phospho-JNK, c-Jun, phospho-c-Jun and AP-1 DNA binding activity. The enhanced expression of ADPRC and followed alterations of phospho-JNK, phospho-c-Jun and AP-1 DNA binding activity as well as VSMC proliferation and migration were absent in NC group and C57BL/6J control mice. Treatment with SP600125 or NDGA decreased ADPRC expression and reversed VSMC proliferation and migration in HFD+SP and HFD+Cur group. These data shows that high-fat Western-type diet induced ADPRC expression in aorta of ApoE<sup>-/-</sup> mice may be via RasGRP1 and downstream of Ras/JNK pathway to enhance AP-1 DNA binding activity.

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## ABSTRACTS

### Abstracts for Posters:

#### P57.

##### PROTECTIVE EFFECTS OF PHYSION ON CARDIOVASCULAR FUNCTION VIA ACCENTUATION OF ATRIAL NATRIURETIC PEPTIDE SECRETION

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**Objectives:** Physion, an active anthraquinone constituent isolated from the rhubarb, a traditional Chinese herbal medicine which is widely used in clinical treatment, has cardiovascular protective properties. However, the effects of physion on the cardiovascular system and its pharmacological mechanisms of action have not been studied. The purpose of the present study was to explore the effects of physion on the cardiovascular function, and whether the cardiovascular effects of physion are related to accentuation of the atrial natriuretic peptide (ANP) secretion.

**Methods:** The changes in systolic blood pressure and heart rate of rats in vivo were measured using pressure transducer. To explore the effect of physion on the secretion of ANP and its mechanisms involved, experiments were performed in isolated perfused beating rabbit atria allowing measurement of ANP secretion, atrial pulse pressure, and stroke volume.

**Results:** Physion decreased systolic blood pressure and heart rate in a concentration-dependent manner. Physion increased ANP secretion concomitantly with a decrease in atrial pulse pressure and stroke volume in a concentration-dependent manner. Furthermore, the physion-induced changes in ANP secretion and atrial dynamics were attenuated by inhibition of L-type Ca<sup>2+</sup> channels with nifedipine.

**Conclusions:** The present study demonstrates that physion induces hypotensive effect via accentuation of atrial natriuretic peptide secretion through inhibition of L-type Ca<sup>2+</sup> channels.

**Acknowledgements:** This work was supported by research grants from the National Natural Science Foundation of China (Nos. 81270920, 31571175), the Natural Science Foundation of Shandong Province (No. ZR2015HM037)

#### P59.

##### THE MECHANISMS OF VASORELAXATION INDUCED BY ETHANOL EXTRACT OF *Rumex acetosa* IN RAT AORTA

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**Objectives:** *Rumex acetosa* L. (RA) is an important traditional Chinese medicine (TCM) commonly used in clinic for a long history in China and the aerial parts of RA has a wide variety of pharmacological actions such as diuretic, anti-hypertensive, anti-oxidative, and anti-cancer effect. However, the vasorelaxant effect of RA has not been previously evaluated. The present study was to evaluate the vasorelaxant effect and define the mechanism of action of the ethanol extract of *Rumex acetosa* L. (ERA) in rat aorta.

**Methods:** ERA was examined for their vascular relaxant effects in isolated phenylephrine-precontracted rat thoracic aorta. In addition, human umbilical vein endothelial cells (HUVECs) were used to examine nitric oxide (NO) synthase (NOS) activity by measuring NO production in the culture medium.

**Results:** The phosphorylation level of Akt and eNOS were assessed by Western blot analysis in the cultured HUVECs. ERA-induced endothelium-dependent relaxations were abolished by L-NAME (an NOS inhibitor) or ODQ (a sGC inhibitor), but not by indomethacin. Extracellular Ca<sup>2+</sup> depletion, diltiazem (an inhibitor of L-type Ca<sup>2+</sup> channel), and modulators of the store-operated Ca<sup>2+</sup> entry (SOCE), thapsigargin, 2-aminoethyl diphenylborinate and Gd<sup>3+</sup>, markedly attenuated the ERA-induced vasorelaxation. Inhibition of PI3-kinase/Akt signaling pathway markedly reduced the ERA-induced vasorelaxation. Furthermore, the ERA-induced vasorelaxation was

#### P58.

##### HOMOCYSTEINE AND FOLIC ACID ON THE EFFECT OF HYPERTENSIVE CEREBRAL HEMORRHAGE

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**Objective:** Study the different outcome on the C57BL/6 mice fed with different concentration of homocysteine and folate acid.

**Method:** The mouse were divided into six groups. One: Homocysteine + Angiotensin II (20); Two: Homocysteine+Folic Acid+angiotensin II (18) Three: Homocysteine+L-NAME+angiotensin II (20); Four: Homocysteine+Folic Acid+L-NAME+Angiotensin II (17); Five: L-NAME + angiotensin II (20); Six: angiotensin II (19). Five group was positive control group of cerebral hemorrhage and six groups was hypertension control. Concentration: Homocysteine 1.8 g/L. Folic Acid 0.071 µg/g/dag and L-NAME 100 ng/kg/day in water; Angiotensin II 1000 ng/kg/min in osmotic pumps. Feeding time is 16 weeks.

**Results:** Mice fed homocysteine one week after later, blood homocysteine concentration reached 23.4 µmol/L, folic acid concentration reached 19.8 ng/ml. Mice fed 16 weeks later: Homocysteine + Angiotensin II group died two; Homocysteine+Folic Acid+angiotensin II group died three, one died of cerebral hemorrhage; Homocysteine+L-NAME+angiotensin II group died fourteen, thirteen died of cerebral hemorrhage; Homocysteine+Folic Acid+L-NAME+Angiotensin II group died sixteen, thirteen died of cerebral hemorrhage; L-NAME + angiotensin II group died fifteen, ten died of cerebral hemorrhage; angiotensin II group died one not for cerebral hemorrhage. we found angiotensin II + homocysteine + L-NAME group and Ang II + L-NAME group: p=0.77 >0.05; Angiotensin II + homocysteine group Ang II group: p=0.174 >0.05 from the survival curves. We also compared the mortality of mice and found no significant difference (p>0.05). We found that homocysteine may have a certain impact on the hypertensive intracerebral hemorrhage in mouse and the impact of folic acid on cerebral hemorrhage did not reflect.

**Conclusion:** Mouse have hypertension, fed homocysteine maybe have some positive effect on intracerebral hemorrhage; folic acid to reduce the hypertensive intracerebral hemorrhage was not observed. The quantity of the mouse in our study is limited and we need for further research.

significantly attenuated by BaCl<sub>2</sub>, an inward rectifier K<sup>+</sup> channels (K<sub>IR</sub>) blocker, but not by tetraethylammonium, glibenclamide, or 4-aminopyridine. In HUVECs, ERA increased NO formation, which was prevented by L-NAME and by removing extracellular Ca<sup>2+</sup>. In addition, ERA promoted phosphorylation of Akt and eNOS, which were prevented by wortmannin and LY294002, indicating that ERA induces eNOS phosphorylation through the PI3-kinase/Akt pathway.

**Conclusions:** The ERA-induced vasorelaxation was dependent on endothelial integrity and NO production, and was mediated by endothelial PI3-kinase/Akt- and Ca<sup>2+</sup>-eNOS-NO signaling. The activation of K<sub>IR</sub> channels are also involved in the ERA-induced vasorelaxation.

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## ABSTRACTS

### Abstracts for Posters:

#### P60.

##### ADIPONECTIN AMELIORATES ENDOTHELIAL INFLAMMATION FOLLOWED BY INSULIN RESISTANCE VIA BLOCKING ROS/IKK $\beta$ /IRS-1 PATHWAYS

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Endothelial inflammation, triggered by free fatty acids, endotoxins and others, has a close relationship with insulin resistance (IR). Herein, we investigated the effect of adiponectin (APN), an adipocytes derived hormone, on palmitic acid (PA)-induced inflammation followed by IR in human umbilical vein endothelial cells (HUVECs). PA induced significant up-regulation of TNF- $\alpha$ , IL-6, and ICAM-1 mRNA expression, which was inhibited by APN. APN dramatically suppressed PA-induced protein expression of ICAM-1, NOX2, phosphorylated IKK $\beta$ , NF- $\kappa$ B p65, p38MAPK. It also inhibited PA-induced ROS formation. Furthermore, both APN and NAC attenuated PA-induced serine phosphorylation (S307) of IRS-1 and restored IRS-1 tyrosine phosphorylation (PY99) in response to insulin. In addition, APN restored insulin-mediated PI3K, Akt and eNOS phosphorylation and NO production in the presence of PA. These results showed that APN improves PA-induced IR. The beneficial effect of APN was possibly mediated by regulating inflammatory cytokines expression, inhibiting of ROS formation and promoting NO production *via* modulating NOX2, p38MAPK, NF- $\kappa$ B, IRS-1 and eNOS in endothelial cells.

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#### P61.

##### OXYGEN-SENSITIVE MITOCHONDRIAL ACCUMULATION OF CYSTATHIONINE $\beta$ -SYNTHASE MEDIATED BY LON PROTEIN

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**Objectives:** Cystathionine  $\beta$ -synthase (CBS) is a nuclear encoding heme protein, playing a key role in homocysteine and cysteine metabolism and endogenous H<sub>2</sub>S production. Lon protease is a major protease in mitochondrial matrix in mammalian cells, being engaged in the degradation of proteins to prevent protein aggregation. The mechanisms by which Lon protease recognizes and regulates the degradation of CBS proteins are unknown to date. This study aimed at addressing the mechanisms for the interaction of CBS and Lon.

**Methods:** Mitochondrial and cytosolic localization of CBS under normoxia and hypoxia were detected by Western blotting. The mechanisms for heme-dependent recognition and degradation of mitochondrial CBS by Lon protease were determined using DNA transfection, short interfering RNA transfection and co-immunoprecipitation assay.

**Results:** CBS exists in both the cytosol and mitochondria in liver cells under normoxic conditions. Hypoxia triggers the accumulation of CBS inside mitochondria and reperfusion normalizes CBS level and H<sub>2</sub>S production in mitochondria. The oxygenation status of the heme group contained in CBS protein is the determining factor for CBS recognition and degradation by Lon protease in mitochondrial matrix.

**Conclusions:** Our findings provide a fundamental and general mechanism for oxygen-sensitive regulation of mitochondrial functions by linking oxygenation level to the accumulation/degradation of mitochondrial heme proteins.

#### P62.

##### FALL IN BLOOD LEAD LEVEL IN THE US POPULATION 1999-2012

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**Objective:** Chronic low-level exposure to lead is thought to be associated with increased blood pressure in adults. Therefore, we analyzed the latest trends in blood lead level in the US population.

**Method:** We used the 1999-2012 data on blood lead in the US National Health Nutrition and Examination Survey (NHANES). Participants with blood lead measurements were included. They were stratified according to sampling year, age and gender. Data were analyzed using the complex sampling functions of SPSS version 22.

**Results:** There were 7970, 8946, 8373, 8407, 8266, 8793 and 7920 participants in NHANES 1999-2000, 2001-2002, 2003-2004, 2005-2006, 2007-2008, 2009-2010 and 2011-2012, respectively. Blood lead levels were (geometric mean [95% confidence interval]) 1.77 [1.72-1.81], 1.57 [1.54-1.61], 1.52 [1.48-1.55], 1.41 [1.38-1.44], 1.38 [1.35-1.41], 1.23 [1.21-1.25] and 1.09 [1.06-1.21] for adults aged 20 or above, and 1.43 [1.39-1.47], 1.16 [1.13-1.19], 1.18 [1.15-1.21], 0.99 [0.96-1.01], 0.98 [0.96-1.01], 0.81 [0.80-0.83], 0.66 [0.64-0.68] for children, respectively. Both decreasing trends were significant ( $p < 0.001$ ). Compared to children aged 7 or above, children aged 6 or below had significant higher blood lead levels (1999-2000: 1.24 [1.20-1.28] vs. 2.08 [2.01-2.26]; 2001-2002: 1.03 [1.00-1.06] vs. 1.65 [1.57-1.73]; 2003-2004: 1.03 [1.01-1.06] vs. 1.69 [1.61-1.78]; 2005-2006: 0.86 [0.84-0.88] vs. 1.41 [1.35-1.48]; 2007-2008: 0.85 [0.83-0.88] vs. 1.46 [1.40-1.54]; 2009-2010: 0.72 [0.70-0.74] vs. 1.15 [1.10-1.19]; 2011-2012: 0.59 [0.57-0.61] vs. 0.93 [0.88-0.99];  $p < 0.001$ ).

**Conclusions:** Blood lead level has been decreasing in the US population during the period 1999-2012. The blood lead level in children aged 6 or below is of concern.

#### P63.

##### COMP DEFICIENCY AGGRAVATES VASCULAR SENEESCENCE

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**Rationale:** Vascular smooth muscle cells (VSMCs) undergo senescence after their limited proliferation during lifespan. The dysfunction of senescent cells contributes to vascular morbidity and promotes vascular diseases. Cartilage oligomeric matrix protein (COMP), one kind of vascular extracellular matrix mostly expressed in VSMCs, plays a critical role in maintaining vascular homeostasis.

**Objective:** We aimed to explore whether COMP affected VSMCs senescence and subsequent vascular dysfunction.

**Methods and Results:** COMP was significantly decreased in the aortas from C57BL/6J mice with increasing ages (2, 5, 12, 20 months). Moreover, COMP was also downregulated in the aortas from senescent mouse models as ApoE<sup>-/-</sup> and Klotho<sup>-/-</sup> mice compared with their wide type (WT) littermates. It indicated COMP expression was positively related with senescence. In vitro, COMP knockdown by small interfering (si) RNA led to the elevation of  $\beta$ -gal staining and the upregulation of senescence markers including p53, p21, p16 and  $\gamma$ H2AX in VSMCs. Intriguingly, COMP overexpression by infection of adenovirus transduction could inhibit the COMP deficiency-increased senescence markers expression. Furthermore, COMP<sup>-/-</sup> mice also displayed the senescent characteristics in the aortas compared with their WT littermates. In accordance, COMP deficiency induced the vascular dysfunction, as evidenced by the significantly reduced phenylephrine-induced contraction and acetylcholine-induced relaxation. Finally, using SM22-COMP transgenic mice, we observed that COMP overexpressed in VSMCs retarded the calcification, an important indicator of vascular senescence.

**Conclusion:** Our data revealed that COMP decelerated the vascular senescence, which may shed light on the new strategies for vascular physiology and pathology.

## ABSTRACTS

### Abstracts for Posters:

#### P64.

##### IMPACT OF END-STAGE RENAL DISEASE ON LONG-TERM SURVIVAL AFTER FIRST-EVER MECHANICAL VENTILATION: A POPULATION-BASED STUDY

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**Objectives:** Patients with end-stage renal disease (ESRD) usually have multi-comorbidities, predisposing to acute organ failures and in-hospital mortality. We aimed to assess the impact of ESRD on long-term risk of death after first-ever mechanical ventilation (MV) for acute respiratory failure, which remains poorly understood.

**Method:** Retrospective comparison of patients with or without ESRD after first-ever MV between 1999 and 2008. Patients were followed from the index admission date to death or the end of 2011. Primary outcome was death after MV. The effect of ESRD on risk of death after MV was assessed using a Cox proportional hazard regression model.

**Setting:** The patients after first-ever MV from Taiwan's National Health Insurance Research Database (NHIRD).

**Patients:** We identified all patients (n=38,659) receiving first-ever invasive MV between 1999 and 2008 from 1,000,000 beneficiaries who were randomly selected from Taiwan's NHIRD. Patients with ESRD (n=1,185; mean age 65.9 years, men 51.5%) were individually matched to those without ESRD in a 1:8 ratio using propensity score matching method.

**Results:** After the propensity score matching, the baseline characteristics of the two cohorts were well balanced. The incidence of death was higher in patients with ESRD than in those without ESRD (342.30 vs. 179.67 per 1,000 person-years,  $p < 0.001$ ), representing a covariate-adjusted hazard ratio of 1.43 (95% confidence interval 1.31-1.51). Among those who survived to discharge, patients with ESRD were not associated with long-term mortality (more than 4 years).

**Conclusions:** ESRD increases the risk of death after first-ever MV, but a long-term tracing seemed a similar survival.

#### P65.

##### TBX5 GENE MUTATION IN VENTRICULAR SEPTAL DEFECT

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**Purpose:** Ventricular septal defect (VSD) is the most frequently occurring congenital heart disease (CHD) in newborns. A number of genetic studies have linked TBX5 mutations to cardiac abnormalities. Here, we identify potential pathogenic mutations for TBX5 and provide insights into the etiology of isolated VSD in Chinese patients.

**Methods:** Case-control mutational analysis was performed in 354 patients with isolated VSD and 341 healthy controls. First, all the coding exons and intron-exon boundaries of TBX5 were sequenced in VSD patients and controls. Sanger sequencing with high-resolution melting (HRM) curve analysis was then combined to detect new TBX5 mutation and identify its frequency.

**Results:** A novel heterozygous missense mutations (c. 40C>A) was identified in TBX5 gene exon-2. This mutation leads to proline to threonine substitution at position 14, which is highly conserved among many species. The damaging and disease causing of this mutation is predicted by Polyphen2, SIFT and Mutation Taster. No more c. 40C>A mutation was found in enclarger cases and controls by HRM analysis.

**Conclusion:** We identified a novel heterozygous missense mutation in TBX5 gene exon-2 in isolated VSD patients in Chinese population and found that this missense mutation probably causes the disease. Further, our results showed the important role of HRM as a reliable and efficient method to determine disease-related gene mutation in congenital heart disease.

#### P66.

##### SELECTIVE STIMULATION OF LARGE-CONDUCTANCE $Ca^{2+}$ -ACTIVATED $K^{+}$ CHANNELS BY EQUAL

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**Objectives:** Equol [7-hydroxy-3-(4'-hydroxyphenyl)-chroman], an active metabolite of the soy isoflavone daidzein, is produced by intestinal microbial flora in some individuals consuming daidzein, and is considered to be responsible for the cardiovascular benefits of soy. However, it is unclear whether equol would affect cardiovascular  $K^{+}$  currents. The present study was designed to investigate the effects of equol on cardiovascular  $K^{+}$  channel currents.

**Methods:** A whole-cell patch clamp technique was employed to recorded transient outward  $K^{+}$  current ( $I_{to}$ ) and ultra-rapid delayed rectifier  $K^{+}$  current ( $I_{Kur}$ ) in isolated human atrial myocytes, and Kv4.3, Kv1.5, hERG channel current ( $I_{hERG}$ ) and human cardiac KCNQ1/KCNE1 channel current ( $I_{Ks}$ ) stably expressed in HEK 293 cells, as well as human large-conductance  $Ca^{2+}$ -activated  $K^{+}$  ( $BK_{Ca}$ ) channel current expressed in HEK 293 cells.

**Results:** We found that equol at 10  $\mu$ M inhibited cardiac channel currents including human atrial native  $I_{to}$  and  $I_{Kur}$ , Kv4.3, Kv1.5 currents as well as  $I_{hERG}$  and  $I_{Ks}$  expressed in HEK 293 cells in concentration-dependent manner with  $IC_{50}$  of 30.1  $\mu$ M for  $I_{to}$ , 36.1  $\mu$ M for  $I_{Kur}$ , 30.3  $\mu$ M for Kv4.3, 16.2  $\mu$ M for Kv1.5, 31.7  $\mu$ M for  $I_{hERG}$  and 25  $\mu$ M for  $I_{Ks}$ , respectively. It is interesting to note that equol at 1  $\mu$ M induced a remarkable activation of  $BK_{Ca}$  current in HEK 293 cells expressing both  $\alpha$  and  $\beta$ 1 subunits, but not the sole  $\alpha$ -subunit.

**Conclusion:** These results demonstrate the first information that equol selectively stimulates  $BK_{Ca}$  channel current by acting on its  $\beta$ 1 subunit, which probably contributes to the equol-mediated vasodilation action reported in early study.

## ABSTRACTS

### Abstracts for Posters:

#### P67.

##### SEX DIFFERENCES IN EXPRESSION OF 5-HYDROXYTRYPTAMINE RECEPTORS IN NODOSE GANGLIA POTENTIAL IMPACT ON MYOCARDIAL INFARCTION WITH CHEST PAIN

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**Background:** Accumulated evidences have shown that 5-HT activates vagal afferents and transmits pain in baroreflex afferent pathway, and plays a noticeable role in the cardiac pain. The current investigation aims to explore the gender difference in expression profiles of 5-HT receptors, including 5-HT<sub>1A</sub>R, 5-HT<sub>1B</sub>R, 5-HT<sub>1D</sub>R, 5-HT<sub>2A</sub>R, 5-HT<sub>3A</sub>R and 5-HT<sub>3B</sub>R.

**Methods:** Whole-cell patch clamp, RT-PCR, western-blot, and immunostaining were performed using ganglion tissue or in fluorescently and electrophysiologically identified baroreceptor neurons (BRNs) from adult male, age-match female and ovariectomized rats.

**Results:** 5-HT induced membrane depolarization and inward currents in Ah-types concentration-dependently. The mRNA expression of 5-HT<sub>1A</sub>R, 5-HT<sub>1B</sub>R and 5-HT<sub>1D</sub>R showed no difference among sexes ( $P>0.05$ ). The mRNA and protein for 5-HT<sub>2A</sub>R, 5-HT<sub>3A</sub>R and 5-HT<sub>3B</sub>R showed significant difference among groups ( $P<0.05$ ). Immunohistochemical data indicated that, in A-type or Ah-types, 5-HT<sub>2A</sub>R was located on the nucleus and a little on the cell membrane, 5-HT<sub>3A</sub>R was on the cell membrane and cytolymph, and 5-HT<sub>3B</sub>R was on the nucleus and cell membrane. In C-types, 5-HT<sub>2A</sub>R and 5-HT<sub>3A</sub>R were mostly in cytolymph. The protein expressions for 5-HT<sub>3A</sub>R and 5-HT<sub>3B</sub>R showed a higher level in male MI model rats compared with sham control

( $P<0.05$ ) and females as well ( $P<0.05$ ), whereas, the protein expression for 5-HT<sub>2A</sub>R had no difference in both males ( $P>0.05$ ) and females ( $P>0.05$ ).

**Conclusions:** 5-HT could participate, at least partially, in sex differences in MI-mediated cardiac pain through visceral afferent reflex/baroreflex afferent pathway. Converging lines of observations suggest that 5-HT<sub>3A</sub>R may play a key role in the fact that women are less likely to present chest pain than age-matched men. The RT-PCR and western blot data suggested that 5-HT<sub>1</sub>R and 5-HT<sub>2A</sub>R make a little or no effects on gender differences of chest pain.

#### P68.

##### SEXUAL DIMORPHISM IN EXPRESSION PROFILE OF SUBSTANCE P RECEPTORS (NKs) IN VICSERAL AFFERENT REFLEX PATHWAY

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**Background:** Substance-P, a tachykinin (NK), distributes in nodose ganglia and near coronary vessels in the heart. NK receptors (NK1-3) express different levels in nodose ganglia and heart between sexes, suggesting that it could be one of the reasons why blood pressure in women is different from age-matched men. Importantly, three types of aortic baroreceptor afferent neurons (BRNs) have been identified in nodose ganglia, i.e., myelinated A-type, Ah-type, and unmyelinated C-type. A unique and functionally distinct class of low-threshold Ah-type BRNs is observed only in adult female rats, rather than males. Therefore, we hypothesises that the sex-specific expression of NK receptors in Ah-type BRNs in females may lead to a gender differences in neurocontrol of circulation and blood pressure regulation.

**Methods:** Real-time RT-PCR, western blot, immunostaining, whole-cell patch technique, and single-cell RT-PCR were performed on fluorescently and electrophysiologically identified BRNs isolated from adult male, age-matched female, and ovariectomized rats.

**Results:** RT-PCR analysis showed that the mRNA expression for NK1 was dramatically difference among groups ( $P<0.01$ ;  $n=4$ ), while no differences were observed in both NK2 and NK3; Western bolt results indicated that the protein expression for NK1 was also up-regulated significantly among groups ( $P<0.01$ ;  $n=10$ ); Immunohistochemical data showed that NK1 receptors

distributed on both on the cell membrane and cytoplasm; Single-cell RT-PCR showed that myelinated Ah-type BRNs expressed a relatively higher level of NK1 mRNA (87.5%) compared with A-type (57.1%) and C-type neurons (42.8 %).

**Conclusions:** These data have demonstrated for the first time that NK1 receptor, rather than NK2 and NK3, plays a potential role in neurocontrol of circulation by transmitting baroreceptor afferent reflex information; and presumably, Ah-type BRNs are key players more likely in sexual dimorphism of baroreflex and blood pressure regulation under physiological and disease condition.

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