Results: Results showed that treatment with AVE 0991 could reduce AngII-induced proliferation, migration, and ROS production. However, treatment with AVE 0991 could attenuate AngII-induced activation of Smad signaling pathways. Furthermore, the beneficial effects and Smad pathways’ changes of AVE0991 were abolished by A-779. Conclusion: Our data showed that AVE 0991 attenuated proliferation of cardiac fibroblast, which may be due to the inhibition of Smad pathways.

GW25-e1651
Identification of Stim1 as a Candidate Gene for Exaggerated Sympathetic Response to Stress in the Stroke-Proximal Spontaneously Hypertensive Rat
Xiao Bing1,2,3, Liu Fan1, Ohara Hiroki1, Nakiba Toru1
1Department of Cardiology, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei province, China, 2Department of Functional Pathophysiology, Shimane University School of Medicine, Izumo, Japan

Objectives: The stroke-proximal spontaneously hypertensive rat (SHRSP) is known to have exaggerated sympathetic nerve activity to various types of stress, which might contribute to the pathogenesis of severe hypertension and stroke observed in this strain. Previously, by using a congenic strain (called SPwch1.72) constructed between SHRSP and the normotensive Wistar-Kyoto rat (WKY), we showed a 1.8-Mb fragment on chromosome 1 (Chr1) of SHRSP harbored the response gene (s) for the exaggerated sympathetic response to stress. To further narrow down the candidate region, in this study, another congenic strain (SPwch1.71) harboring a smaller fragment of Chr1 including two functional candidate genes, Phox2a and Sip2, was generated.

Methods: Sympathetic response to cold restraint stress was compared among SHRSP, SPwch1.71, SPwch1.72 and WKY by three different methods (urinary norepinephrine excretion, blood pressure measurement by the telemetry system and the power spectral analysis on heart rate variability).

Results: The results indicated that the response in SPwch1.71 did not significantly differ from that in SHRSP, excluding Phox2a and Sip2 from the candidate genes. As the stress response in SPwch1.72 was significantly less than that in SHRSP, it was concluded that the 1.2-Mb congenic region covered by SPwch1.72 (and not by SPwch1.71) was responsible for the sympathetic stress response. The sequence analysis of 12 potential candidate genes in this region in WKY/Izm and SHRSP/Izm identified a nonsense mutation in the stomatoid interaction molecule 1 (Stim1) gene of SHRSP/Izm among 4 substrains of SHRSP. A western blot analysis confirmed a truncated form of STIM1 in SHRSP/Izm. In addition, the analysis revealed that the protein level of STIM1 in the brainstem of SHRSP/Izm was significantly lower when compared with WKY/Izm.

Conclusions: Our results suggested that Stim1 is a strong candidate gene responsible for the exaggerated sympathetic response to stress in SHRSP.

GW25-e1653
SIRT1 enhances Therapeutic Efficacy of Aged Mesenchymal Stem Cells in Rat Myocardial Infarction via lightening MSCs Aging and heightening Stress Resistance
Huixiang Chen, Jian-an Wang
Cardiovascular Key Lab of Zhejiang Province, the Second Affiliated Hospital, School of Medicine, Zhejiang University

Objectives: Mesenchymal stem cells (MSCs) hold great promise for stem cell therapy and are gradually used in clinical trials in recent years. However, advanced age is a negative factor for autologous MSCs transplantation.

Methods: For in vitro study, we evaluated cell senescence using SA-β-gal method while for evaluated the stress response we conducted H2O2-induced hypoxia/ischemia model in MSCs. The conditioned medium was collected and was added to HUVEC to observe the alteration of tube-formation between vectot-aged MSCs and while for evaluated the stress response we conducted H2O2-induced hypoxia/ischemia model in MSCs. For experiments in vivo, we first conducted ischemic femoral heart model induced by left anterior descending (LAD) ligation. At day 3 after male cell transplantation, the cell survival was evaluated by SRY detection. At day 28 after cell transplantation, heart function and fibrosis were analyzed by ECHO and Mason staining respectively. Moreover, vascular density was evaluated by CD31 and SMA staining.

Results: In the present study, we evaluated the effect of SIRT1 overexpression in MSCs from aged rat in vivo and in vitro. Compared with vector-aged MSCs, SIRT1-modified aged MSCs significantly promote cellular survival in the ischemic heart induced by left anterior descending ligation at day 3 after cell transplantation. Moreover, SIRT1 overexpression in aged MSCs could down-regulate fibrosis and up-regulate vascular density, consequently contribute to the improvement of heart function. In vitro data demonstrated that forced expression of SIRT1 inaged MSCs could ameliorate the phenotypes and function of aged MSCs, consequently contribute to the improvement of cell-based therapy for myocardial infarction.

GW25-e2290
Effect of Sini Decoction on the expression of SOD and MDA in EAHy926 cells injured by homocysteine
Liu Yong, Song Zhihong, Zhou Bin, Yu Shujie, Liu Denglai, Hua Baoshan, Wu Lin, Wang Min, Chen Lin, Qian Xiaoqian
Department of Cardiology, The Third Affiliated Hospital of Sun Yat-sen University

Objectives: To detect the effect of Sini Decoction on the expression of SOD and MDA in EAHy926 cell injured by homocysteine.

Methods: Model of EAHy926 cell injured by homocysteine was made, the protection on the EAHy926 cell of Sini Decoction with different dosages were observed. SOD and MDA concentration of cell culture fluid was detected, effect of Sini Decoction on the expression of protein of SOD1 in EAHy926 cells were observed by Western-blot, and effect of Sini Decoction on the expression of mRNA of SOD1 in EAHy926 cell were observed by fluorescent quantitation PCR.

Results: After model of EAHy926 cell injured by homocysteine was made, we found that cultured with 0.5, 1.0, 2.0, 4.0, 8.0μmol/L homocysteine, cells grew less than cultured with normal culture medium, as culturing with homocysteine 4.0μmol/L for 24h did lower damage to cells and could induce effective cell injury, it was made to be the model of injury. To detect the effect of Sini Decoction on EAHy926 cell injured by homocysteine, well growing EAHy926 cells were cultured in culture plate. 24h later, cells were cultured with medium containing Sini Decoction 0, 0.25, 0.5, 1.0μg/ml for 30 min respectively. Furthermore, the concentration of SOD1 protein of SOD1 in EAHy926 cells increased obviously, while in Sini Decoction groups, SOD concentration of cell culture fluid increased and MDA concentration of cell culture fluid decreased, in Sini Decoction 1.0μg/ml plus homocysteine 4.0μmol/L group was the most obvious (P<0.05). Detected by Western-blot, it was found that, compared with control group, there was no obvious change of protein of SOD1 in Sini Decoction 1.0 g/ml group, but in homocysteine 4.0μmol/L model group, SOD (2.64±0.576 μmol/L) concentration of cell culture fluid decreased obviously compared with control group (3.83±1.169 μmol/L) (P<0.05). detected by fluorescent quantitation PCR.

Conclusions: Sini Decoction could ameliorate the phenotypes and function of aged MSCs, consequently contribute to the improvement of cell-based therapy for myocardial infarction.

GW25-e2345
MIR-211 mediated hypoxia induce bone marrow mesenchymal stem cells migration through STAT5A
Hu Xinyang, Jian-an Wang
Cardiovascular Key Lab of Zhejiang Province, the Second Affiliated Hospital, School of Medicine, Zhejiang University

Objectives: Efficacy of intravenous administration of mesenchymal stem cells (MSCs) for myocardial infarction (MI) is limited by low cell migration to the damaged myocardium. Our previous study demonstrated that migration ability of MSCs enhanced by hypoxia preconditioning (HPC). miRNA microarray displayed that miR-211 exhibited the most significant change between HPC and normoxia cultured MSCs. The aim of this study is to identify whether miR-211 regulates MSCs migration and the potential mechanism.

Methods: In vitro, transwell assay were used to assess the migration ability of MSCs. The aim of this study is to identify whether miR-211 regulates MSCs migration and the potential mechanism.

Results: Quatitative RT-PCR showed that miR-211 expression of MSCs upregulated significantly after HPC (P<0.05). Detected by Western-blot, it was found that, compared with control group, there was no obvious change of protein of SOD1 in Sini Decoction 1.0 g/ml group, but in homocysteine 4.0μmol/L model group, expression of SOD1 protein weakened obviously, and in Sini Decoction groups, expression of SOD1 protein enhanced, and in Sini Decoction groups, expression of SOD1 mRNA enhanced, and in Sini Decoction 1.0μg/ml Plus homocysteine 4.0μmol/L group, it was the most obvious (P<0.05). Detected by fluorescent quantitation PCR.

Conclusions: Homocysteine may injure EAHy926 cell by suppressing the expression of SOD, while Sini Decoction may protect EAHy926 cell by enhancing the expression of SOD.
STAT5A can combine to the promoter of miR-21, which lead to the regulation of miR-21 transcription. In vivo data showed that miR-21 overexpression enhanced MSC's homing to ischemic myocardium, the long-term experiment illustrated miR-21 overexpressing MSCs improved cardiac function 28days post-MI. However, miR-21 knockdown decreased MSCs homing and hampered cardiac function recovery. Conclusions: These results indicate that miR-21 has important role in regulating MSCs migration through targeting STAT5A, meanwhile STAT5A regulated miR-21 transcription.

GW25-e2347

Hyposia preconditioned mesenchymal stem cells prevent cardiac fibroblast activation and collagen production via leptin

Chen Panpan, Jian-an Wang

Cardiovascular Key Lab of Zhejiang Province, the Second Affiliated Hospital, School of Medicine, Zhejiang University

Objectives: Activation of cardiac fibroblasts into myofibroblasts constitutes a key step in cardiac remodeling after myocardial infarction (MI), due to interstitial fibrosis. Mesenchymal stem cells (MSCs), especially hyposia preconditioned MSCs (H-MSCs), have been shown to be able to improve post-MI remodeling. Leptin has been shown to promote cardiac fibrosis. The expression of leptin is significantly increased in MSCs after hyposia preconditioning. However, it is still unknown whether and how leptinparticipates in the therapeutic effects by MSCs, especially the fibroblasts process. The objective of this study was to identify the role of leptin from H-MSCs in cardiac fibroblastactivation.

Methods: The activation of cardiac fibroblasts was induced by hyposia (0.5% O₂). The effects of MSCs on the fibroblast activation were analyzed by co-culturing MSCs with CFs, and determining the expression change of 5-SMA and S22α, as well as collagenIIα in CFs with western blot, immunofluorescence and Sirius red staining.In vivo MSCs fibrotic effects on left ventricular remodeling were investigated using an acute MI model that was induced by permanent ligation of the left anterior descending coronary artery (LAD).

Results: Co-cultured MSCs decreased fibroblast activation, hyposia preconditioned MSCs showed stronger effects on preventing fibroblast activation than normoxic treated MSCs. However, leptin deficient MSCs from Ob/Ob mice did not exhibit such effect. The in vivo study also showed that H-MSCs significantly inhibited cardiac fibrosis after MI, along with the decrease of TGFB-β-Smad2 and MRTF-A in CFs, which again were absent when leptin in MSCs was deficient.

Conclusions: Our data demonstrate that activation of cardiac fibroblasts can be inhibited by MSCs, hyposia preconditionedMSCs enhanced the inhibition effect. Leptin in MSCs played a key role in inhibiting cardiac fibrosis, possibly through blocking both TGFB-β-Smad2 and MRTF-A signal pathways.

GW25-e2454

DNA Damage and Cell Cycle Arrest Induced by Protoporphyrin IX-mediated sonodynamic therapy in MCF-7

Gao Weimei, Tian Ye

Harbin Medical University Pathology and Pathophysiology Department

Objectives: Protoporphyrin IX (PpIX), a well-known hematoporphyrin derivative component, have shown great potential to enhance light induced tumor cell damage. In addition, PpIX-mediated sonodynamic therapy (PpIX-SDT) could also exert anti-tumor effects. However, the mechanisms underlying those effects are still incompletely understood. This study thus investigated the putative mechanisms underlying the anti-tumor effects of PpIX-SDT on MCF-7.

Methods: MCF-7 cells incubated with PpIX (1 µg/ml) were exposed to ultrasound for different time. Following the treatment, cell viability was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay; Loss of mitochondrial membrane potential was measured by flow cytometry; The translocation of apoptosis inducing factor (AIF) from mitochondria to nucleus was visualized by confocal laser scanning microscopy; DNA damage was detected by single phase gel electrophoresis; Cell cycle distribution was analyzed by DNA content with flow cytometry; Cell cycle related proteins were detected by Western blotting.

Results: PpIX-SDT (≥ 1 minutes) significantly inhibited proliferation and reduced viability of MCF-7 cells in a time-dependent manner. PpIX-SDT rapidly and significantly triggered mitochondrial membrane depolarization, AIF translocation from mitochondria to nucleus and DNA damage, effects partially abolished by the specific inhibitor of MPTP (mitochondrial permeability transition pore). Furthermore, S phase arrest and upregulation of the related proteins of P53 and P21 were observed following 3 and 5 minutes PpIX-SDT treatment.

Conclusions: Tumor cell proliferation could be inhibited by PpIX-SDT through the induction of DNA damage and cell cycle arrest in the S phase.

GW25-e3107

Protective Effects of Jishen Prescription on Ventricular Remodeling in the Early Period of Myocardial Infarction in Rats

Xie Shiyang, Youping Wang, Bin Li, Xiaoli Nan, Yanyan Liu, Yuan Gao, Zhu Mingjun

First Affiliated Hospital, Henan University of Traditional Chinese Medicine, Zhengzhou, 450000, China

Objectives: This study was designed to determine the effects of Jishen prescription on left ventricular remodeling and TGFB-βSmads signaling in the early period of myocardial infarction (MI) in rats.

Methods: MI was induced by the ligation of left anterior descending coronary artery in male Sprague-Dawley rats. The rats were divided into five groups: sham-operated group; MI vehicle group; MI+JSP-3g (3g/kg/day) group; MI+JSP-6g (6g/kg/day) group and MI+losartan (10mg/kg/day) group. The vehicle, JSP, or losartan was given for 4 weeks by oral gavage once a day after MI. The echocardiographic parameters were examined to determined cardiac function. The distribution and level of collagen were determined using Masson’s trichrome staining and hydroxyproline assay, respectively. The myocardial expression of transforming growth factor β-1 (TGF-β1), phosphorylated Smad 2 (P-Smad 2), and phosphorylated Smad 3 (P-Smad 3) was determined by the use of Western blot.

Results: Compared to MI vehicle rats, JSP at the dose of 6 g/kg/day attenuated the increased left ventricular end-diastolic dimension (0.91±0.05 vs. 0.71±0.03 cm, P<0.05) and left ventricular end-systolic dimension (0.72±0.06 vs. 0.44±0.02 cm, P<0.05), and the decreases in ejection fraction (44.6±4.6% vs. 74.1±1.3%, P<0.05) and fractional shortening (19.8±2.5% vs. 39.0±1.2%, P<0.05) at week 4 after MI. Left ventricular weight index was decreased in MI+JSP-6g rats compared to MI vehicle rats (1.92±0.13% vs. 2.17±0.06%, P<0.05). In addition, treatment with JSP at the dose of 6 g/kg/day increased the levels of collagen (120±1.07 vs. 18.2±0.70 µg/mg protein, P<0.05) and expression of TGF-β1, P-Smad 2 and P-Smad 3 compared to MI+vehicle rats at week 4 after MI (TGF-β1: 0.58±0.01 vs. 0.78±0.01 %GAPDH arbitrary units; P-Smad 2: 0.58±0.01 vs. 0.88±0.02 %GAPDH arbitrary units; P-Smad 3: 0.50±0.02 vs. 0.78±0.01 %GAPDH arbitrary units, P<0.05). Losartan treatment has the similar results with JSP at the dose of 6g/kg/day. Conclusion: Our studies showed that JSP administered after MI improved cardiac function, and inhibited left ventricular dilatation and the increase in the level of collagen. The results were associated with the decreased expression of TGF-β1 and P-Smad 2/3. Our data suggest that JSP may inhibit left ventricular remodeling possibly via attenuating TGF-β/Smad's signal pathway in the early period of MI.

GW25-e3176

Co-expression and Effects of TK1 and TIMP1 Genes on the Proliferation of Vascular Smooth Muscle Cells

Pengzi Zhu, Huizhen Yu, Shujie Huang, Hong Xiang, Weiiping Zheng, Fan Liu, Feng Huang

Fujian Provincial Hospital

Objectives: Tissue kalikrein (TK1) and inhibitor of matrix metalloproteinase 1 (TIMP1) play important role in inhibiting vascular smooth muscle cells (VSMCs) proliferation, migration and improving vascular remodeling, respectively. Therefore, we hypothesis that combination with double TK1 and TIMP1 gene mediated by a recombinant adenovirus vector could greatly augment or synergy the effects of suppressing vascular remodeling.

Methods: The human TIMP1 cDNA in pMD-hTIMP1and pDC316 plasmid was digested by endonuclease, respectively, and cloned to construct a plasmid pDC316-mCMV-hTIMP1-EGFP. The promter mCMV carrying hTIMP1 cDNA full length DNA was digested with endonuclease BglII and SalI, and was subcloned to the plasmid pDC316-hTK1 to construct a recombinant plasmid carrying hTK1 and hTIMP1 double genes. Then the double gene plasmid and adenovirus backbones plasmid were co-transfected and packaged in 293A cells. The gene transcription and protein expression were respectively examined by real-time PCR and western-blotting assays.VSMCs proliferation was detected by cell counting and methyl thiazolyl blue (MTT) method.

Results: The plasmid containing hTK1 and hTIMP1 double gene was proved and constructed correctly by means of PCR, double digestion and sequencing analysis. Ad5-hTK1-hTIMP1 was successfully packaged in 293A cell. When VSMCs were co-transfected and packaged in 293A cells. The gene transcription and protein expression were respectively examined by real-time PCR and western-blotting assays.VSMCs proliferation was detected by cell counting and methyl thiazolyl blue (MTT) method.

Results: The plasmid containing hTK1 and hTIMP1 double gene was proved and constructed correctly by means of PCR, double digestion and sequencing analysis. Ad5-hTK1-hTIMP1 was successfully packaged in 293A cell. When VSMCs were co-transfected and packaged in 293A cells. The gene transcription and protein expression were respectively examined by real-time PCR and western-blotting assays.VSMCs proliferation was detected by cell counting and methyl thiazolyl blue (MTT) method.

Conclusions: Our studies showed that double hTK1 and hTIMP1 gene mediated by a recombinant adenovirus vector could greatly augment or synergy the effects of suppressing vascular remodeling.

GW25-e3385

Soluble Receptor for Advanced Glycation End-Products Inhibits Hypoxia-/ Reoxygenation Myocardial Oxidative Stress in Rat

Guo Caihua, Guo Caihua, Chen Buxing

Beijing TianTan Hospital, Capital Medical University

Objectives: To elucidate the influence of sRAGE on hypoxia / reoxygenation myocardial oxidative stress.

Methods: Primary cultured rat cardiac myocytes were isolated from neonatal rats with the modified 2-step collagenase digest method, Hypoxia for 3-h followed by 2-h reoxygenation injury model was reduced. Cells were randomly divided into four