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S5.1

Bone marrow mesenchymal stem cells reversed potassium channels remodeling in post-infarcted myocardium via regulating calcineurin pathway

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Aim: Bone marrow mesenchymal stem cells (BMSCs) emerge as a new promising approach for treating heart diseases. However, the effects of BMSCs-based therapy on cardiac electrophysiological remodeling following myocardial infarction were largely unclear. This study was aimed to investigate whether BMSCs transplantation reverse cardiac potassium channels remodeling in post-infarcted hearts. **Methods:** Myocardial infarction was established in male SD rats, and BMSCs were then intramyocardially transplanted into the infarcted hearts after 3 d. Cardiac electrophysiological remodeling in the border zone was evaluated by Western blotting and whole-cell path clamp technique after 2 weeks. **Results:** We found that BMSCs transplantation significantly ameliorated the increased heart weight index, the impaired left ventricular functions, and the enhanced cardiac fibrosis in infarcted hearts. The survival ratio of infarcted rats was also improved after BMSCs transplantation. Importantly, electrical stimulation-induced arrhythmias were seldom observed in BMSCs-transplanted infarcted rats compared with rats without BMSCs treatment. Furthermore, BMSCs transplantation effectively inhibited the prolongation of action potential duration and the reduction of transient and sustained outward potassium currents in ventricular myocytes from post-infarcted rats. Consistently, BMSCs-transplanted infarcted hearts exhibited the more expression of $K_V4.2$, $K_V4.3$, $K_V1.5$, and $K_V2.1$ proteins than infarcted hearts. Moreover, intracellular free calcium level, calcineurin and nuclear NFATc3 protein expression was shown activated in infarcted hearts, which was inhibited after BMSCs transplantation. **Conclusion:** Collectively, BMSCs transplantation prevented ventricular arrhythmias and reversed cardiac potassium channels remodeling in post-infarcted heats via attenuating intracellular calcium overload and calcineurin/NFATc3 signal pathway, which help to advance our understanding of mesenchymal stem cells pharmacology and experimental therapeutics.

Keywords: bone marrow mesenchymal stem cells; myocardial infarction; arrhythmias; potassium channels; calcineurin

S5.2

Expression and function of B cell-activating factor of the TNF family in T cell-mediated autoimmune arthritis

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Aim: To investigate the expression and function of B cell-activating factor (BAFF) and its receptors (BCMA, TACI, and BAFF-R) in T cell-mediated inflammatory immune responses of animal model for rheumatoid arthiritis. **Methods:** The model of rat adjuvant-induced (AA) was induced by a single intradermal injection of 0.1 mL of CFA into the right hind metatarsal footpad of rat. The rats with experimental arthritis were randomly separated into different groups and then treated with TACI-Ig (0.7, 2.1, and 6.3 mg/kg) respectively once per day from d 16 to 34 after immunization. The degree of arthritis was evaluated by radiographic and histological examination. Levels of BAFF were assessed by enzyme-linked immunosorbent assay. Cluster of differentiation (CD)3, CD20, CD68, CD21, CD138, BAFF, and PNAd expression were detected by immunohistochemical analysis. ($CD3^+CD4^+$), ($CD4^+CD25^+$), ($CD4^+CD44^+$), and ($CD4^+CD62L^+$) markers on $CD4^+$ T cells were detected by flow cytometry. Double-labeled immunofluorescent staining was used to identify the expression of CD68 and BAFF receptor. Expression of BAFF and its receptors were analysed by immunohistochemical analysis, quantitative real-Time PCR and Western blot analysis. **Results:** Compare with the control group, AA rats developed severe arthritis. This was characterized by marked ankle joint radiographic and histological manifestation. BAFF level in macrophage and joint homogenate elevated significantly in AA group, and the expression of BAFF in spleen and synovial tissue increased significantly by immunohistochemical analysis and quantitative real-Time PCR. Correlation analysis showed joint histopathological manifestation significantly correlated with the level of joint BAFF. The expression of total and activated $CD4^+$ T cells significantly decreased in blood and spleen in AA group compared with those in control group. The expression of CD21, CD138, and PNAd in synovial tissue and expression of CD3, CD20, and CD68 in spleen increased significantly in AA group. Immunofluorescent staining showed the number of $CD68^+$ macrophage coexpressed TACI, BCMA, BAFF-R in control and AA group. TACI, BCMA, BAFF-R mRNA expression in spleen increased apparently in AA group. The expression of TACI and BCMA in synovial tissue increased significantly, while the expression of BAFF-R decreased significantly in AA group. Subcutaneous administration of TACI-Ig significantly decreased BAFF level and attenuated progression of experimental arthritis, with reductions in inflammatory response and bone and joint destruction. **Conclusion:** Local production of BAFF may foster survival and/or expansion of B cells that produce pathogenic autoantibodies and/or promote local T cell activation and consequent joint destruction. Together, BAFF-receptors interactions are important not only for B cell function but for T cell-mediated immune responses.

Keywords: BAFF; receptor; arthritis; autoimmune; T cell

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S5.3

PPARδ mediates the vascular benefits of metformin in diet-induced obese mice

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Aim: 5′ Adenosine monophosphate-activated protein kinase (AMPK) was reported to form transcriptional complex with peroxisome proliferator-activated receptor delta (PPARδ) to induce gene expression synergistically. This study investigated whether PPARδ mediates the effects of anti-diabetic drug metformin (AMPK activator) in ameliorating ER stress and endothelial dysfunction in high-fat diet-induced obese (DIO) mice. **Methods:** Endothelium-dependent relaxation (EDR) and protein expressions in aortae were measured by wire myograph and Western blotting, respectively. Fluorescence imaging determined the levels of reactive oxygen species (ROS) and nitric oxide (NO) under confocal microscopy. **Results:** DIO mice showed impaired EDR and elevated levels of ER stress markers and ROS in aortae. Chronic metformin treatment reversed the above-described effects in DIO *PPARδ* wild-type littermates but not in knockout mice. Metformin and PPARδ agonist GW1516 alleviated the tunicamycin (ER stress inducer)-induced impairment of EDR, ER stress and oxidative stress in mouse aortae as well as NO production in endothelial cells. Effects of metformin were abolished by the co-treatment of GSK0660 (PPARδ antagonist) whilst those of GW1516 were not affect by compound C (AMPK inhibitor). **Conclusion:** The present study supports that metformin curtails ER stress and oxidative stress and increases NO bioavailability upon activation of AMPK/PPARδ pathway, and subsequently combats against vasculopathy in obese diabetic mice.

Keywords: metformin; PPARδ; endothelial dysfunction; ER stress; obesity

S5.4

Anti-hyperlipidemic effect of ZBH, a novel compound constructed by incorporating the pharmacorphore of fibrates into resveratrol, in hyperlipidemic hamster

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Aim: Hyperlipidaemia, a prevalent disease, is a high risk factor of cardiovascular disease. However, effective and safe pharmacological option for its treatment remains limitation. In order to acquire more excellent anti-hyperlipidemic drug, a novel structural compound ZBH was constructed by incorporating the key pharmacophore of fibrates into natural scaffold of resveratrol. In present study, the hypolipidemic action of ZBH was systematically evaluated in hyperlipidemic hamster and its mechanism of action was further investigated. **Methods:** The action of ZBH at PPAR was characterized by using transactivation assay. It's binding to

human PPARα was corroborated by surface plasmon resonance (SPR) biosensor technology. Hypolipidemic action of ZBH was evaluated in high fat diet induced hyperlipidemic hamsters after 5 weeks treatment. Tissue-based gene expression assays were performed after 3-d and 5-week ZBH treatment. **Results:** ZBH showed stronger PPARα agonism *in vitro* relative to PPARδ and PPARγ, its activation potency to PPARα is 5.5 fold over than that of bezafibrate. Biacore SPR revealed that ZBH bind to the ligand binding domain (LBD) of PPARα with high affinity. ZBH significantly lowered triglycerides, TC, FFA, proatherogenic lipoproteins LDL-C, hyperinsulinemia and improved insulin sensitivity, which is considerably stronger than bezafibrate. Gene expression disclosed that ZBH markedly decreased endogenous fatty acid synthesis in liver and increased fatty acid uptake and oxidation in liver and skeletal muscle, besides up-regulated SIRT1 expression. **Conclusion:** ZBH could significantly ameliorated dyslipidemia and insulin resistant of the hyperlipidemic hamsters by activating PPARα through interactions with the LBD of the receptor and promoting the expression of SIRT1.

Keywords: PPARα; SIRT1; dyslipidemia; insulin resistance

S5.5

Cucurbitacin B induced ATM-mediated DNA damage causes G_2/M cell cycle arrest in a ROS-dependent manner

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Aim: Cucurbitacin B (Cuc B), a natural triterpenoid with potent anti-cancer activities both *in vitro* and *in vivo*, induces cancer cells cycle arrest at S or G_2/M phases while the detailed mechanisms remain to be clear. This study was designed to precisely dissect mechanisms responsible for Cuc B-induced cell cycle arrest in human lung adenocarcinoma epithelial A549 cells. **Methods:** The effect of Cuc B on A549 cell cycle distribution was analyzed with flow cytometry. The effect of Cuc B on DNA damage was determined with Comet assay and protein expression of γH_2AX. The intracellular reactive oxygen species (ROS) was determined with flow cytometry using fluorescence probe $DCFH_2$-DA. The role of ATM, Chk1 was evaluated with specific siRNA. Related protein expressions were determined with Western blotting. **Results:** Cuc B dramatically induced G_2/M phase arrest. Cuc B treatment caused DNA damage without affecting the signal transducer and activator of transcription 3 (STAT3). Cuc B triggered ATM-activated Chk1-Cdc25C-Cdk1 pathway, which could be reversed by both ATM siRNA and Chk1siRNA treatment. Cuc B also triggered ATM-activated p53-14-3-3-σ pathway, which could be reversed by ATM siRNA. Cuc B treatment increased intracellular ROS formation, which was inhibited by N-acetyl-l-cysteine (NAC) pretreatment. Furthermore, NAC pretreatment inhibited Cuc B induced DNA damage and G_2/M phase arrest. **Conclusion:** Taken together, these results suggested that Cuc B induced DNA damage in A549 cells mediated by increasing intracellular ROS formation, which lead to G_2/M cell phase arrest through ATM-activated Chk1-Cdc25C-Cdk1 and p53-14-3-3-σ parallel branches. These observations provide novel mechanisms and potential targets for better understanding of the anti-cancer mechanisms of cucurbitacins.

Keywords: Cucurbitacin B; ROS; DNA damage; G_2/M arrest

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S5.6

Novel monofunctional platinum (II) complex Mono-Pt induces apoptosis-independent autophagic cell death in human ovarian carcinoma cells distinct from cisplatin

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Aim: Failure to engage apoptosis appears to be a leading mechanism of resistance to traditional platinum drugs in patients with ovarian cancer. Therefore, an alternative strategy to induce cell death is needed for the chemotherapy of this apoptosis-resistant cancer. Here we report that autophagic cell death, distinct from cisplatin-induced apoptosis, is triggered by a novel monofunctional platinum (II) complex named Mono-Pt in human ovarian carcinoma cells. **Methods:** Cytotoxic effects were assessed by MTT and typan blue assay; Cell cycle was detected with PI staining; Autophagic and apoptotic cell death were confirmed by MDC staining, Western blot and EM analysis. **Results:** Mono-Pt-induced cell death has the following features: cytoplasmic vacuolation, caspase-independent, no nuclear fragmentation or chromatin condensation, and no apoptotic bodies. These characteristics integrally indicated that Mono-Pt, rather than cisplatin, initiated a nonapoptotic cell death in caov-3 ovarian carcinoma cells. Furthermore, incubation of the cells with Mono-Pt but not with cisplatin produced an increasing punctate distribution of microtubule-associated protein 1 light chain 3 (LC3), and an increasing ratio of Lc3-II to Lc3-I. Mono-Pt also caused the formation of autophagic vacuoles as revealed by monodansylcadaverine staining and transmission electron microscopy. In addition, Mono-Pt-induced cell death was significantly inhibited by the knockdown of either Beclin-1 or ATG7 gene expression, or by autophagy inhibitors 3-methyladenine, chloroquine and bafilomycin A1. Moreover, the effect of Mono-Pt involved the AKT1-MTOR-RP s6KB1 pathway and ERK1/2 signaling, since the MTOR inhibitor rapamycin increased, while the ERK1/2 inhibitor U0126 decreased Mono-Pt-induced autophagic cell death. **Conclusion:** Taken together, our results suggest that Mono-Pt exerts anticancer effect via autophagic cell death in apoptosis-resistant ovarian cancer. These findings lead to increased options for anticancer platinum drugs to induce cell death in cancer.

Keywords: autophagy; monofunctional platinum complex; cisplatin; AKT; ERK

S5.7

Compound *Astragalus* and *Salvia Miltiorrhiza* Extract suppresses cell migration of keloid fibroblasts: involvement of MAPK signaling pathway

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Aim: To investigate the effects of Compound *Astragalus* and *Salvia Miltiorrhiza* Extract (CASE) on cell migration of keloid fibroblasts (KFs) and its mechanisms involving mitogen-activated protein kinase (MAPK) pathways including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 kinase. **Methods:** KFs were cultured and then treated with transforming growth factor beta 1 (TGF-β_1) with or without CASE (7.5, 15, and 30 μg/mL) treatment. *In vitro* scratch-wound assays were performed to observe cell migration; Phosphorylation of ERK, JNK, or p38 and their intracellular localization in KFs were detected by Western blot and immunofluorescence assay, respectively. **Results:** Cell migration induced by TGF-β_1 in KFs was significantly inhibited by CASE in a dose-dependent manner. ERK, JNK and p38 were moderately phosphorylated and mainly localized in the cytoplasm in KFs under control conditions. TGF-β_1 enhanced the phosphorylation of ERK, JNK and p38, and promoted their nuclear translocation and which were significantly suppressed by CASE treatment. **Conclusion:** CASE significantly suppressed cell migration induced by TGF-β_1 in keloid fibroblasts indicating its anti-keloid effects, the mechanisms might involve the inhibition on MAPK signaling pathway.

Keywords: keloid; compound *Astragalus* and *Salvia miltiorrhiza* extract; cell migration; transforming growth factor beta 1; mitogen activated protein kinase

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S5.8

Synthesis and antibacterial activity evaluation of three novel biscoumarin derivatives

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Aim: The treatment failure of methicillin resistant *Staphylococcus aureus* (MRSA) infections and emergence of vancomycin-resistant *Staphylococcus aureus* (*S aureus*) urgently requires developing new antimicrobials. **Methods:** Three new biscoumarin derivatives, 3,3′-(4-nitrobenzylidene)-*bis*-(4-hydroxycoumarin)

(NBH), 3,3′-(4-methoxybenzylidene)-*bis*-(4-hydroxycoumarin) (MBH), and 3,3′-(4-chloromethylbenzylidene)-*bis*-(4-hydroxycoumarin) (CBH), were synthesized and characterized by FT-IR, ^{1}H NMR, HRMS and single crystal X-ray crystallography. The minimum inhibitory concentration and time–kill curves were observed for the three title compounds and antibiotics. **Results:** Our results showed that two classical asymmetrical intramolecular O–H···O hydrogen bonds were formed in the three compounds. The compound NBH exerted potent bactericidal effects against almost all *S aureus* tested, including LAC, a highly virulent and wide-spread clinical isolate responsible for the recent epidemic of MRSA infections. NBH exhibited almost completely growth inhibition on all *S aureus* tested at 128 μg/mL and partly bactericidal effect at 32 μg/mL. **Conclusion:** The formation of intramolecular hydrogen bonds was considered as an important factor in assisting the molecule to attain the correct configuration for biological activity. The different substituents in the phenyl ring of biscoumarins had different antibacterial activities against *S aureus*.

Keywords: biscoumarin; single crystal; *Staphylococcus aureus*; minimum inhibitory concentration

S5.9

Trp64Arg (rs4994) polymorphism of β3-adrenergic receptor gene is associated with hyperuricemia in a Chinese male population

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Aim: β3-Adrenergic receptor (β3-AR) gene is associated with insulin resistance and may affect serum uric acid levels. Our aim was to determine the possible association between β3-AR gene Trp64Arg polymorphism (rs4994) and hyperuricemia in a Chinese male population. **Methods:** A total of 410 hyperuricemic and 420 normouricemic male subjects were genotyped in this study. The genotypic and allelic frequencies were compared between the two groups. Body mass index (BMI), waist to hip ratio (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP), serum uric acid, urea nitrogen, creatinine, triglyceride, total cholesterol, low-density lipoprotein-cholesterol (LDL-c), high-density lipoprotein-cholesterol (HDL-c) and fasting plasma glucose (FPG) were determined. **Results:** The frequencies of *CC* genotype and *C* allele for Trp64Arg polymorphism were higher in hyperuricemic group than in normouricemic group (P<0.01 and P<0.05, respectively). In both hyperuricemic and normouricemic groups, subjects with mutated *C* allele of Trp64Arg polymorphism showed significantly higher average uric acid levels than *TT* genotype carriers (P<0.01 and P<0.01, respectively). Univariate and multivariate logistic regression showed that carrier of mutated *C* allele of Trp64Arg polymorphism was significantly associated with hyperuricemia occurrence (P=0.003, OR=1.587, 95% CI 1.175–2.145 and P=0.003, OR=1.676, 95% CI 1.051–3.617). **Conclusion:** Trp64Arg polymorphism was associated with hyperuricemia in a Chinese male population and should be an independent risk factor for hyperuricemia.

Keywords: β3-adrenergic receptor; genetic polymorphism; hyperuricemia; serum uric acid; association analysis

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S5.10

Effect of NR2B antagonist against $A\beta_{25-35}$ induced acute synaptic plasticity disruption in rat hippocampal slices

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Aim: Amyloid beta (Aβ)-induced synaptic dysfunction is thought to be an important event in early stage of Alzheimer's disease (AD). In this study, the effect of NR2B antagonist against $A\beta_{25-35}$ induced acute synaptic plasticity disruptionwas observed. **Methods:** Hippocampus slices from adult rats were used for LTP experiments. **Results:** The results shown that treating hippocampal slices with $A\beta_{25-35}$ or NMDA suppressed the induction of long-term potentiation (LTP) significantly. Slices treated with $A\beta_{25-35}$ by incubation could be reserved by ifenprodil incubation. LTP deficits induced by NMDA could also be reserved by ifenprodil. It was interesting that ifenprodil perfusion could not prevent the disruption of LTP in slices treated with $A\beta_{25-35}$ by perfusion. Co-applying ifenprodil and NMDA has protective effect on LTP against $A\beta_{25-35}$ perfusion. It was well established that Aβ could increase glutamate release and Aβ incubation or application *in vivo* might induce a high level of glutamate in incubation fluids or Aβ treated tissue. So perfusion of Aβ and NMDA mimics the situation of Aβ incubation. As the NMDARs are mainly consisting of NR2B and NR2A in the hippocampal tissues of adult rats, co-applying ifenprodil with NMDA means mainly activating NR2A. **Conclusion:** Our results indicated that blocking NR2B and activating NR2A might be a potential treatment strategy for AD in early stages.

Keywords: amyloid beta; NR2A; NR2B; long-term potentiation; hippocampus

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S5.11

Angiotensin II type I receptor-mediated oxidative stress in rostral ventrolateral medulla underlies the elevated blood pressure after stroke

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Stroke is a major health problem throughout the world. The elevated blood pressure (BP) after stroke is a common complication associated with poor outcome and is less understood. Since the crucial role of angiotensin II (Ang II) and rostral ventrolateral medulla (RVLM) in central regulation of BP is well-known, the present study delineated the role of Ang II type I receptor (AT1R) at RVLM in the elevation of BP after transient stroke. The stroked Sprague-Dawley rats received 2-h left middle cerebral artery occlusion (MCAO) operation and BP were measured by tail-cuff plethysmography method in conscious conditions before and 24-h after stroke. The levels of Ang II, AT1R mRNA or protein, superoxide production, and activities of NADPH oxidase (Nox) or mitochondrial respiratory chain in RVLM were determined by ELISA, RT-PCR or chemiluminence method. The elevation of BP was observed at 24 h after MCAO, accompanied by an increase of Ang II and the mRNA or protein levels of AT1R in RVLM. Moreover, the elevated BP, increased superoxide levels, augmented Nox activity and decreased activities of mitochondrial respiratory chain in RVLM at 24 h after stroke were significantly reduced by bilateral microinjection of AT1R antagonist candesartan (5 nmol), Nox inhibitor apocynin (10 nmol) or mitochondrial superoxide anion scavenger mitoTEMPO (500 pmol) into RVLM immediately after MCAO. We concluded that the AT1R-mediated oxidative stress in the RVLM participates in the elevation of BP after stroke.

Keywords: MCAO; stroke; AT1R; RVLM; oxidative stress

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S5.12

Id proteins are critical downstream effectors of BMPR-II in pulmonary arterial smooth muscle cells

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Aim: Bone morphogenetic protein type II receptor (BMPR-II) mutations are responsible for over 70% of cases of heritable pulmonary arterial hypertension (PAH) and up to 15%–40% of sporadic idiopathic disease. Loss of BMP signalling promotes pulmonary vascular remodelling via modulation of pulmonary artery smooth muscle cell (PASMC) migration, differentiation and proliferation. Id proteins (Id1-4), are endogenous inhibitors of basic helix-loop-helix transcription factor binding to DNA, and are major downstream transcriptional targets of BMP signalling. However, the impact of BMPR-II mutation on the expression of the range of Id proteins and the contribution of individual Id proteins to abnormal PASMC function remains unclear. **Methods:** Human PASMCs were incubated with BMP4, 6, 9 or with a range of cytokines. Expression of Id proteins (Id1-4) was determined using real-time PCR and Western blotting. The responses in control cells were compared with PASMCs harboring BMPR-II mutations and cells in which BMPR-II was knocked down by siRNA transfection. Id3 expression in pulmonary

vessel was also investigated in the lungs of wild type mice and BMPR-II mutant mice. Furthermore, lentiviral over-expression of Id3 was employed to investigate the role of Id3 protein on cell cycle regulation and proliferation in PASMCs. **Results:** BMP4 and BMP6, but not BMP9, induced mRNA expression of all four Id genes in PASMCs, though Id1 and Id3 demonstrated higher levels of induction compared to Id2 and Id4. Other cytokines and growth factors tested had minimal effect on Id gene expression. The time course and dose responsiveness of Id1 and 3 induction by BMPs was confirmed by immunoblotting. The BMP stimulated induction of Id1 and Id3 was markedly reduced in BMPR-II mutant PASMCs and in control PASMCs following siRNA silencing of BMPR-II. Pulmonary arteries in BMPR-II mutant mice demonstrated a lower expression of Id3 than in wild type mice. Lentiviral overexpression of Id3 reduced cell cycle progression and inhibited proliferation of PASMCs. **Conclusion:** Id proteins, and particularly Id1 and Id3, are critical downstream effectors of BMP signaling in PASMCs. Loss of BMPR-II function reduces the induction of Id genes in PASMCs, and Id3 regulates the proliferation of PASMCs via cell cycle inhibition.

Keywords: pulmonary hypertension; BMPR-II; Id proteins; PASMCs; cell cycle
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S5.13

The analgesic and anticancer effects of minimally toxic fraction from Liu-Shen-Wan and the underlying mechanisms

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Aim: Liu-Shen-Wan (LSW), an ancient preparation used to treat localized infection with pain, was reported to possess anticancer activity. We obtained a LSW supernatant (LSWS) fraction from ultrasound-assisted ethanol extraction (yield 15.9%) which proved to be safer than LSW in terms of hepatotoxicity. The mechanism responsible for LSW's analgesic and anticancer activity was investigated. **Methods:** Involvement of substance P/neurokinin-1 receptor (NK-1R) in these effects was studied in mouse dorsal root ganglion (DRG) cells and HL-60 and HepG2 cancer cells. **Results:** The LSWS (1 and 10 μg/mL) exhibited a potent inhibitory effect on the bradykinin-evoked rapid release of substance P from DRG cells. At concentrations of 0.1 μg/mL and higher, the LSWS resulted in a significant increase in the percentage of apoptotic HL-60 and HepG2 cancer cells. The LSWS also exerted a concentration-related inhibitory effect on HepG2 cells. The LSWS significantly downregulated the NK-1R expression in both HepG2 and bradykinin-treated DRG cells. In addition, it suppressed the caspase-dependent apoptotic pathway, but induced mitochondria-mediated apoptosis in HepG2 cells by downregulating the Bcl-2/Bax expression ratio. **Conclusion:** The substance P/NK-1R system was partly responsible for the analgesic and anticancer activity of LSWS. Our findings will be useful for developing more effective and less toxic LSW preparations.

Keywords: Liu-Shen-Wan; anticancer; analgesic; substance P; neurokinin-1 receptor
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S5.14

N8-regulated entry of CD8$^+$ T cells into the CNS is required for the initiation of experimental autoimmune encephalomyelitis

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Aim: In contrast to T cell priming in the periphery, therapeutic strategies targeting the initiation step of T cell trafficking into the central nervous system (CNS) have not been extensively investigated. In this study, the effect of N8 on experimental autoimmune encephalomyelitis (EAE) and the unique mechanism were elucidated. **Methods:** C57BL/6 mice were immunized with MOG35-55. Mice were monitored for clinical severity of disease and histopathologic features in the CNS. Levels of cytokines in serum were measured by ELISA. Effects of N8 on expressions of chemokines and cytokines in the CNS were determined by quantitative PCR. **Results:** N8-treated mice developed conventional T helper 1 (T_H1) and T helper 17 (T_H17) responses but were highly resistant to the induction of EAE. Treatment with N8 resulted in decreased CNS accumulation of lymphocytes and increased functional expression of the chemokine receptor CXCR7 on CD8$^+$ T cells. Moreover, adoptive transfer of T cells from 2D2-transgenic mice reconstituted EAE susceptibility in N8-treated mice, indicating that N8 only targets the initial migration of pioneer T cells. **Conclusion:** N8 almost completely abolished the development of EAE by blocking the initial infiltration of pioneer CD8$^+$ T cells into the uninflamed CNS. Taken together, these results support N8 as a potential lead compound for the treatment of relapsing-remitting multiple sclerosis.

Keywords: N8; pioneer CD8$^+$ T cells; CXCR7; EAE; multiple sclerosis

S5.15

Pharmacological study of a novel urea transporter inhibitor PU-48 as a diuretic

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Aim: The purpose of this study is to determine the feasibility of urea transporters (UT) as novel diuretic drug targets and to discover urea transporter inhibitors with diuretic activity. **Methods:** *In vitro* erythrocyte lysis model was used to get the potential urea transporter inhibitors. Inhibition activity of hits on UT-A was assayed in an MDCK cell line that stably expresses rat UT-A1. Cytotoxicity was assayed with MDCK cell viability after incubation with compounds for 72 h by cell counting kit-8 (CCK-8) assay. Diuretic activity of the compounds was determined in rats. Urine output, blood and urinary osmolality and urea concentration were measured to evaluate the diuretic role of compounds. **Results:** Structure-activity analysis indicated that the most potent compound, thienoquinolin PU-48, reversibly inhibited UT-B-facilitated urea transport in human, rabbit, rat and mouse erythrocytes with IC_{50} of 0.18, 0.68, 0.35, and 0.77 μmol/L, respectively. PU-48 did not affect urea transport in UT-B null mouse erythrocytes. PU-48 showed no significant cellular toxicity at concentrations up to 80 μmol/L. Subcutaneous delivery of PU-48 (at 3.12, 12.5, and 50 mg/kg) to rats produced an increase of urine output and a decrease of the urine urea concentration and osmolalities without electrolyte disturbance and liver or renal damages, which indicates that PU-48 has significant diuretic effect by urea-selective diuresis. Moreover, there was no significant difference in UT-A1-A3, UT-B, and AQP1-4 protein abundance in the medulla between PU-48 treated rats and control. **Conclusion:** These results indicate that PU-48 or its analogues might be developed as a new diuretic to increase renal fluid clearance in diseases associated with water retention without causing electrolyte imbalance, as well as a tool drug to establish 'chemical knockout' analysis of the physiological functions of UTs.

Keywords: diuretics; urea transporter; urine concentrating mechanism; drug discovery
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S5.16

Low expression of β-arrestin2 correlated with hepatocellular carcinoma progression

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Aim: β-Arrestins are ubiquitous cytosolic proteins which initially be regarded as potential characters in G protein-coupled receptors (GPCR) desensitization, sequestration, and internalization. Recently, it has been documented that β-arrestins play a vital role in many tumors growth and metastasis. The present study aimed to explore the function and prognostic role of β-arrestins in hepatocellular carcinoma (HCC). **Methods:** The expression of β-arrestin1 and β-arrestin2 in paraffin-embedded human liver tissues and in tumors from HCC patients who underwent surgery resection were detected by immunohistochemistry (IHC), qPCR and Western blot, respectively. The prognostic significance of β-arrestin2 was validated using the Kaplan-Meier survival estimates and the log-rank tests. The

expression of β-arrestin1 and β-arrestin2 in stepwise metastatic human HCC cell lines (HCCLM3, MHCC97H, MHCC97L, SMMC-7721, and HepG2) and normal liver cell line L-02 were measured by RT-PCR and Western blot analysis. pcDNA3 expression plasmids encoding HA-tagged β-arrestin2 was used to investigate the role of β-arrestin2 in the metastasis and invasion of HCC. **Results:** IHC analysis showed that expression of β-arrestin2 was higher in paraffin-embedded normal liver tissues than in HCC tumor tissues, but the expression of β-arrestin1 was not significantly changed. The similar results were presented in tumors from HCC patients who underwent surgery resection. Univariate and multivariate analysis revealed that β-arrestin2 was a significant predictor for overall survival. *In vitro*, the RT-PCR and Western blot analysis showed that β-arrestin2 was low expressed in the highly metastatic HCC cell lines and high expressed in normal liver cell line L02, but there was no difference of β-arrestin1 expression among human HCC cell lines and L02. Furthermore, HCCLM3 cells were transfected with plasmid pcDNA3/β-arrestin2-HA. Cell scratching and transwell experiments showed that overexpression of β-arrestin2 reduced the ability of cell migration and invasion. Moreover, overexpression of β-arrestin2 significantly attenuated the pAkt activation and enhanced the level of E-cadherin. However, ERK activation had no significant change. **Conclusion:** β-Arrestin2 upregulation inhibits HCC cell invasion. And β-arrestin2 might to be a novel target in HCC diagnosis and therapy.

Keywords: hepatocellular carcinoma; β-arrestins; invasion; metastasis; prognosis
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S5.17

Vascular endothelial growth factor receptor 2 maintains resting cardiac vagal baroreflex and heart functions as revealed by $Kdr^{+/-}$ mice

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Aim: The nucleus tractus solitarii (NTS), the principal terminal site of the baroreceptor afferents, contributes to cardiac vagal baroreflex by its connection with the nucleus ambiguus (NA), the origin of the vagus nerve. We investigated the role of vascular endothelial growth factor receptor 2 (VEGFR2), also known as Kdr/Flk-1, in cardiac vagal baroreflex and heart function under resting conditions. **Methods:** Blood pressure and heart rate (HR) of heterozygous ($Kdr^{+/-}$) and wild-type ($Kdr^{+/+}$) mice were recorded under conscious state by radiotelemetry. Magnetic resonance imaging/diffusion tensor imaging (MRI/DTI) was performed using a 9.4 T Animal MR scanner. Echocardiography was obtained using a 40-MHz linear-array transducer. **Results:** Flk-1 mRNA and protein in the NTS and heart, power spectrum of HR, an indicator of overall functionality of brain stem cardiovascular regulation, were significantly reduced in $Kdr^{+/-}$ mice. DTI of the brain stem revealed that the connectivity between the NTS and NA was reduced in $Kdr^{+/-}$ mice, concurrent with a decrease in cardiac vagal baroreflex and an increase in HR. Echocardiographic analysis further showed that $Kdr^{+/-}$ mice were inferior to $Kdr^{+/+}$ mice in terms of ejection fraction , fractional shortening, circumferential strain, and radial strain.

Conclusion: We conclude that VEGFR2 expression in the NTS is required for the maintenance of cardiac vagal baroreflex manifested in the form of connectivity between the NTS and NA, and for sustained heart functions under resting conditions.

Keywords: VEGFR2; cardiac vagal baroreflex; MRI/DTI

S5.18

Nicotinamide phosphoribosyltransferase-mediated NAD^+ biosynthesis contributes to post-ischemic neovascularization: role of endothelial progenitors

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Aim: The aim of the present study was to determine whether nicotinamide phosphoribosyltransferase (NAMPT)-mediated nicotinamide adenine dinucleotide (NAD^+) biosynthesis is important for post-ischemic neovascularization. **Methods:** EPCs were isolated from bone-marrow (BM) for *in vitro* study. Two strains of transgenic mice overexpressing NAMPT (Tg mice) and H247A-mutant dominant negative NAMPT (ΔTg mice) were generated for *in vivo* and mechanism study. Diabetic *db/db* mice and patients with EPC dysfunction were administrated with a substrate of NAMPT (vitamin B_3) to test the potential translational therapeutic value. **Results:** In mice, ischemia increased the circulating and BM NAD^+ levels and EPCs number, which were abolished by NAD^+ synthesis blockade with NAMPT inhibition. NAMPT inhibition also inhibited EPCs function *in vitro*. EPCs obtained from Tg mice, but not ΔTg mice, demonstrated enhanced proliferation, migration and tube formation. Furthermore, post-ischemic neovascularization after hind-limb or cerebral ischemia was improved in Tg mice but not in the ΔTg mice. NAMPT-overexpression increased the protein levels of endothelial nitric oxide synthase (eNOS) in EPCs in a SIRT1 deacetylase-dependent manner. Lastly, administration of vitamin B_3 increased the number of circulating EPCs in diabetic *db/db* mice as well as in patients, and accelerated wound healing in *db/db* mice. **Conclusion:** These findings suggest that NAMPT-mediated NAD^+ metabolic pathway plays a critical role in biological function of EPCs and contributes to post-ischemic neovascularization.

Keywords: endothelial progenitor cells; NAD^+; NAMPT; neovascularization; SIRT1
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S5.19

Tumor necrosis factor-α regulates the signaling of prostaglandin E_2 receptor 4 through the interaction between TRAF2 and GRK2 in human fibroblast-like synoviocytes

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Aim: In our previous work, we established that upon tumor necrosis factor-α (TNF-α) stimulation, the prostaglandin E_2 (PGE_2)- PGE_2 receptor (EP)- guanine nucleotide-binding protein (G protein)- adenylyl cyclase (AC)-cyclic adenosine monophosphate (cAMP) signal transduction pathway of synoviocytes in collagen-induced arthritis (CIA) rats was unbalanced. The present study was designed to explore how TNF-α regulated the signaling of EP4 receptor through its downstream molecules in human fibroblast-like synoviocytes (hFLS). **Methods:** TNF-α-induced endogenous PGE_2 production was inhibited by Indometacin (INN), a COX-2 selective inhibitor. The concentration of PGE_2 in culture supernatants was determined with a human-specific enzyme linked immunosorbent assay (ELISA) kit. Proliferation of hFLS was detected by thiazolyl blue tetrazolium bromide (MTT) assay. Cellular cAMP level was measured using ^{125}I-cAMP radioimmunoassay (RIA) kit. The levels of TNF receptor (TNFR) 1 and TNFR2 were quantified by flow cytometry. The expressions of total TNF receptor-associated factor 2 (TRAF2), stimulatory G protein (Gαs), EP4, as well as the levels of EP4 and G protein coupled receptor kinase 2 (GRK2) in plasma membrane and cytoplasm were analyzed by Western blotting. The ability of TRAF2 to interact biochemically with GRK2 or β-arrestin2 and the level of activated Gαs were determined by co-immunoprecipitation. The gene expression of TRAF2 was silenced by small interfering RNA (siRNA) transfection. **Results:** We were surprised to find that TRAF2 could interact with GRK2, which contributed to the regulation of EP4 signaling by TNF-α. Further studies revealed that silencing TRAF2 gene could significantly decrease the membrane translocation of GRK2 resulted in an up-regulated expression of membrane EP4 and intracellular cAMP. **Conclusion:** These results suggest a novel form of cross-talk between TNF-α and G protein coupled receptors (GPCRs) EP4. TNF-α promotes desensitization of EP4 by regulating the compound of TRAF2-GRK2; furthermore, TRAF2 could control the translocation of GRK2.

Keywords: tumor necrosis factor-α; TNF receptor-associated factor 2; G protein coupled receptor kinase2; EP4; human fibroblast-like synoviocytes
Acknowledgements: This work was financially supported by the National Natural Science Foundation of China (30973543, 81173075, 81202541, and 81202596), the Anhui Provincial Natural Science Foundation (1208085QH146), the Anhui Province Natural Science Foundation in University (KJ2011Z181 and KJ2011Z180), the Specialized Research Fund for the Doctoral Program of Higher Education of China (20113420120002), Grants for Scientific Research of BSKY (XJ201213), and the Foundation for Key Teacher by Anhui Medical University (2013).

S5.20

Proliferation effects of dorsolateral prostate induced by exposure to low-dose bisphenol A in adult rats

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Aim: Since low-dose bisphenol A (BPA) could aggravates symptom of benign prostatic hyperplasia (BPH) in rat BPH model, we hypothesized that it will has the same effect on prostate of adult rats, thus conducted present study and further investigated its underlying mechanisms. **Methods:** 50 adult male rats were divided into 5 groups at random, 10 per group, and were ig 10 mL/kg vehicle, 200 μg/kg Tamoxifen (TAM), 10.0, 30.0, and 90.0 μg/kg BPA for 4 weeks. Animal were terminated on the next day after the last treatment. Blood were collected to detect levels of testosterone (T), estradiol (E_2), prolactin (PRL) and prostate specific antigen (PSA). The dorsolateral prostate (DLP) lobes were dissected and weighed, then fixed in formalin solution for histological examination, and further detected gene expression by microarray analysis and RT-PCR analysis**. Results:** 1) There were no significant differences in animal body weight between control and BPA groups, while it was lower in TAM group. 2) Between control and BPA groups, there were no differences in levels of E_2 and PRL, but T levels in BPA groups were decreased, and PSA levels in 10.0 μg/kg (P<0.05) and 90.0 μg/kg BPA (P<0.01) groups were increased. In TAM group, levels of E_2, PRL (P<0.05) and PSA (P<0.01) were increased than that of control, but T level was lower than that of control. 3) Compared with control, total prostate weight, volume and prostate index in BPA groups were increased, and weight and index of DLP were increased than that of control (P<0.01). While in TAM group, all indexes were decreased than that of control. 4) In BPA groups, the height of DLP epithelium were higher than that of control (P<0.01), but it decreased with BPA dose increasing, while in TAM group, it was lower than that of control (P<0.01). 5) Microarray analysis and RT-PCR analysis results showed that BPA induced up-regulation of *Pcna* and *Egf*, and down-regulated expression of *Cdh2* in DLP, especially down-regulated the gene *Cdh2* expression. **Conclusion:** Environment exposure to low dose of BPA may induce DLP of adult rats to proliferate through activating DNA replication and biosynthesis of steroids pathways.

Keywords: bisphenol A; proliferation; benign prostatic hyperplasia; genome oligo microarray analysis; RT-PCR; rat

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S5.21

P-glycoprotein mediated efflux of aconitine *in vitro*, in situ and *in vitro*

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Aim: Aconitine (AC) is a toxic alkaloid from some Chinese medicinal herbals with origin of genus *Aconitum*, which have been widely used for analgesic, cardiotonic and anti-rheumatism treatment. To assess the mechanism of its low and variable oral bioavailability, the role of P-glycoprotein (P-gp) in AC absorption was evaluated in this study. **Methods:** The bidirectional transport of AC across Caco-2 and MDR1-MDCKII cells was investigatedwith or without P-gp inhibitors (verapamil and cyclosporin A). This was followed by an *in situ* single-pass intestinal perfusion study in rat to evaluate the effect of verapamil on the intestinal permeability of AC and determination of pharmacokinetic profile of AC following oral administration with or without pre-treatment of verapamil in rats. **Results:** The efflux of AC across Caco-2 and MDR1-MDCKII cells was greater than its influx and both P-gp inhibitors significantly decreased the efflux of AC. The *in situ* study indicated verapamil co-perfused with AC exhibited significant increase in intestinal permeability by 12.9-fold, from $(0.22\pm0.12)\times10^{-5}$ cm/s to $(2.85\pm0.85)\times10^{-5}$ cm/s. After oral dosing, the mean area under the plasma−concentration curve (AUC) of AC significantly increased from 150.73±29.62 h·ng/mL without verapamil to 1002.75±105.9 h·ng/mL with verapamil (P<0.001). **Conclusion:** This work updates the involvement of P-gp in low oral bioavailability of AC and provides significant information for clinical application of aconite and their formulations containing AC.

Keywords: aconitine; P-glycoprotein; permeability; oralabsorption

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S5.22

Translational regulation of RPA2 via internal ribosomal entry site and by eIF3a

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Aim: RPA2 is a subunit of a trimeric RPA complex important for DNA repair and replication. Although it is known that RPA activity is regulated by post-translational modification, whether RPA expression is regulated and the mechanism therein is currently unknown. eIF3a, the largest subunit of eIF3, is an important player in translational control and has been suggested to regulate translation of a subset of mRNAs important for tumorigenesis, metastasis, cell cycle progression, drug response, and DNA repair. In the present study, we aimed to explore if RPA2 expression is regulated at translational level via internal ribosome entry site (IRES)-mediated initiation in response to DNA damage. **Methods:** Dual luciferase reporter assay, *in vitro* transcription and translation were used to identify and locate IRES element of RPA2. Realtime PCR and Western blotting were used to detect RPA2 mRNA and protein expression level, respectively. UV crosslinking and pull down assay was performed to explore the binding of eIF3a and RPA2 IRES. **Results:** In the present study, we show that RPA2 expression is regulated at translational level via internal ribosome entry site (IRES)-mediated initiation in response to DNA damage. We also found that eIF3a suppresses RPA2 synthesis and inhibits its cellular IRES activity by directly binding to the IRES element of RPA2 located at -50 to -150 bases upstream of the translation start site. **Conclusion:** RPA2 expression is translationally regulated via IRES and by eIF3a and that this regulation is partly accountable for cellular response to DNA damage and survival.

Keywords: eIF3a; replication protein A (RPA); cellular internal ribosome entry sites (IRES); DNA repair; DNA damage; translational control

S5.23

Traditional Chinese medicine with reinforcing kidney activity ameliorate experimental autoimmune encephalomyelitis in rats by modulating neuroinflammatory and neurotrophic responses

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Aim: The present study was designed to determine whether traditional Chinese medicine with reinforcing kidney activity such as cornel iridoid glycoside (CIG) and epimedium flavonoids (EF) had effect on the development of experimental autoimmune encephalomyelitis (EAE) in rats and to elucidate its underlying mechanisms. **Methods:** EAE model was induced by immunization of adult female Lewis rats with purified guinea-pig myelin basic protein (MBP) 68–86. CIG and EF were administrated intragastrically once a day after immunization until d 21 post immunization (PI). Histopathological staining, enzyme-linked immunosorbent assay (ELISA), biochemical methods and Western blotting approaches were used to evaluate the disease incidence and severity, neuroinflammatory and neurotrophic response in the central nervous system (CNS). **Results:** CIG or EF intragastrical administration significantly reduced clinical score of neurological deficit in EAE rats; alleviated demyelination and inflammatory infiltration; and inhibited glias activation, nuclear transcription factor (NF-κB) expression in the spinal cord of EAE rats. Treatment with CIG or EF also enhanced neurotrophic factors such as nerve growth factor (NGF) or brain-derived nerve factor (BDNF) expression, increased the number of oligodendrocytes and protected the ultrastructure of myelin sheaths and axons in the spinal cord of EAE rats. **Conclusion:** Our results showed that CIG and EF could inhibit the development of MBP-induced EAE in rats and our findings suggest that traditional Chinese medicine with reinforcing kidney activity may be useful for the treatment of multiple sclerosis. This effect involved reducing neuroinflammation and enhancing myelination and neurotrophins.

Keywords: experimental autoimmune encephalomyelitis; cornel iridoid glycoside; epimedium flavonoids; neuroinflammation; neurotrophin

S5.24

Berberine improves glucose consumption in cardiomyocytes via activation of 5'-adenosine monophosphate-activated protein kinase

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Aim: Insulin resistance plays an important role in the pathogenesis of diabetic cardiomyopathy. Berberine (BER) is a plant alkaloid which promotes hypoglycemia via increasing insulin sensitivity in peripheral tissues. Little is known of BBR's role in regulating glucose metabolism in heart. **Methods:** We examined the effect and mechanism of BER on glucose consumption and glucose uptake in insulin sensitive or insulin resistant rat H9c2 cardiomyocyte cells. H9c2 myoblast cells were differentiated into cardiomyocytes and incubated with insulin for 24 h to induce insulin resistance. **Results:** BER-treatment of H9c2 cells increased glucose consumption and glucose uptake compared to controls. In addition, BER-treatment attenuated the reduction in glucose consumption and glucose uptake in insulin resistant H9c2 cells. Compound C, an inhibitor of AMP-activated protein kinase (AMPK), abolished the enhancement of glucose consumption and glucose uptake mediated by BBR in both insulin sensitive and insulin resistant H9c2 cells compared to controls. **Conclusion:** BER significantly increased AMPK activity, but had little effect on the activity of protein kinase B (AKT) in insulin resistant H9c2 cells, suggesting that berberine improves insulin resistance in H9c2 cardiomyocytes at least in part via stimulation of AMPK activity.

Keywords: berberine; glucose consumption; cardiomyocyte; AMPK

S5.25

Lycium barbarum polysaccharide LBPF4-OL, a new Toll like receptor 4-MD2 complex activator and inducer, can increase the p38 MAPK phosphorylation and decrease JNK and ERK1/2 phosphorylation

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Aim: LBPF4-OL is a polysaccharide from traditional Chinese medicine *Lycium barbarum*. In this research, we investigated the molecular mechanism of LBPF4-OL on macrophages. **Methods:** RT-PCR and ELISA were used in the analysis of cytokine level. Lymphocyte proliferation was analyzed with ^{3}H-TdR incorporation method. TLR4 gene mutation mice C3H/HeJ and antibodies block were used to verify the binding sites. Flow cytometry (FCM) and Western-blot (WB) were used to analysis TLR4 level and MAPK signal pathway, respectively. **Results:** We found that LBPF4-OL induced the TNF-α and IL–1β secretion in concentration dependent manner. Anti-TLR4 significantly inhibited LBPF4-OL induced TNF-α and IL-1β secretion. Anti-TLR2 (0.31, 0.62, 1.25, and 2.5 μg/mL) concentration dependently inhibited IL-1β secretion, but only in the concentration of 0.62 μg/mL inhibited TNF-α secretion. Anti-CR3 did not affect the TNF-α and IL-1β secretion. In C3H/HeJ mice, the results showed that LBPF4-OL significantly induced TNF-α and IL-1β secrete on C3H/HeJ mice macrophage, but had no influence on those of C3H/HeN mice. FCM results showed that LBPF4-OL significantly induced the expression of TLR4/MD2 both on peritoneal macrophage and on Raw264.7 cells. WB results showed that LBPF4-Ol could strengthen p38 MAPK phosphorylation, but inhibited ERK1/2 and JNK phosphorylation. **Conclusion:** *Lycium barbarum* polysaccharide LBPF4-OL is a new Toll like receptor 4-MD2 complex activator and inducer, which can increase p38-MAPK and decrease JNK and ERK1/2 phosphorylation level.

Keywords: *Lycium barbarum* polysaccharide; TLR4/MD2; MAPK

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S5.26

Upregulation of TRPM7 channels by angiotensin II triggers phenotypic switching of vascular smooth muscle cells of ascending aorta

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Aim: The present study was designed to explore whether the Ca^{2+}-permeable transient receptor potential melastatin 7 (TRPM7) channel is involved in Ang II-induced phenotype switching of ascending aortic VSMCs, and to dissect the molecular mechanisms by which TRPM7 modulates VSMC phenotype.

Methods: Whole-cell and single-channel patch clamping was carried out to measure TRPM7 currents in mouse aortic smooth muscle cells. TRPM7-mediated Ca^{2+} entry was assessed by Fura-2/AM loading. Cell cycle distribution was analyzed by flow cytometry. **Results:** As revealed by current recording, Ang II infusion increased TRPM7 whole-cell currents in ascending aortic VSMCs. The increase in TRPM7 currents was found to result from enhanced expression of TRPM7 protein, rather than elevated single channel activity (open probability and slope conductance) and/or reduced Mg^{2+}-mediated channel block. Mechanistically, Ang II elevated TRPM7 expression via Ang II type 1 receptor-mediated ERK1/2 signaling. As indicated by the expression levels of VSMC differentiation marker genes, phenotypic switching of ascending aorta occurred during Ang II infusion. Meanwhile, ERK1/2-Elk-1 signaling pathway known to suppress VSMC differentiation was activated in Ang II-infused ascending aorta. Knockdown of TRPM7 with small interfering RNA (siRNA) established a causative role of TRPM7 in Ang II-induced phenotypic change and promotion of cell proliferation. Moreover, TRPM7 was shown to be required for Pyk2-ERK1/2-Elk-1 pathway activation by Ang II, which potentiated TRPM7 channel function and thus activated the Ca^{2+}-sensitive kinase Pyk2. Finally, TRPM7 knockdown attenuated Ang II-induced displacement of myocardin from *SM22* promoter, but the effects could be reversed by expression of constitutively active c-Src. **Conclusion:** Our data establish that upregulation of TRPM7 channels by Ang II contributes to the development of the proliferative phenotype of ascending aortic VSMCs, and TRPM7 channel suppresses VSMC gene expression via Ca^{2+} influx-mediated activation of Pyk2-ERK1/2-Elk-1 pathway.

Keywords: angiotensin II; TRPM7; phenotypic modulation; smooth muscle cell; Ca^{2+} signal

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S5.27

Effects of Jin Chai antiviral capsule *in vivo* approach of influenza virus infection through inhibition of virus-induced NF-κB activation

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Aim: This study is to investigate the treatment of Jin Chai antiviral capsule for influenza virus PR8/34 (H1N1) infection. **Methods:** The model of pneumonia was established by dropping influenza virus into the nose of normal mice, using specific IKK inhibitors at non-toxic concentrations. This study analyzed the expression of NF-κB virus load cytokinen in mouse model at the 1st, 3th, 5th, and 7th d after affected. **Results:** 1) By immunohistochemical assay that NF-κB pathway are activated upon PR8/34 (H1N1). By real-time PCR assay that both IKK inhibitors at non-toxic concentrations and Jin Chai antiviral capsule dose groups lead to decrease in signaling virus load and reduced cytokine (ELISA). Jin Chai antiviral capsule can decrease the expression of NF-κB. IKKα, large dose groups are significantly decrease the expression of NF-κB. IKKαcompared with model group at each time point (P<0.05, P<0.01). Middle dose groups are significantly decrease the expression of NF-κB. IKKα compared with model group at the 3th d and the 5th d (P<0.05), small dose groups are significantly decrease the expression of NF-κB. IKKαcompared with model group at the 3th d (P<0.05). Specific IKK inhibitors used in non-toxic concentration, also were given the Jin Chai antiviral capsule (large dose groups), assay that there was no obvious difference between the IKK inhibitors group and IKK inhibitors+Jin Chai antiviral capsule group (virus load cytokinen). **Conclusion:** Effects of Jin Chai antiviral capsule approach of influenza virus infection through inhibition of virus-induced NF-κB activation.

Keywords: Jin Chai antiviral capsule; influenza virus H1N1; NF-κB pathway

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