**Objective** We have previously shown that genetic deletion of the transcription factor E2F1 increases the expression of vascular endothelial growth factor (VEGF) and enhances blood flow recovery in the ischemic limb (Qin et al., PNAS 2006). However, the physiological significance of this regulation in ischemic heart disease and the molecular mechanisms of E2F1-mediated VEGF regulation are still unknown. The purpose of this study is to understand the role of E2F1 in cardiac neovascularization following ischemic injury.

**Methods and results** Myocardial infarction (MI) was induced by surgical ligation of the Left Anterior Descending (LAD) coronary artery in wild-type (WT) and E2F1−/− mice. At day 5 after surgery, angiogenic factors at the infarct border zone were analysed by qRT-PCR and Western blotting. At day 28, the vascular density and infarct size were evaluated histologically. VEGF mRNA and protein levels were significantly higher in E2F1−/− than in WT mice (p < 0.01, n = 5). E2F1−/− mice displayed a greater vessel density in the infarct border area (p < 0.01, n = 5) and a smaller infarct size (p < 0.01, n = 15). In vitro, hypoxia treatment (0.5% O2 for 24 h) increased VEGF mRNA expression to a higher level in E2F1−/− cardiac fibroblasts than in WT control cells (p < 0.01, n = 5). Overexpression of E2F1 suppressed the hypoxia-induced VEGF promoter activity in WT cells, however, (del) but not in p53−/− cells, suggesting that p53 is required for E2F1 to suppress VEGF transcription. Hypoxia treatment (0.5% O2) for 24 h dramatically increased the level of both E2F1 and p53 proteins; overexpression of E2F1 further enhanced the hypoxia-induced accumulation of p53. To understand whether E2F1 regulates p53 protein stability, we treated WT and E2F1−/− cardiac fibroblasts with hypoxia for 6 h, pulsed the cells with cyclohexamide (40 mg/ml) and chased p53 degradation. The p53 protein level declined gradually in WT cells (half-life: ~4 h), but, significantly faster in E2F1−/− cells (half-life: ~1 h) (p < 0.01 at 1, 2, and 4 h, n = 4). Interestingly, addition of Lactacyclin significantly delayed the rates of p53 degradation in both WT and E2F1−/− cells and eliminated the difference between the two groups of cells, suggesting that under hypoxia, E2F1 promotes p53 accumulation by attenuating its ubiquitin-proteasomal degradation. Furthermore, co-immunoprecipitation (co-IP) experiments indicated that hypoxia treatment induced physical associations between E2F1 and p53. In the E2F1−/− cells, suggesting that under hypoxia, E2F1 stabilizes p53 accumulation by attenuating its ubiquitin-proteasomal degradation.

**Conclusions** The E2F1 stabilizes p53 protein, thereby suppressing VEGF expression and new vessel formation in the ischemic heart. Targeting E2F1-p53 interaction (eg, by E2F1 N-terminal peptide) may protect heart from ischemic injury.

*Note: Results and Methods were mixed.*
Methods 36 Wistar rats were divided randomly into three groups: control group (n=12), angioplasty group (n=12) and Bosentan (BA) with angioplasty group (n=12). Bosentan was administrated del to rats of BA with angioplasty group. The balloon catheter injury was performed on left common carotid artery of rat by imitating the process of angioplasty. After 7 days and 14 days, neointimal and media area of BA with angioplasty group was higher than that of the angioplasty group (p<0.01). The serum VEGF level 7 days after injury had linearly negative correlation with SI. The in second experiment, arterial neointima hyperplasia reached delsummit at 28 days in the angioplasty group and 14 days in the BA with angioplasty group. Neointimal and media area of BA with angioplasty at different times (14th, 28th, 45th day) were significantly decreased compared with angioplasty group (p<0.001). The rate of PCNA positive cell increased statistically in BA with angioplasty group than in angioplasty group at 14th day (p<0.01). The rat of a2-action positive cell increased significantly in BA with angioplasty group compared with angioplasty group (p<0.01) at 14th day.

Conclusions Bosentan may be effective on artery restenosis by inhibiting neointimal hyperplasia, increasing the serum VEGF level, reducing the proliferation, migration and transconformation of vascular smooth muscle cells.

Note: Combination of paper e0367 and e0371.

e0051 MIR214 IS UPREGULATED DURING VENTRICULAR REMODELLING POST MI

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Objective MicroRNAs (miRNAs) are endogeneous, single-stranded non-coding RNA molecules about 22 nucleotides long, del regulating target gene expression post-transcriptionally by base pairing with specific binding sites located in the 3' untranslated regions (UTRs) of downstream target mRNAs. miRNAs play important roles in the regulation of a multitude of physiological functions such as cell differentiation, proliferation, apoptosis and immune response. Recent studies suggest that aberrant expression of miRNAs is associated with cardiovascular diseases. Those miRNAs exhibit unique spatial expression patterns that might become biomarker of diagnosis and target of treatment of ventricular remodelling. In present study, the expression level of miR-214 during ventricular remodelling post MI was detected.

Methods Rats underwent left descending coronary ligation or sham surgery. Rats with MI was assigned to two groups (n=5). Realtime PCR was developed to detect the expression of miR-214 in myocardium and plasma.

Results The expression level of miR-214 in both myocardium and plasma were up-regulated in the 14th and 28th day post MI. Compared to sham group, the expression level of miR-214 in myocardium increased by 33% (1.38±0.12 vs 1.00±0.02, p<0.01) in the 14th day and by 88% (1.85±0.08 vs 1.00±0.02, p<0.01) in the 28th day post MI. Compared to sham group, the expression of miR-214 in plasma increased by 60% (1.60±0.09 vs 1.00±0.06, p<0.01) in the 14th day and by 116% (2.16±0.13 vs 1.00±0.06, p<0.01) in the 28th day post MI.

Conclusions The expression level of miR-214 in myocardium and in plasma up-regulated in the progress of ventricular remodelling post MI in rat. The dynamic change of miR-214 may potentially become a new biomarker in ventricular remodelling post MI.

e0052 VALSARTAN REVERSED VASCULAR FIBROSIS THROUGH THE BLOCKADE OF THE AT1-MEDIATED TGF-β/SMAD SIGNAL PATHWAY IN THE FAT-FED, STREPTOZOTOCIN-TREATED RATS

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Objective Angiotensin II (All) and transforming growth factor-β (TGF-β) are closely involved in the pathogenesis of diabetic complications. The aim of this study was to clarify the role of All in the regulation of the TGF-β system in diabetic vascular dysfunction.

Methods Male Wistar rats were randomly divided into three groups: normal control, diabetic rats and valsartan group. Diabetes was induced by high-calorie diet for 4 weeks and a single intraperitoneal injection of streptozotocin (STZ) thereafter. The expression of TGF-β1/Smads signalling was analysed by real-time reverse transcriptase-PCR and immunohistochemistry in aorta of three groups.

Results Compared with control group, the expression of both TGF-βI (27.4013±10.49256 vs 15.1259±6.64343, p<0.01), TGF-β receptor types II (20.5209±7.82756 vs 31.4029±10.44721, p<0.01) and activated of the smad2/3 (31.4029±10.44721 vs 12.8769±6.98547, p<0.001) signalling pathway were up-regulated in the vasculature in diabetic rats. Compared with diabetic group, active TGF-βb (18.5622±10.29359 vs 27.4013±10.49256, p<0.05) and Smad2/3 (20.5209±7.82756 vs 31.4029±10.44721, p<0.01) protein levels were reduced in the aorta after the treatment of valsartan.

Conclusions Our results suggest that AT1 receptor antagonist has reversed vascular fibrosis through the blockade of the AT1-mediated TGF-β/Smad signal pathway in the diabetic rats with vascular dysfunction. These observations may del support additional, benefical effects of angiotensin receptor antagonists observed during del diabetic vascular complications.

Note: n=? to be sent back.

e0053 ELECTROPORATION-MEDIATED ANGIOTENSIN II TYPE 2 RECEPTOR GENE TRANSFECTED INTO RAT CAROTID ARTERIES AND THE EFFECTS OF AT2R GENE TRANSFER ON NEointimal HYPERPLASIA IN RAT CAROTID ARTERIES AFTER BALLOON ANGIOPLASTY

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Aim To study the effects of Electroporation on the angiotensin II type 2 receptor (AT2R) transfected into rat carotid arteries and study the effects of AT2R gene transfer on neointimal hyperplasia in rat carotid arteries after balloon angioplasty.

Methods Electroporation-mediated AT2R gene transfected into rat carotid arteries after the establishment of rat carotid balloon injury restenosis model. The arteries were harvested at 5 days, 14 days and 21 days after gene transfer. The expression of AT2R in arteries and morphology analysis were evaluated by fluorescence microscope, immunohistochemistry, HE staining and in situ hybridisation.

Results Electroporation-mediated AT2R gene delivered into injured rat carotid arteries significantly up—regulated the levels of AT2R

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