e0048 CYP2E1 INCREASES OXIDATIVE STRESS AND INDUCES APOPTOSIS OF CARDIO MYOCYTES IN TRANSGENIC MICE

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Objective Cytochrome P450 2E1 (CYP2E1) is an effective generator of reactive oxygen species, such as the superoxide anion radical and hydrogen peroxide. The expression of CYP2E1 varies with the progression of myocardial ischaemia and cardiomyopathy. This paper examined the heart specific CYP2E1 transgenic mice to study the effect of CYP2E1 on DCM.

Methods The expression of CYP2E1 in both hypertrophic cardiomyopathy (HCM) and DCM mice were analysed using RT-PCR and western blot on cTnTR92Q and cTnTR141W transgenic mice. The cDNA of CYP2E1 was amplified by RT-PCR from the mice heart, and del inserted into the downstream of α-MHC promoter to construct the CYP2E1 expression vector. The transgenic mice were created by the method of microinjection. And they were del crossed with cTnTR141W transgenic mice. The cardiac structure and function were analysed with M-mode echocardiography. Survival data of the experimental mice were recorded. Pathologic changes were observed by light microscopy. The contents of hydrogen peroxide (H2O2), malonaldehyde malondialdehyde (MDA), reduced glutathione (GSH) as well as the total anti oxidation capable (T-AOC) were detected by spectrophotometry. Myocyte apoptosis was analysed by in situ terminal dUTP nick end-labelling (TUNEL) stain.

Results In the current paper, it was indicated that expression of CYP2E1 was lightly up-regulated in HCM hearts from cTnTR92Q transgenic mice and was strongly down-regulated in DCM hearts from cTnTR141W transgenic mice at 3 months old. The transgenic expression of CYP2E1 reduced mortality of CYP2E1 transgenic mice by 7.5% and increased the mortality of cTnTR141W transgenic mice from 10% to 45% (n=40, p<0.01). The expression of CYP2E1 also brought about increases in left ventricular volumes, diameters, and decreases in left ventricular walls, ejection fraction and fractional shortening, as well as increases in myocyte disarray and interstitial fibrosis for both of CYP2E1 and CYP2E1×cTnTR141W transgenic mice. The levels of H2O2 and MDA were increased and the levels of GSH and T-AOC were strongly reduced in both of CYP2E1 and CYP2E1×cTnTR141W transgenic mice. In addition, myocyte apoptosis del increased 7-fold in the CYP2E1 transgenic mice compared with WT mice (n=3, p<0.01) and increased 1.7-fold in the CYP2E1×cTnTR141W transgenic mice compared with cTnTR141W transgenic mice (n=3, p<0.01).

Conclusions Expression of CYP2E1 was regulated in the progression of transgenic cardiomyopathy mice. CYP2E1-mediated oxidative stress and myocyte apoptosis may play an important role in aggravating the DCM phenotype.

e0049 E2F1 STABILISES P53 TO SUPPRESS VEGF EXPRESSION AND NEOVASCULARIZATION IN THE ISCHAEMIC MYOCARDIUM

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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 ischaemic limb (Qin *et al*, PNAS 2006). However, the physiological significance of this regulation in ischaemic 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 ischaemic 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=3). 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 6h, 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: ~1h) (p<0.01 at 1, 2, and 4 h, n=4). Interestingly, addition of Lactacystin 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−/− fibroblasts transiently transfected with HA-tagged E2F1 or E2F1 truncation mutants, p53 co-precipitated with the ectopically expressed WT E2F1 and the E2F1 mutants with deletions in the transactivaton domain and/or DP-dimerisation domain, but not with the E2F1 mutant with a deletion in the N-terminus (amino acids 1−109), suggesting that the N-terminal region is essential for E2F1 to interact with p53.

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

*Note: Results and Methods were mixed.

e0050 EFFECT OF BOSENTAN ON CAROTID ARTERY RESTENOSIS IN RAT AND CORRELATION OF SERUM VEGF

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Objective To investigate the effect of Bosentan on carotid artery restenosis in rat and correlation of serum vascular endothelia growth factor (VEGF).