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The Improved Cell-Autonomy Role of Bay60 2770 in Doxorubicin-Cardiotoxicity Mediated by Up-Regulated Mitochondrial Ferritin and Balancing p-P53ser15: An omen of a New Hypothesis of Innovative Antitumor Approach to Cancer Therapy with Doxorubicin

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OBJECTIVES The cardiotoxicity induced by the highlyeffective anticancer agent doxorubicin (DOX) involves increased oxidative stress, mitochondrial iron overload, DNA damage, autophagy, necrosis and apoptosis, underlining which also associated with its secondary tumorigenicity. For the development of seriously cardiotoxicity, doxorubicin is limited clinically in patients. Previous studies have attributed the cause of DOX mediated cardiotoxicity to mitochondrial iron accumulation and the ensuing production of reactive oxygen species (ROS) which seems to be independent to its DNA damage effect on antitumor. Interestingly, chemosensitization effect on cancer of soluble guanylate cyclase to cyclic guanosine monophosphate (sGC-cGMP)pathway induced tumor cells death, yet dramaticly, during heart failure sGC-cGMP signals protected cardiomyocytes survival. The present study investigates the effect of Bay60 2770, a more effective activator of oxidized soluble guanylate cyclase (sGC), and its role in alleviating DOX mediated cardiotoxicity.

METHODS SD rats administrated DOX (3.33mg/kg, 3 dose per week within 2 weeks, subsequently 2 weeks absence of treatment) with (w/o) Bay60 2770 pretreatments (5mg/kg, 3 dose per week, 1 hours prior DOX), then displayed heart dysfunction.H9C2 cardiomyoblast cells were treated with Bay60 2770 24 hours prior DOX. We used routine method to detecte echocardiography, cell ability, mitochondrial iron transfer and regulative proteins level, apoptosis, autophay, ROS and mitochondrial membrane potential.

RESULTS Heart dysfunction was observed by impaired left ventricular hemodynamic performance parameters in DOX rats, but lowering apoptosis and increasing cardiac function in Bay60 2770 pretreated group marked with significantly high MtFt and ABCB8 expression, meanwhile with a trend of mitigatory p-P53 ser15 protein level. Further, it also revealed significant reduction in P53 activation and 3-Nitrotyrosine (3-NT) formation in Bay60 2770 pretreated H9C2 cardiomyoblast cell lines resulting in increased cell viability and the ensuing decreased apoptosis associated with recovery of mitochondrial membrane potential and decreased mitochondrial ROS. To better understand the cardioprotective role of Bay60 2770 against DOX, by using siRNA construction, we found Bay60 2770 amazingly inversed the decreased level of autophagysomes in our MtFt knock down (MtFt-KD) DOX cells.

CONCLUSIONS Bay60 2770 could improve cell-autonomous mechanism to attenuate DOX induced cardiotoxcity and have potential viability to decrease DOX antitumor resistance and its secondary tumorigenicity. Together our findings reveal novel insights into the Bay60 2770 against the development of DOX mediated cardiotoxicity.

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ET-1 siRNA inhibits myocardial fibrosis induced by aldosterone in the cardiac fibroblasts in vitro

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OBJECTIVES The morbidity and mortality of cardiovascular diseases are the highest in the world, with approximately 17.3 million cases of death each year. Myocardial fibrosis is an important pathology characteristic for the chronic cardiovascular diseases, which is the cause of heart failure, malignant arrhythmia and sudden cardiac death. At present the precise mechanism of myocardial fibrosis is not clear. It is well known that aldosterone(Ald) antagonists either spironolactone or eplerenone have long been used to reduce myocardial fibrosis. Endothlin-1(ET-1)could promote myocardial fibrosis. We hypothesized that aldosterone promote myocardial fibrosis might be through Endothlin-1 pathway and siRNA might inhibit Endothlin-1 to reduce myocardial fibrosis.

METHODS To test our hypothesis, the following experiments were performed,

1. Endothelin-1 small interfering RNA transfection of cardiac fibroblasts, the transfection efficiency and time curve were evaluated by using green fluorescence intensity and fluorescence expression rate under the fluorescent inverted microscope.

2. The expression levels of endothelin-1 were determined with ELISA, MTT, and Hydroxyproline assay in transfected cells as well as in cell culture supernatant,

RESULTS 1. No statistical difference was found in the numbers of cardiac fibroblasts between control cells and cells with siRNA transfection by using LipofectamineTM RNAiMAX at any time points, no green fluorescent cells in the green fluorescent field, indicating that siRNA transfection was low toxicity to cells and specified.

2.Transfection efficiency and integrated optical density of green fluorescent protein were increased in a dose-dependent manner at 6h and 12h in cardiac fibroblasts. Transfection efficiency was significantly higher at 12 h, 24 h, 48 h and 72 h time points than at 6h point ($P<0.05$, respectively).

3.ELISA results showed that ET-1 content significantly increased in Ald+control-siRNA group compared with control group ($P<0.01$), whereas significantly decreased in Ald+ endothelin -1 siRNA 1/siRNA 2/siRNA 3 groups ($P<0.01$, respectively), and obviously lower than control group ($P<0.01$, respectively). Compared with Ald+control siRNA group, ET-1 content was significantly higher in supernatants of three Ald+ ET-1 siRNA groups than in respective cells.

4.MTT results showed that the average absorbance value in Ald+control siRNA group visibly increased compared with control group ($P<0.01$), decreased in Ald+ ET-1 siRNA 1/siRNA 2/siRNA 3 groups significantly ($P<0.01$, respectively).

5.Hydroxyproline content assay showed that collagen content significantly increased in Ald+control siRNA group than in control group ($P<0.01$), decreased in three Ald+ ET-1 siRNA groups ($P<0.01$, respectively).

CONCLUSIONS The study showed that siRNA could be efficiently and specifically transfected into cultured cardiac fibroblasts with low toxicity. Endothlin-1 siRNA inhibited myocardial fibrosis induced by aldosterone, indicating that Endothlin-1 plays a vital role in development of myocardial fibrosis.

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Circular RNA Related to PPARγ Function as ceRNA of microRNA in Human Acute Myocardial Infarction

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OBJECTIVES Circular RNAs (circRNAs) has been implicated in the development of various diseases, but there is little knowledge of circRNAs in acute myocardial infarction (AMI). Previous study have demonstrated that PPARγ can protect heart from AMI. The aim of this study was to identify circRNA expression in human AMI and to explore the function of PPARγ-related circRNAs in AMI.

METHODS To identify circRNAs that are specifically expressed in AMI, we compared plasma expression of circRNAs in AMI patients with healthy volunteers (AMI group vs. Con group, Arraystar CircRNA Microarray). PPARγ expression and activity were also detected by western blot. Bioinformatics analysis was employed to predict the interaction of circRNAs and PPARγ mRNAs in AMI. Loss-of-function and rescue experiments were then performed in vitro.

RESULTS 7326 circRNAs were detected in the plasma samples, of which 160 were differentially expressed between AMI group and Con group (73 up-regulated and 87 down-regulated, >2 folds, $p<0.05$). Especially, circRNA_081881 was significantly down-regulated in AMI group (12.5 fold, $p<0.05$). What's more, PPARγ expression (156±12%, $p<0.05$) and activity were significantly lower in AMI group than in Con group. Bioinformatics analysis (mirWalk database) show that hsa-miR-548 is related to PPARG gene ($p<0.01$). Meanwhile, miRNA GO analysis show that 7 different binding sites of miR-548 can be found in circRNA_081881 sequence. Loss-of-function and rescue experiments show that circRNA_081881 expression increased with interleukin-1 (IL-1) and tumor necrosis factor (TNF-α) levels in macrophages ($n=6$, $p<0.05$). Silencing of circRNA_081881 using small interfering RNA suppressed PPARγ expression, decreased M2 polarization and increased foam cell formation ($n=6$, $p<0.05$). CircRNA_081881 could compete for miR-548 with PPARγ.