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## ERK5 KNOCK DOWN AGGRAVATES DETRIMENTAL EFFECTS OF HYPOTHERMAL STIMULATION ON CARDIOMYOCYTES VIA BIM UPREGULATION

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**Objectives** Our study was designed to investigate role of ERK5/Bim pathway in hypothermal stimulation-induced damage or apoptosis of cardiomyocytes.

**Methodology** Neonatal rat cardiomyocytes (CMs) were cultured from one to two days old Sprague–Dawley rats. Rat specific siRNA were used to knock down expression of ERK5 or Bim in CMs. Hypothermal stimulation was performed as CMs incubation in 35, 5% $CO_2$ cell incubator for 8 h per day. Whole procedure of intervention lasted four days. Control group was persistently incubated in normal 37, 5% $CO_2$ cell incubator for 4 days. Groups were set as control group (C); hypothermal stimulation group (S); hypothermal stimulation combined with non-coding siRNA transfection group (SN); hypothermal stimulation combined with Bim siRNA transfection group (SB); hypothermal stimulation combined with ERK5 siRNA transfection group (SE); hypothermal stimulation combined with ERK5 and Bim double siRNA transfection group (SBE). Bim or ERK5/p-ERK5 protein expression in each group was detected by western blot. Level of intracellular calcium was measured by Fluo-3AM probe loading method. ROS was detected using carboxy-H2DCFHDA with flow cytometry analysis. The mitochondrial membrane potential ($\Delta\Psi$m) was evaluated using JC-1 staining with fluorescent microscope and flowcytometry analysis. Detection of apoptosis was performed using the Annexin V-FITC/PI apoptosis detection kit.

**Results** In CMs which under hypothermal stimulation, ERK5 siRNA transfection suppresses the elevated expression of Bim protein (S: 1.04±0.09 vs SE: 1.54±0.14, $p<0.05$), and Bim siRNA transfection doesn't influence the ERK5 protein expression but attenuates the production of phosphoratred ERK5. ERK5 siRNA induced increased apoptosis rate in hypothermia stimulated CMs (S: 32.5±2.01% vs SE: 41.3±4.21%, $p<0.01$), while Bim siRNA effected oppositely and cancelled pro-apoptotic effect of ERK5 siRNA (SB: 15.9±1.02%; SBE: 27.01±2.82%). Intracellular $Ca^{2+}$ overload and ROS activity were more severe in SE group while lighter in SB group, when compared with S or SN group. Bim siRNA rescued the higher intracellular $Ca^{2+}$ overload and ROS activity that induced by ERK5 siRNA. Mitochondrial membrane potential was more significantly damaged in SE group. Bim siRNA attenuated inhibition to $\Delta\Psi$m that induced by hypothermal stimulation, and canceled the detrimental effect of ERK5 siRNA.

**Conclusion** ERK5 knock down releases inhibition to Bim expression, induces aggravated apoptosis in CMs under hypothermal stimulation, which related to higher intracellular $Ca^{2+}$ overload, ROS activity, and more severe mitochondrial membrane potential damage. Results revealed important regulative role of ERK5/Bim pathway in hypothermal stimulation-induced injure or apoptosis of cardiomyocytes.