Abstract: P489

Geldanamycin-induced IkBa cleavage potentiates autophagy in angiotensin II-mediated hypertrophy

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Topic(s):
Basic Science - Cardiac Diseases: Cardiac Hypertrophy

Citation:
Cardiovascular Research (2018) 114 (Supplement 1), S118

Objective

The heat shock protein 90 (Hsp90) has been found to be a regulator of NF-κB cascade. However, it is still unclear whether Hsp90 is associated with NF-κB cascade in the modulation of cardiac hypertrophy induced by angiotensin II (Ang II). Here we investigated a novel mechanism of Hsp90 in Ang II-induced cardiac hypertrophy.

Methods

Mini osmotic pumps (model 2002, Alzet) were implanted subcutis of 200-250g Male Sprague Dawley rats. The pumps release AngII (Sigma; 1mg/kg/day) or GA (A.G Scientific; 1mg/pump) for 2 weeks at the rate of 0.5µl/hr. The cDNA encoding human wild type IκBa (WT) and cleavage-deficient mutant IκBa (D31A) was constructed into pcDNA3.1/v5-His-TOPO vector, which were transfected into H9c2 cells by Lipofectamine2000TM (Invitrogen). H9c2 cells at approximately 50% confluence were transfected with caspase-3, -8, -9 siRNA duplex (100 nM) or scrambled sequence. The siRNA was transfected by Lipofectamine RNAimax (Invitrogen) reagent.

Results

Ang II-induced cellular hypertrophy was suppressed by a specific inhibitor of Hsp90, geldanamaycin (GA), treatment in cultured rat neonatal cardiac myocytes in a concentration-dependent manner. In rat animal model, GA administration inhibited Ang II-induced LV hypertrophy. The cardiac cells treated with GA showed that nuclear translocation of p65 NF-κB was decreased. Interestingly, GA induced caspase-8 activation and IκBa cleavage at amino-terminal specific region in cultured cardiac cells and angiotensin II infused rat model. When cardiac cells were treated with a caspase-8 inhibitor, z-IETD-fmk, GA-mediated IκBa cleavage was inhibited and the IκBa ubiquitination and NF-κB activity were also reversed. Cleavage at N-term of IκBa (WT) under GA treatment did not occur in IκBa (D31A). In addition, IκBa (D31A) overexpressed cells show resistance against GA-mediated autophagy and hypertrophy.

Conclusion

GA activates caspase-8 to cleave N-term of IκBa, leading to decrease of NF-κB activity and hypertrophy, consequently induces autophagy, thereby providing the molecular basis by which Hsp90 regulates apoptosis in hypertrophied heart.