Regulatory mechanism of 17β-estradiol and/or estrogen receptor β on hypoxia-induced autophagic and apoptotic pathways in H9c2 cardiomyoblast cells.

Yi-Ping Lai[1], Wei-Wen Kuo[2] and Chih-Yang Huang[1, 3]

[1]Graduate Institute of Basic Medical Science, China Medical University, Taichung
[2]Department of Biological Science and Technology, China Medical University, Taichung
[3]Graduate Institute of Chinese Medical Science, China Medical University, Taichung

2012 The 27th Joint Annual Conference of Biomedical Sciences
第27屆 生物醫學聯合學術年會

Abstract

Myocardial infarction (MI) is the common cause of cardiomyocyte apoptosis and hypoxia alone is sufficient to induce apoptosis of cardiomyocytes. In hearts, autophagy might play important roles in hypoxia-mediated cardioprotection or myocardial damage effects. To date, hypoxia-inducible factor-1α (HIF-1α) transcriptional factor and BH3-only bcl-2 family protein (BNIP3) are known to play fundamental roles in adaptive or death process in response to hypoxia. In addition, hypoxia can induce insulin-like growth factor binding protein 3 (IGFBP-3) to block the IGF1R/PI3K/Akt survival pathway. Therefore, we would like to investigate the molecular mechanism and the interaction of IGFBP-3, HIF-1α and BNIP3 in hypoxia-induced cell injury of H9c2 cardiomyoblast cells. Moreover, 17β-Estradiol (E2) has been reported recently to prevent cardiac apoptosis via estrogen receptors (ERs). Previous studies have ever revealed the novel cardioprotective role of ER β in myocardial ischemia. Therefore, our studies aim to reveal the regulatory mechanism of ER β on hypoxia-induced cell death. Heart-derived H9c2 cells were incubated in normoxic or hypoxic (<1% oxygen) conditions for 24 h after ER β overexpression. Results showed the hypoxia primarily caused HIF-1α expression highly increase, then activated downstream genes such as BNIP3 and IGFBP-3, and further triggered autophagic and apoptotic pathways. However, all phenomena were recovered by E2/ER β overexpression. E2/ER β overexpression also further promoted the cardiac survival pathway related proteins, p-IGF1R and p-Akt activation. Taken together, ER β exerts the protective effect through repressed hypoxia-inducible BNIP3 and IGFBP-3 levels to restrain the hypoxia-induced autophagy and apoptosis effects in H9c2 cardiomyoblast cells.

Results

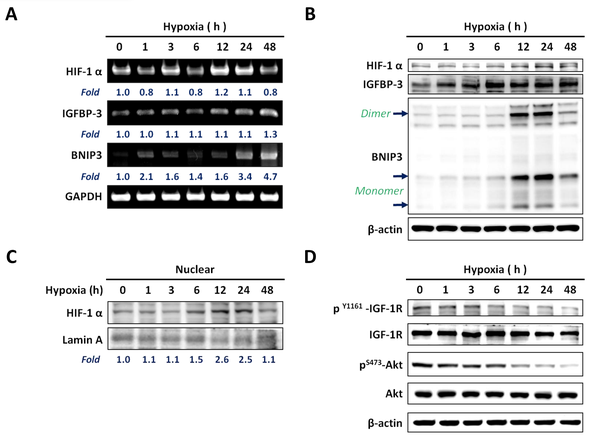

Fig. 1. Effects of hypoxia on (A) the mRNA expression of HIF-1 α, IGFBP-3 and BNIP3 was measured by RT-PCR, (B) the protein level of HIF-1 α, IGFBP-3 and BNIP3, (D) the phosphorylation of IGF-1R and Akt, and (C) HIF-1 α nuclear translocation were measured by Western blot in H9c2 cardiomyoblast cells.

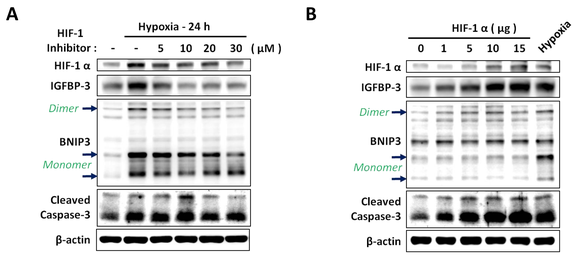

Fig. 2. Role of HIF-1 α in the IGFBP-3, BNIP3 and cleaved caspase-3 expression under normoxia and hypoxia. (A) H9c2 cells were treated with 5-30 μM HIF-1 inhibitor and were exposed to hypoxia for 24 h. (B) Rat cardiomyoblasts were transient transfected with 1-15 μg HIF-1 α plasmid under normoxia for 24 h.

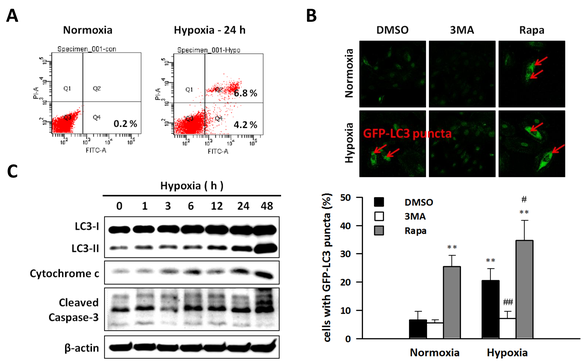

Fig. 3. Effects of hypoxia on (A) apoptosis was detected by annexin-V/PI, (B) the basal autophagosome formation was detected by GFP-LC3 puncta, and (C) the protein level of LC3, cytochrome c and cleaved caspase-3 was detected by Western blot. Data are presented as the mean ± SD (n=3). $^{**}P$<0.01 vs. normoxia-treated cells. $^{\#}P$<0.05 and $^{\#\#}P$<0.01 vs. hypoxia-treated cells.

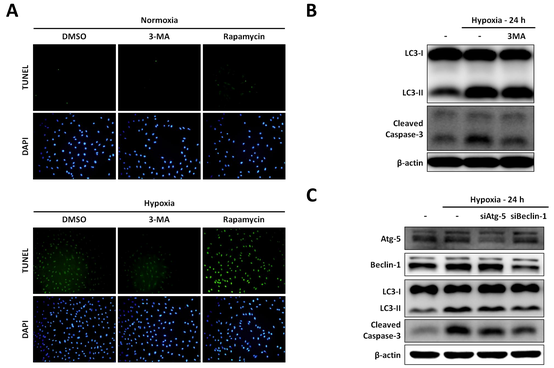

Fig. 4. Effects of autophagy inhibitor (3-MA), siRNA such as Atg-5 and Beclin-1 and autophagy inducer (rapamycin) under hypoxia for 24 h on apoptosis were measured by TUNEL assay (A) and were measured by Western blot (B and C).

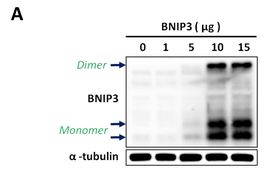

Fig. 5. Role of BNIP3 in the LC3 and cleaved caspase-3 protein level under normoxia were measured by Western blot. (A and B) H9c2 cardiomyoblast cells were transient transfected with 1-15 μg BNIP3 plasmid for 24 h.

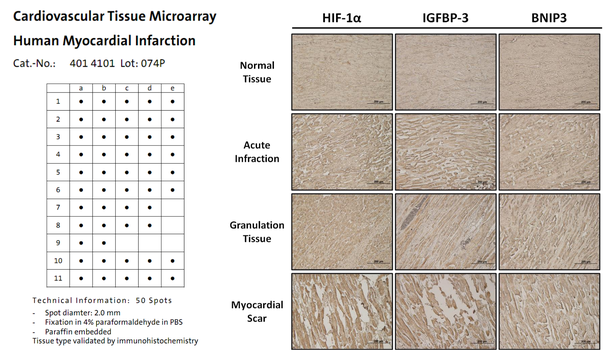

Fig. 6. Immunohistochemical analysis for HIF-1 α and transcriptional targets in sections from the human cardiovascular tissue with myocardial infarction disease. Immunohistochemical analysis, with the indicated antibodies, of serial sections of representative lesions: a normal tissue, an acute infraction, a granulation tissue and a myocardial scar. Final magnifications: × 200.

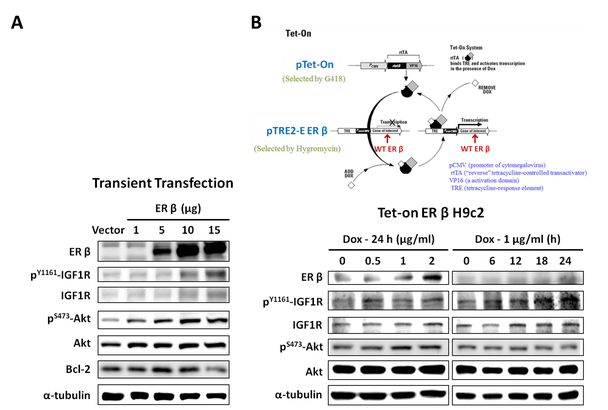

Fig. 7. Effects of ER β on the phosphorylation of IGF1R and Akt survival pathway were measured by Western blot in Rat cardiomyoblast cells. (A) H9c2 cells were transient transfected with 1-15 μg ER β plasmid for 24 h. (B) Tet-on ER β H9c2 cells were treated with 0.5-2 μg/ml doxcyclin (Dox) for 24 h or stimulated with 1 μg/ml Dox in time course to overexpress ER β.

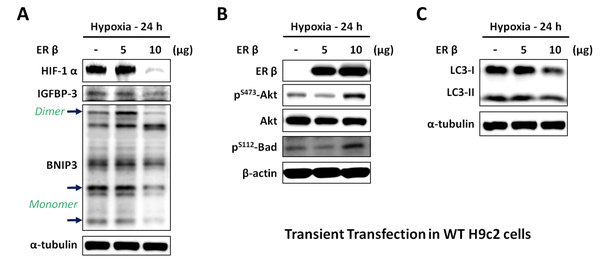

Fig. 8. Effects of ER β overexpression by transient transfection under hypoxia on (A) the protein level of HIF-1 α, IGFBP-3 and BNIP3, (B) the phosphorylation of Akt, Bad, and (C) the autophagy expression of LC3-II/LC3-I were measured by Western blot in H9c2 cardiomyoblast cells.

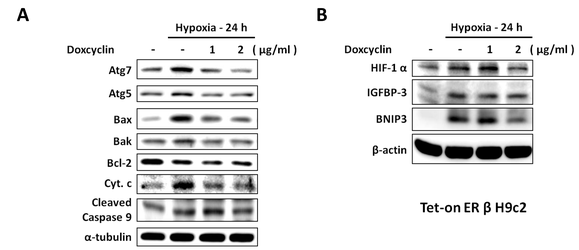

Fig. 9. Effects of ER β overexpression under hypoxia on the protein level of (A) Atg7, Atg5, Bax, Bak, Bcl-2, cytochrome c, cleaved caspase 9, and (B) HIF-1 α, IGFBP-3 and BNIP3 were measured by Western blot in Tet-on ER β H9c2 cells.

Summary

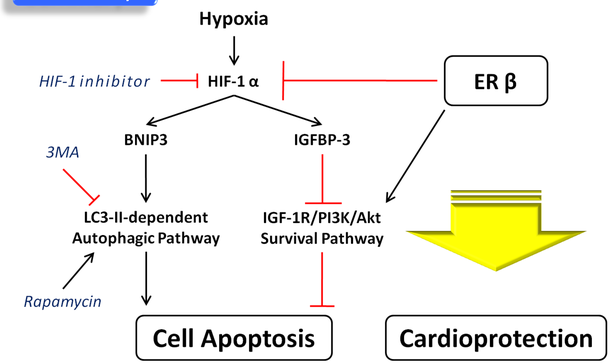

Fig. 9. Hypoxia could stabilize HIF-1 α protein accumulation to trigger downstream IGFBP-3 and BNIP3. And then IGFBP-3 could block the IGF1R survival pathway to further enhance cell apoptosis. And BNIP3 could activate caspase 3 to induce apoptosis through LC3-dependent autophagy induction in H9c2 cardiomyoblast cells. However, under hypoxia ER β could activate the IGF-1R survival pathway and totally abolish hypoxia related proteins such as HIF-1 α, IGFBP3 and BNIP3 to inhibit cell apoptosis indicated that ER β has a protection role in H9c2 cardiomyoblast cells.