Unfolded protein response exerts cytoprotection and promotes the proliferation of nucleus pulposus cells in TNF-α stimulation by activating NF-κB

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Abstract

Background: Intervertebral disc (IVD) degeneration is a degenerative disease closely related to inflammation of nucleus pulposus (NP) cells. Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine which induces NP cells (NPCs) apoptosis and accelerate IVD degeneration (IDD). The endoplasmic reticulum (ER) serves several important cell functions, which are essential for normal cell function and survival. Nuclear factor-kappaB (NF-κB) is important for genes involved in cell survival, adhesion, and proliferation. However, the roles of ER stress and NF-κB in IDD remain to be elucidated. This study aims to clarify the roles of NF-κB and ER stress related unfolded protein response (UPR) in TNF-α-induced biological changes in rat NP cells and IVD degeneration.

Methods: We cultured rat NPCs with different concentrations of TNF-α, with or without UPR and NF-κB pathway small interfering RNA (siRNA). The protein levels of UPR markers (XBP1s and *p*-eIF2α) and *p*-p65 were measured by immunofluorescence staining and western blot analysis and were used to monitor UPR and NF-κB, respectively. Cell proliferation was evaluated by CCK-8 assay, cell-cycle analysis and cyclin proteins expression. Apoptosis was detected by flowcytometry, TUNEL staining and Western blot analyses. All the data were expressed as mean ± SD and from multiple independent experiments. The results were compared among different groups by using unpaired student's-test. *P*-values<0.05 were considered significant.

Results: TNF-α induced the apoptosis of some NPCs in the early stage and then accelerated the proliferation of surviving cells. In addition, TNF-α stimulus up-regulated XBP1s, *p*-eIF2α and *p*-p65 at the protein level, which indicated that TNF-α activated UPR and NF-κB signals in rat NP cells. However, these effects could be reversed by UPR and NF-κB siRNA, and UPR interference decreased the expression of *p*-p65 notably. In parallel, both UPR and NF-κB interference reduced cell proliferation and enhanced apoptosis.

Conclusions: Our study demonstrated that UPR reinforces the survival and proliferation of NPCs in TNF-α stimulus by activating NF-κB signaling, which could be an important therapeutic target in the future.

Images:

Fig.1

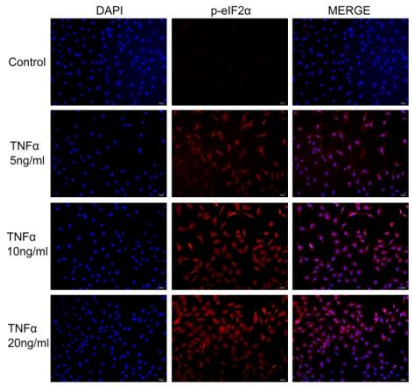


Fig.1. After treatment with different concentrations of TNF-α for 24h, the expression of UPR marker p-eIF2α was significantly upregulated by Immunofluorescence staining.

Fig.2

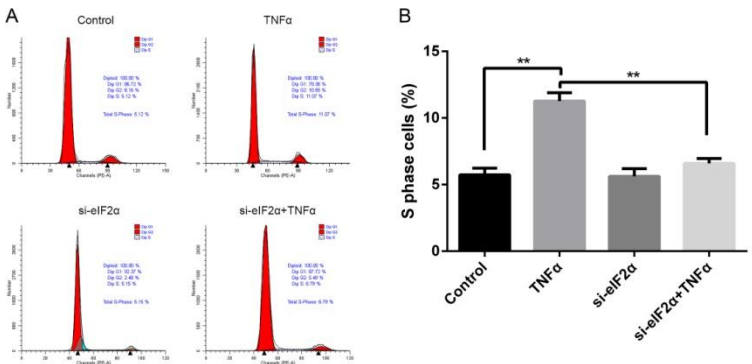
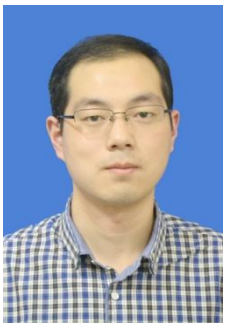


Fig.2. The effect of UPR on NPCs proliferation. (A) The percentage of S phase population cells was measured by flowcytometry after eIF2α silencing under TNF-α stimulus. (B) eIF2α interference reduced cell proliferation in TNF-α significantly. (*$p < 0.05$)

Keywords: *Nucleus pulposus cells, NF-κB, TNF-α, unfolded protein response*

Professional Biography

Lu Chen, male, 29 years old, has got his bachelor's and master's degree in the school of medicine, Southeast University and worked in orthopaedic department as a surgeon for 3 years. Now he is further pursuing his PhD study in Southeast University, and doing the basic research work in the field of degenerative disc disease. He has published more than 5 papers in reputed journals. He has participated in the 12th International Congress of Chinese Orthopedic Association (COA), China- E-Poster Presentation; International Symposium on Life Science & Biological Engineering (ISLSBE), Hong Kong- Oral Presentation.



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