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THE PROTECTIVE EFFECTS OF X-BOX BINDING PROTEIN 1 ON TUMOUR NECROSIS FACTOR-ALPHA INDUCED PRO-INFLAMMATORY RESPONSE

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Objectives Tumour necrosis factor alpha (TNF-α) is a potent pro-inflammatory factor playing a critical role in the initiation and progression of atherosclerosis. Exposure of vascular endothelial cells to TNF-α is known to induce adhesion molecule expression and inflammatory cytokine secretion, leading to endothelial dysfunction and apoptosis. X-box binding protein 1 (XBP1) is an active transcription factor involved in endoplasmic reticulum (ER) stress

response including ER- associated protein degradation (ERAD). This study is to investigate the effect of spliced XBP1 on the expression of pro-inflammatory cytokines induced by tumour necrosis factor alpha (TNF-alpha) in human umbilical vein endothelial cells (HUVECs).

Methods Adenovirus encoding mouse spliced XBP1 (Ad-XBP1s) were infected in cultured HUVECs. The overexpression of spliced XBP1 were examined by western blot analysis. Adenoviruses encoding green fluorescent protein (Ad-GFP) were used as control. Cell viability was measured by MTT assay. HUVECs were infected with Ad-XBP1 or Ad-GFP for 48 h, and then stimulated in the presence or absence of TNF-α (10 ng/ml) for 24 h. XBP1 knockdown was performed by Small RNA interference (siRNA). The knockdown efficiency was monitored by determining the protein level of XBP1 using western blot analysis. HUVECs were transfected with scramble siRNA or XBP1 siRNA using Lipofectamine 2000 for 6 h. The pro-inflammatory cytokines macrophage chemoattractant protein-1 (MCP-1) and intercellular adhesion molecule (ICAM-1) induced by TNF-α were determined at the mRNA and the protein levels. Real Time RT-PCR was used for evaluating mRNA expression. Western blot analysis was used to examine ICAM-1 protein expression and ELISA was used to measure the protein secretion of MCP-1 in cell culture supernatants.

Results The overexpression of XBP1 by adenovirus encoding XBP1 were confirmed by western blot analysis. It had no effect on cell viability in cultured HUVECs. ICAM-1 and MCP-1 mRNA expression were upregulated by 40- and 16-fold respectively after TNF-α treatment. The up-regulation of ICAM-1 and MCP-1 were significantly attenuated in Ad-XBP1-treated cells. In parallel, results from western blot analysis and ELISA further confirmed the reduction of ICAM-1 and MCP-1 protein levels induced by TNF-α in Ad-XBP1 treated cells. Moreover, downregulation of XBP1 by transfection with XBP1 siRNA increased the ICAM-1 and MCP-1 at both mRNA and protein level in HUVECs.

Conclusions XBP1 suppresses TNF-α induced pro-inflammatory response in cultured HUVECs. Ongoing studies are focusing on the signalling pathways underlying the inhibitory effect of XBP1 on pro-inflammatory cytokines.