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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- α) 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 knock-down 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.