Adenoviral Expression of Mitogen Activated Protein Kinase Phosphatase-2 (MKP-2) Abolishes COX-2 and Reduces Apoptosis in Human Endothelial Cells

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Endothelial cell dysfunction is a key event in the development of cardiovascular diseases. This is partly mediated through regulation of the mitogen activated protein kinases (MAPKs). We developed an adenoviral MKP-2 that is able to regulate JNK (Cadalbert *et al.*, 2005) and assessed its effect upon COX-2, ICAM-1, VCAM-1 expression and apoptosis in human umbilical vein endothelial cells (HUVECs).

HUVECs were infected with Adv.MKP-2 and/or Adv.dominant negative IKKβ$^{-/+}$ for 40h prior to stimulation with TNF-α. Proteins expression were determined by Western blotting. Apoptosis was measured by FACs analysis. Following treatment of HUVECs with TNF-α (20ng ml^{-1}), a transient phosphorylation of JNK was observed which was significantly attenuated by overexpression of MKP-2 (% inhibition at 300 p.f.u. = 99.5 ± 0.5, p< 0.001, n=4). Stimulation with TNF-α resulted also in increased expression of COX-2 at 24 h (fold basal ± s.e.m. : 30.0 ± 16.0, n=3), ICAM-1 and VCAM-1 (fold basal ± s.e.m.: ICAM-1 = 102.2 ± 29.9, VCAM-1 = 69.6 ±14.2, n=3). Adv.MKP-2 substantially reduced COX-2 expression (% inhibition= 99.6 ± 0.2, P<0.0001) however, ICAM-1 and VCAM-1 expression were not affected. Indeed, when ICAM-1 and VCAM-1 expression was partly reduced by infection with DN-IKKβ$^{-/+}$, Adv.MKP-2 attenuated this inhibition. Adv.MKP-2 also reversed DN-IKKβ$^{-/+}$ inhibition of TNF-α induced IKB-α loss suggesting cross-talk between MKP-2 and NFkB. We also examined the potential for Adv.MKP-2 to reverse endothelial cell apoptosis. Cells stimulated alone with TNF-α showed no significant increase in apoptosis. However, infecting cells with 300 pfu/ml of Adv. DN-IKKβ$^{-/+}$ prior to stimulation with TNF-α resulted in a significant increase in apoptosis, (% apoptosis: mean ± s.e.m, n=3), Control = 1.7 ± 1.2, β-gal = 3.1 ± 1.2, DN-IKKβ$^{-/+}$ = 6.6 ± 0.2, TNF-α = 5.2 ± 1.0, MKP-2 = 3.4 ± 0.1, TNF-α/DNIKKβ$^{-/+}$ = 28.2 ± 7.1; P< 0.01). Under these conditions co-expression of MKP-2 significantly reduce the death in response to TNF-α/DNI-KKβ$^{-/+}$ (14.2 ± 4.8; P<0.01).

Taken together these results show that Adv.MKP-2 both positively and negatively regulate inflammatory protein expression due to possible cross-talk between NFκB and JNK.This cross-talk may also help protect against endothelial cell death.

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