The Nicotinamide Adenine Dinucleotide Phosphate-Oxidase P22-phox Subunit Is Required For Angiogenesis In Human Endothelial Cells

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Angiogenesis is critical to many physiological and pathophysiological processes, including hypertension and cardiovascular disease, cancer and inflammatory disorders. Reactive oxygen species, mainly generated by NAD(P)H oxidase in human endothelial cells, are involved in angiogenesis [1].

The aim of this study was to determine whether the NADPH oxidase subunit p22phox is required for angiogenesis in human endothelial cells.

To determine the effect of p22phox on angiogenesis, we synthesised double stranded small interference RNA to knock down specifically p22phox expression. Human endothelial cells (HUV-EC-C, ATCC-CRL-1730) were cultured in Ham's F-12K medium (ATCC) containing 10% foetal calf serum (FCS), 100 μg/ml heparin (Sigma) and 75 μg/ml human ECGS (Sigma). Statistical significance was assessed by ANOVA, with post-hoc paired testing on parametric data. Data are means±SEM.

siRNA against p22phox (sip22phox) markedly reduced both p22phox mRNA and protein levels, determined using real time PCR and western blotting, and reactive oxygen generation, measured using dihydroethidium staining ($P<0.001$). sip22phox [10nMol] reduced basal Akt phosphorylation by 58±4 % ($P<0.001$). sip22phox [10nMol] increased caspase-3 activation by 3-fold ($P<0.001$). Furthermore sip22phox suppressed vascular tube formation in Matrigel by endothelial cells by 94±1% ($p< 0.001$). Reduced vascular tube formation was associated with 98±1% decrease in expression of vascular endothelial cadherin ($P<0.001$), 70±1% reduction in the number of migrating cells, and 75±5% decrease in rate of cell proliferation ($P<0.01$). sip22phox also significantly suppressed stimulation by angiotensin II [1uM] of endothelial cell migration ($P<0.01$) and proliferation ($P<0.05$): angiotensin II stimulated endothelial cell proliferation by 18±3% (Ang II vs. control, $p < 0.01$); the stimulatory effect of angiotensin II [1uM] on endothelial cell proliferation was blocked by 5nmol/L sip22phox (Ang II + 5 nmol/L siRNA vs. control, $p = 0.26$).

Taken together, these data demonstrate that the NADPH oxidase subunit p22phox is required for angiogenesis in human endothelial cells.

1. Ushio-Fukai M. Redox signaling in angiogenesis: Role of NADPH oxidase. Cardiovasc Res. 2006;71:226-235.