Abstract: P335

Beta-catenin mediates the anti-apoptotic effects of NO in endothelial cells

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Increased endothelial cell (EC) apoptosis is associated with the development of atherosclerotic plaques that develop predominantly at sites exposed to disturbed flow. Strategies to promote endothelial cell survival may therefore represent a novel therapeutic approach in cardiovascular disease. Nitric oxide (NO) and β-catenin have both been shown to promote cell survival. Recently we showed that pharmacological activation of the endothelial nitric oxide synthase (eNOS), acting through soluble guanylate cyclase and cGMP, can promote nuclear translocation and transcriptional activity of β-catenin in static endothelial cells. Using an orbital shaker system to generate shear stress, we investigated the physiological role of β-catenin as a mediator of NO-induced cell survival in endothelial cells.

In this study we show that β-catenin depleted human umbilical vein endothelial cells (transfected with β-catenin siRNA) exhibit a reduction in eNOS phosphorylation (Ser1177) and intracellular cGMP levels following histamine stimulation compared to non-transfected controls. Incubation of cells with LiCl (that increases β-catenin levels) resulted in increased eNOS phosphorylation (Ser1177). These data suggest that β-catenin regulates eNOS activation and NO production in static ECs. β-catenin depletion also abrogated the protective effects of the NO donor, SNAP (S-Nitroso-N-acetylpenicillamine), during pharmacologically induced apoptosis suggesting that β-catenin mediates the pro-survival effects of NO in static endothelial cells. To study whether β-catenin is a mediator of eNOS induced survival under physiological flow, we confirmed expression of eNOS and β-catenin by quantitative-PCR and immunostaining, as well as the interaction between eNOS and β-catenin by proximity ligation assay in ECs exposed to undisturbed flow (UF) or disturbed flow (DF) for 72h on an orbital shaker. ECs exposed to orbital flow exhibited different degrees of apoptosis between the UF zone in the periphery of the well and DF zone in the centre of the well (1.38% ± 0.13 (mean ± SEM) cleaved caspase positive cells in DF zone compared to 0.21% ± 0.03 in UF zone; n=3 p<0.01) which was increased in the DF area when cells were treated with an inhibitor of β-catenin transcriptional activity (2.04% ± 0.15 cleaved caspase positive cells in iCRT5 treated cells compared to non-treated controls; n=3 p<0.05). The expression of the pro-survival/anti-apoptotic genes Bel-2, Survivin and eNOS was downregulated in ECs exposed to DF compared to UF conditions; and their expression was reduced under both DF and UF when the cells were treated with iCRT5.

Together our data indicate that β-catenin regulates eNOS activity and that β-catenin-dependent transcription is essential to maintain cell survival under disturbed flow in endothelial cells, through regulation of Bel-2 and Survivin levels and suggest that additional non-transcriptional mechanisms contribute to cell survival in endothelial cells under UF.