Intracellular Sphingosine Kinase Activity as a Regulator of Endothelial Cell Inflammatory and Angiogenic Potential.

Vidya Limaye
Department of Medicine
University of Adelaide

Date Submitted: 23-4-2004

Table of Contents

3.5 Over-expression of SK modulates cell junctional proteins 79

ABSTRACT

The normal endothelium is in a non-activated state evidenced by its inability to support leukocyte adhesion and its lack of angiogenesis. I present evidence in this thesis that the intracellular levels and activity of Sphingosine Kinase (SK) regulate the inflammatory potential of the endothelium and are also an important determinant of the ability of the endothelium to undergo angiogenesis. Moderate (three to fivefold) elevations in SK activity in human umbilical vein endothelial cells resulted in endothelial activation, as indicated by the heightened expression of adhesion molecules, and further sensitized the cells to subliminal doses of inflammatory cytokines. This corresponded with enhanced neutrophil binding in the un-stimulated and stimulated states. Over-expression of a dominant-negative SK (G82D, containing the substitution glycine to aspartate and which blocks agonist-induced activation of SK) inhibited the adhesion molecule response to inflammatory cytokine and inhibited leukocyte adhesion. Over-expression of SK increased cell survival under the stressful conditions of serum deprivation and loss of attachment with extracellular matrix, which was associated with suppression of apoptotic mechanisms. Raised SK activity also stimulated cell migration and cellular remodeling, additional measures of angiogenesis. Over-expression of SK enabled activation of the phosphatidyl inositol-3kinase (PI-3K/Akt) pathway in response to serum deprivation, and this pathway was obligatory in mediating SK-induced cell survival. Activation of the PI-3K/Akt pathway in cells with raised SK activity was mediated by the cell junctional molecule platelet endothelial cell adhesion molecule-1 (PECAM-1), which was upregulated and dephosphorylated and critical in SK-induced cell survival. Thus raised intracellular SK activity enables a PECAM-1-dependent activation of the PI-3K/Akt pathway to augment cell survival, thus exposing a hitherto unexplored pathway of endothelial cell survival which may be manipulated therapeutically. The findings suggest a possible role for SK levels in the regulation of angiogenic phenomena as well as in the capacity

of endothelial cells to survive in suspension (circulating endothelial cells). Thus SK could be considered as a novel target for therapeutic manipulation in diseases of aberrant inflammation and angiogenesis.