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Cytokine GRO-α is pivotal in thrombin-induced angiogenesis

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The pro-malignant role of thrombin in tumor adhesion, growth, metastasis and angiogenesis is well recognized.[1-6] However, the mechanism of thrombin-induced angiogenesis is not clear. Vascular regulatory proteins and growth factors, particularly metalloproteinases (MMP-1, MMP-2, MMP-9), vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1), and angiopoietin-2 (Ang-2), are required for the regulation of blood vessel formation. Thrombin-induced angiogenesis in a chick chorioallantoic membrane (CAM) assay is associated with upregulation of the major vascular endothelial growth factor (VEGF) as well as Angiopoietin-2 (Ang-2).[6] Thrombin also upregulates VEGF and the major VEGF receptor KDR in endothelial cells and induces the secretion of VEGF and Ang-1 from platelets. Thrombin upregulates Ang-2, MMP-1 and MMP-2 in endothelial cells. However, the cellular mechanisms responsible for thrombin-induced upregulation of these genes have not been established. The chemokine GRO-α has been implicated in blood vessel formation. GRO-α is a CXC chemokine with oncogenic activity. GRO-α induced metastasis is associated with increased angiogenesis. GRO-α binds to a seven transmembrane receptor, CXCR2 on endothelial cells and neutrophils and promotes their chemotaxis.

It is required for maintenance of wound repair and is necessary for neutrophil recruitment. GRO-α enhances growth, chemotaxis and metastasis of several tumor cell lines. Using a gene chip array to investigate the pro-malignant phenotype of thrombin-stimulated cells we observed that thrombin markedly upregulates GRO-α in 5 different tumor cell lines as well as human umbilical vein endothelial cells (HUVEC) by mRNA and protein analysis. Thrombin enhanced the secretion of GRO-α from tumor cells 25-64 fold. GRO-α enhanced angiogenesis in the chick chorioallantoic membrane (CAM) assay 2.2 fold providing direct evidence for GRO-α as an angiogenic growth factor. Anti-GRO-α Ab completely inhibited the 2.7 fold thrombin-induced upregulation of angiogenesis, as well as the 1.5 fold thrombin-induced upregulation of both endothelial cell cord formation in matrigel, and endothelial cell growth *in vitro*. Thrombin as well as its PAR-1 receptor activation peptide (TRAP) as well as GRO-α all markedly increased vascular regulatory proteins and growth factors in endothelial cells: MMP-1, MMP-2, VEGF, Ang-2, CD31 and receptors KDR and CXCR2.

Similar results were obtained in tumor cells for VEGF and Ang-2. All of the thrombin/TRAP induced gene upregulations were completely inhibited by anti-GRO-α Ab and unaffected by irrelevant Ab. Similar inhibition was noted in GRO-α Knockdown (KD) 4T1 breast tumor and B16F10 melanoma cells. *In vivo* tumor growth studies in wild-type mice with isRNA GRO-α KD 4T1 cells revealed 2-4 fold impaired tumor growth, metastasis and angiogenesis (compared to control vector-transfected cells) which was not affected by endogenous thrombin (treatment with hirudin).

In vivo growth of control vector-transfected cells was affected by endogenous (inhibited by hirudin, 2-4 fold). Thus thrombin-induced angiogenesis requires the upregulation of GRO-α. Thrombin upregulation of GRO-α in tumor cells and endothelial cells contributes to tumor angiogenesis.

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