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Anti-angiogenic Activity of the Recombinant Kringle Domain of Human Urokinase

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Angiogenesis is a complex, multi-step process, which occurs during normal physiology, such as wound healing, pregnancy, and development, as well as under pathological conditions, such as diabetic retinopathy and tumorigenesis. During angiogenesis, endothelial cells need to divide, migrate, invade into the extracellular matrix, and form capillary structures from pre-existing blood vessels. This complex process implies the presence of multiple controls, which can be turned on and off within a short period. There is an increasing body of evidence showing that inhibition of angiogenesis could lead to the suppression of tumor growth and metastasis.

Angiogenesis is tightly regulated by both positive and negative signals, and the switch of the angiogenic phenotype depends upon the net balance between these signals. Several growth factors are known to induce angiogenesis *in vitro* and *in vivo*. The positive regulators, such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) are not only potent mitogens, but also motogens for endothelial cells and can promote neovascularization to sustain the expansion of both primary and metastatic tumors.

Among the family of angiogenesis inhibitors, angiostatin has been demonstrated to be the potent angiogenesis inhibitor. This molecule isolated from serum and urine of tumor-bearing animals, consists of first four kringles of plasminogen. The general feature of kringle structure is composed of 70-80 amino acids inter-connected by a triple disulfide-linked loop. In vitro, angiostatin specifically inhibits endothelial cell proliferation but not proliferation of other cell types including tumor cells. In vivo, it suppresses neovasculization, and suppress tumor growth in animals without toxicity. Each kringle of angiostatin have been demonstrated to display differential effects on endothelial cell proliferation and migration.

Urokinase-type plasminogen activator (uPA) and its receptor are important components of cell surface proteolysis used by tumor cells and capillary endothelial cells for basement membrane invasion and implicated in the progression, and

metastasis of numerous tumors. A multi-domain protein, uPA, also shares a common structural charateristic with plasminogen consisting of growth factor-like domain, kringle domain and protease subunit. Since angiostatin is a cryptic fragment derived from plasminogen which itself has no anti-angiogenic activity, we are interested in whether urokinase kringle domain retains anti-angiogenic activity. In this work we examined the effects of the recombinant kringle domain of urokinase on endothelial cells *in vitro*, and on chorioallantoic membrane angiogenesis *in vivo*, and the internalization of the recombinant kringle domain in endothelial and non-endothelial cells.

The recombinant kringle domain obtained from bacterial expression exhibited potent inhibitory activity on bovine capillary endothelial cell proliferation stimulated by basic fibroblast growth factor, with an ED_{50} of approximately 80 nM, and inhibitied migration of human umbilical endothelial cell induced by vascular endothelial growth factor in a dose dependent manner (IC_{50} of 1 nM). It also inhibited *in vivo* angiogenesis on the chick chorioallantoic membrane. The binding of the recombinant kringle domain to immobilized uPA receptor was not detected in real time interaction analysis, whereas internalization of the kringle domain into cells at 37 °C was specific to endothelial cells and maximal at about 20 min. These results suggest that the kringle domain of uPA retains an anti-angiogenic activity and provide that its endothelial cell specific internalization may be related with the action mechanism of its anti-angiogenic activity.