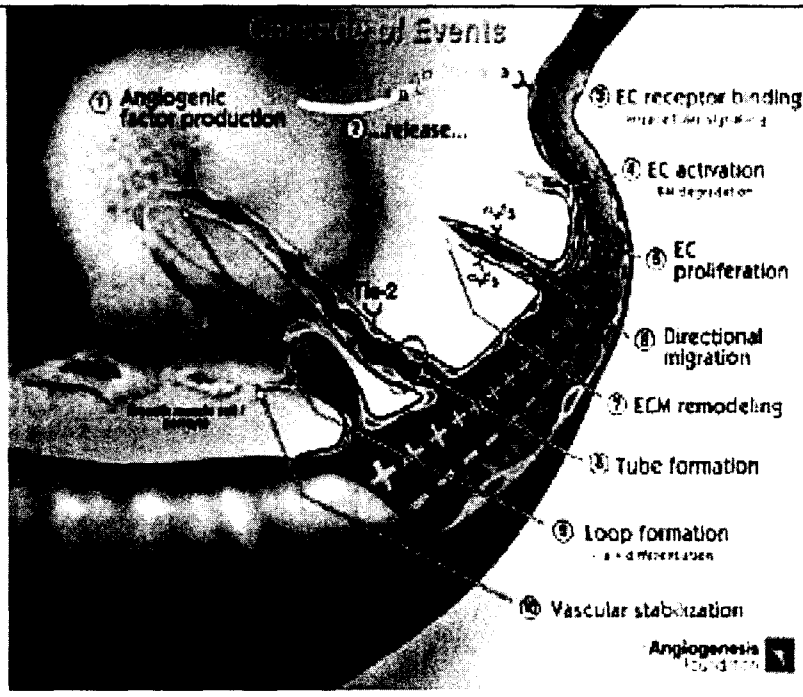
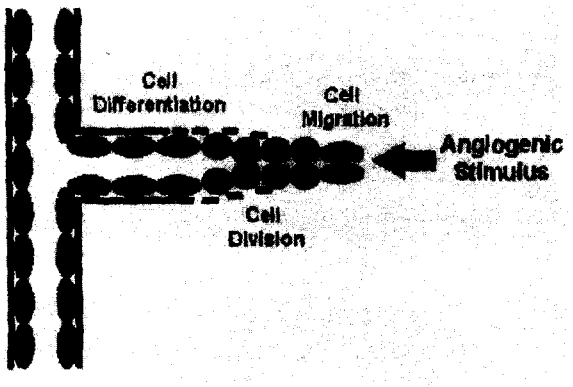
재조합 크링글 도메인 UK-1과 TK1-2의 혈관 신생 억제 작용과 항암효과

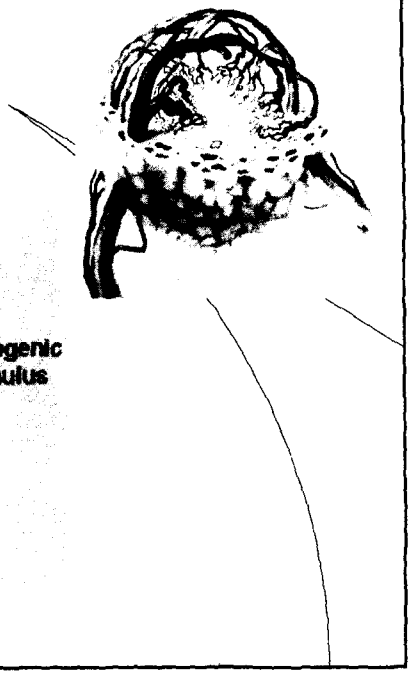
조 영 애

가톨릭대학교 의과학연구원

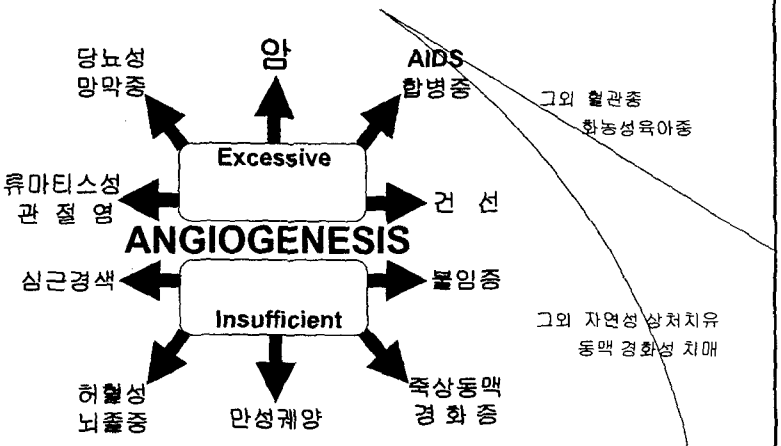


Angiogenesis Process





Angiogenesis 관련 질환

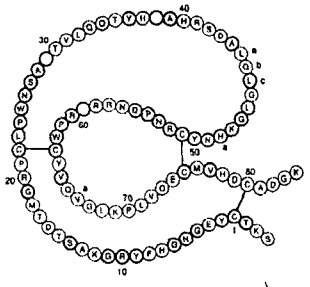


Angiostain (endogenous angiogenesis inhibitor)

- Discovered by Dr. Folkman group in 1994
- Isolated from urine and blood of Lewis Lung Carcinoma animal model
- Plasminogen kringle 1,2,3,4 : 35kDa protein
- Strong inhibition of angiogenesis
- Suppression of Tumor growth
- Clinical Trial: Phase I
- Individual kringle: differential effects on cell proliferation and migration

Kringle domain

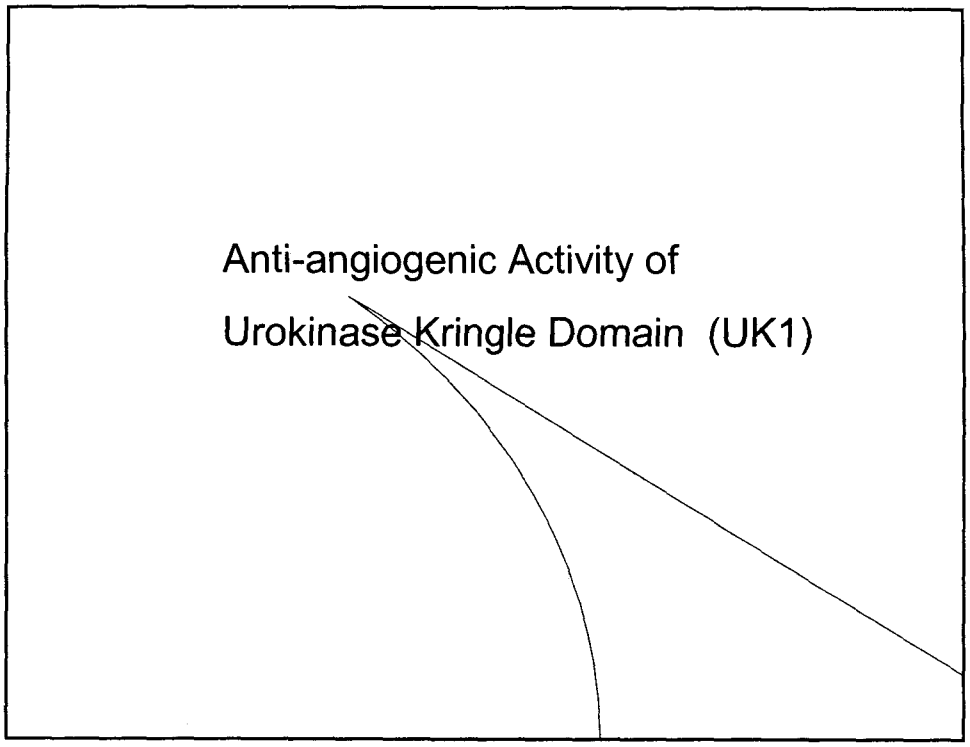
- Approximately 80 amino acids
- Conserved rigid triple disulfide bonds 1-6, 2-4, 3-5
- Plasminogen, t-PA, u-PA, Prothrombin, Apolipoprotein(a), Hepatocyte growth factor



Bokman A. M. (1993) *J. Biol. Chem.*

Anti-angiogenic Activity of

Urokinase Kringle Domain (UK1)

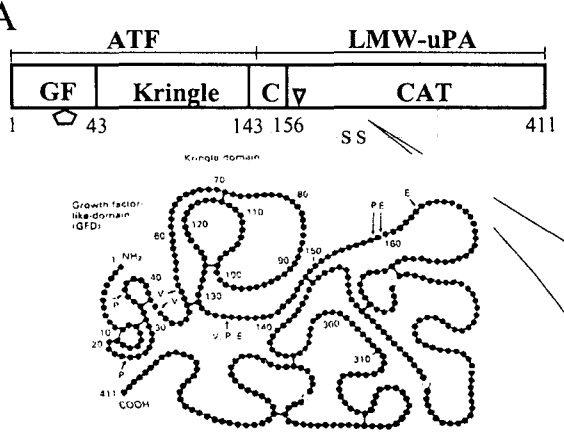


Urokinase Plasminogen Activator (uPA) System

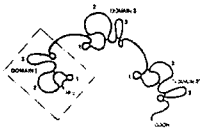
: expressed in tumor cells, angiogenic endothelial cells, macrophages and fibroblast.

- Fibrinolysis
- Neointima and aneurysm formation
- Chemotaxis
- Woond healing
- Angiogensis
- Tumor invasion and metastasis

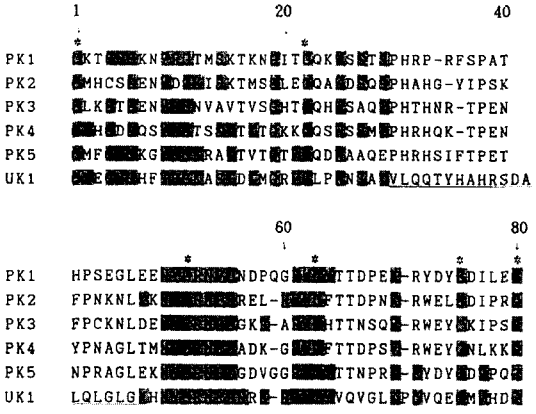
Pro-uPA



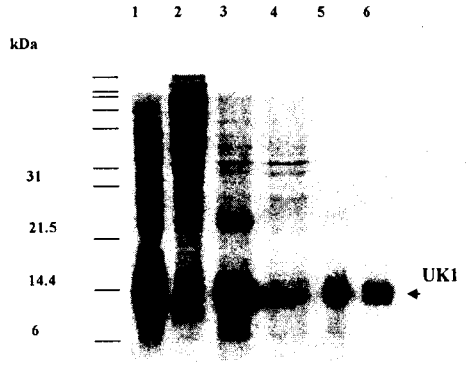
uPA receptor



Comparison of amino acid sequence between UK1 and plasminogen kringle(PK1-PK5)

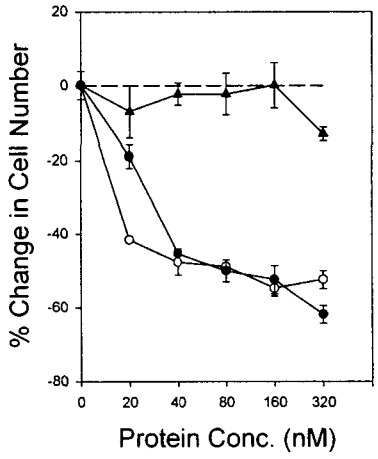


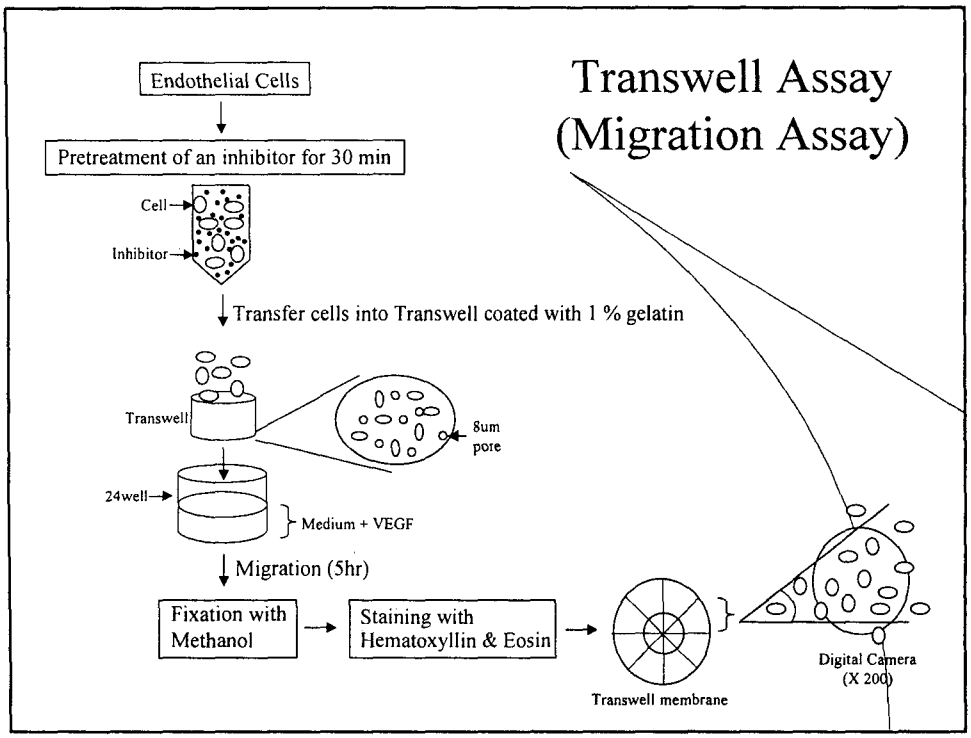
Purification and SDS-PAGE analysis

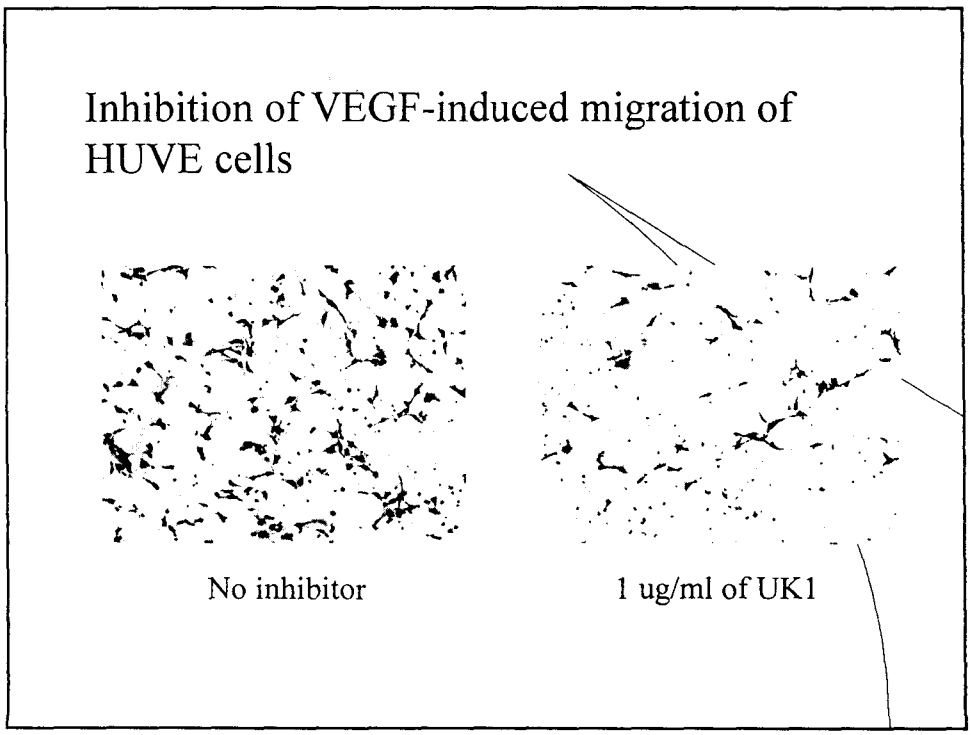


1 : total protein of E. coli
2 : soluble fraction in E. coli
3 : insoluble fraction in E. coli
4 : flow through His-tag affinity column
5 : elution from the His-tag affinity column
6 : purified UK1 through size exclusion column

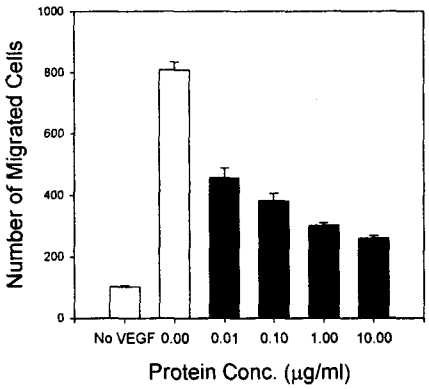
Inhibition of BCE cell proliferation by UK1




Transwell Assay
(Migration Assay)
Endothelial Cells
Pretreatment of an inhibitor for 30 min
Cell
Inhibitor
Transfer cells into Transwell coated with 1 % gelatin
Transwell
8um pore
24well
Medium + VEGF
Migration (5hr)
Fixation with Methanol
Staining with Hematoxyllin & Eosin
Transwell membrane
Digital Camera (X 200)


Inhibition of VEGF-induced migration of HUVE cells
No inhibitor
1 ug/ml of UK1

Inhibition of VEGF-induced endothelial cell migration by UK1



Chorioallantoic Memnrane Assay

Drop the salt free sample (10-20 μg) on the thermanox slip

↓

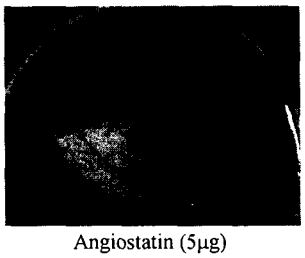
Dry in the clean air

↓

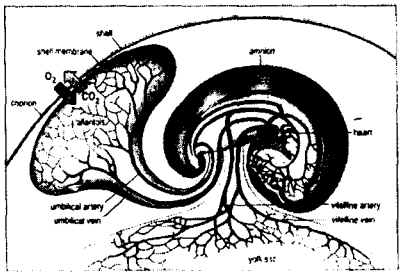
Apply on the CAM of 4.5 day embryo

↓

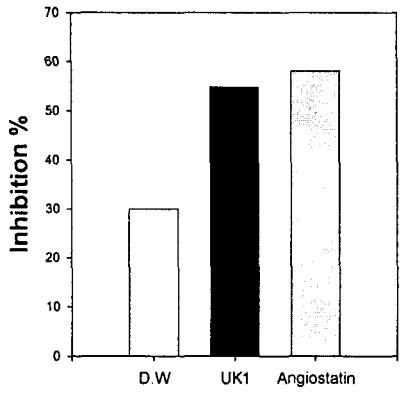
Observe and photograph at 6.5 day



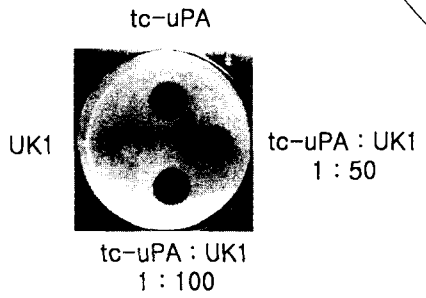
Angiostatin (5μg)



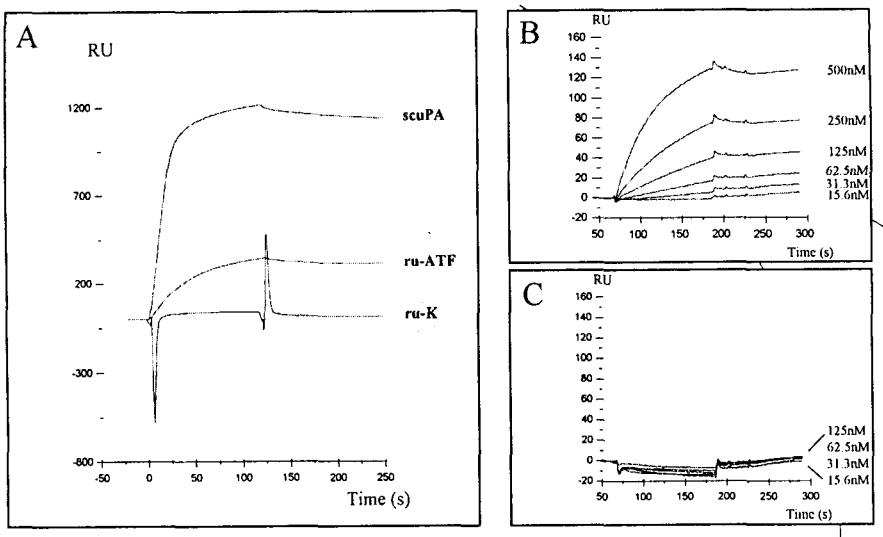
In vivo anti-angiogenic activity of UK1 on the chick CAM



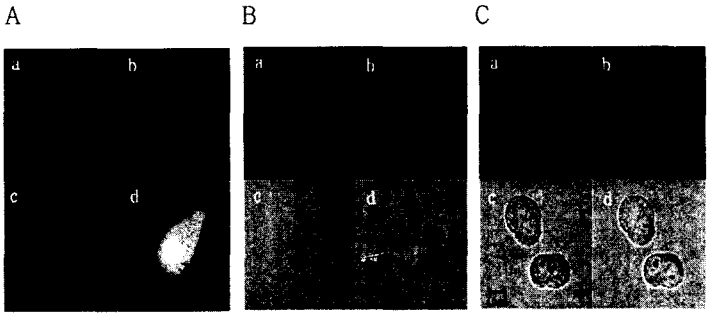
Effect of UK1 on Plasminogen Activation



Real-time Interaction Analysis by Surface Plasmon Resonance



Internalization of UK1 in HUVE cells



CONCLUSION

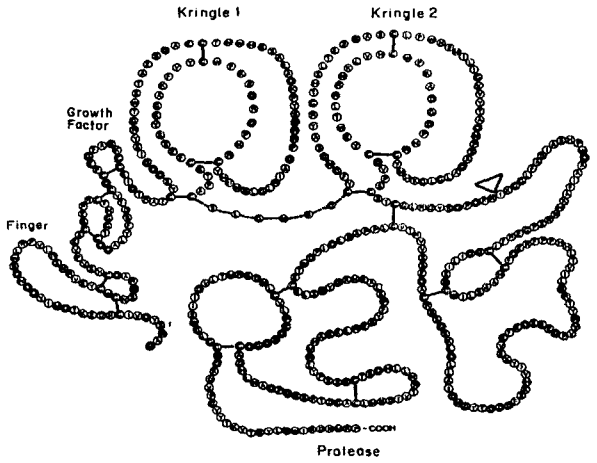
1) The kringle domain of urokinase has an anti-angiogenic activity

2) The anti-angiogenic activity of kringle does not appear to result from inhibition of uPA/uPAR interaction.

3) Internalization of the kringle followed by translocation from cytosol to nucleus is specific to endothelial cells.

Inhibition of human lung tumor growth in vivo by the recombinant kringle domain (TK1-2) of tissue-type plasminogen activator

t-PA ; tissue-type plasminogen activator



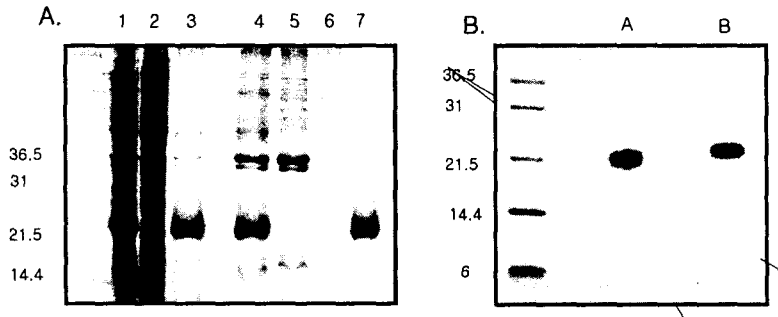
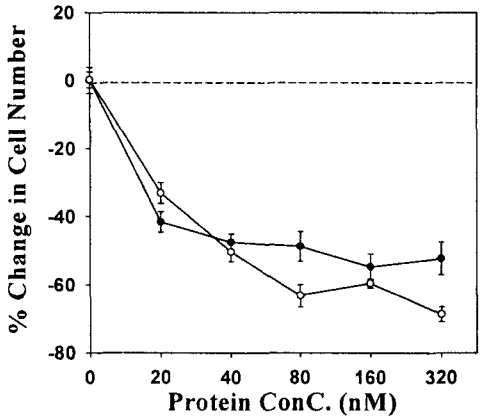
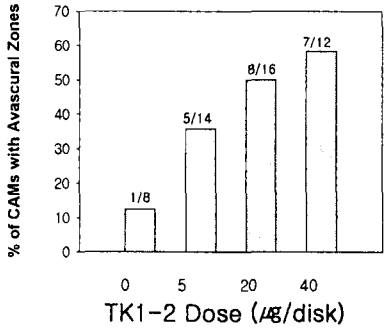
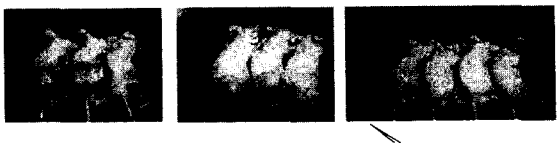


Fig. 1 Purification and SDS-PAGE analysis.
A. Samples obtained from the purification steps were subjected to electrophoresis on a 14% SDS-polyacrylamide-gel in Tris-glycine buffer under *reducing conditions ; the gel was stained* with Coomassie Blue. SDS-PAGE analysis of purified TK1-2 under reducing condition. lane 1; total protein in E.coli; lane 2,soluble fraction of E.coli; lane 3, insoluble fraction from E.coli lysate; lane 4, insoluble fraction dissolved in 6M urea solution;lane 5, flow through fraction from His-tag Ni+ affinity column; lane6, fraction eluted with 20mM imidazole solution; lane 7, sample eluted from the His-tag affinity column. B. purified TK1-2 was analyed under non-reducing condition(lane 1) and reducing condition(lane 2)

Inhibition of BCE cell proliferation by TK1-2







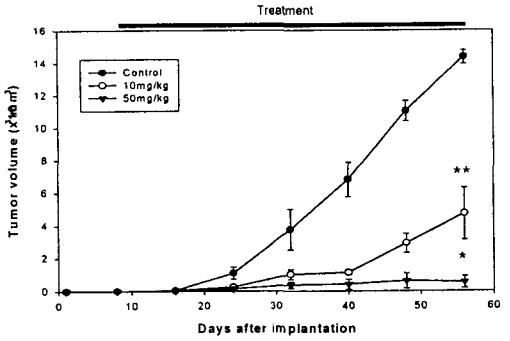


Fig. 6. Suppression of tumor growth of human lung cancer PC14 by recombinant TK1-2. Male BALB/c-nu mice were subcutaneously implanted with 5 X 106 PC14 tumor cells per mouse and systemically treated with PBS(control, A, ●) or recombinant TK1-2 by subcutaneous injection in the abdomen at the dose of 10 mg/kg(B, ○) or 50 mg/kg (C, ▼)everyday at day 8 through day 58. Data represent the tumor volume of ten mice in each group (mean±SE). *, $P < 0.001$; **, $P < 0.005$.

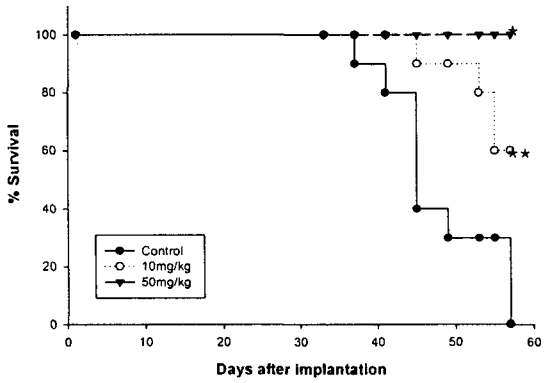
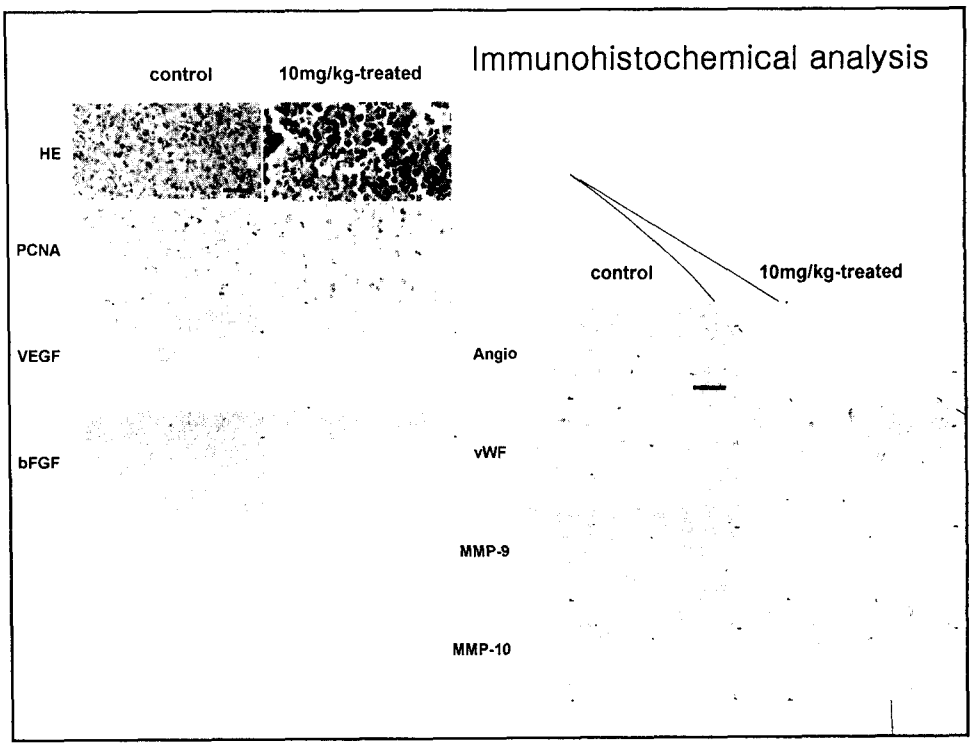


Fig. 7 Survival of TK1-2 - 10mg/kg, TK1-2-50mg/kg or control-PBS treated mice (n=10/group). **, P < 0.005; *, P < 0.08.



CONCLUSION

1) The kringle domain of tissue-type plasminogen activator has an anti-angiogenic activity

2) Low dose TK1-2 treatment (10 mg/kg) suppressed tumor growth by about 76% ($p<0.01$), and high dose TK1-2 treatment (50 mg/kg) almost completely inhibit tumor growth (al least 97%) ($p<0.05$) without any observable signs of toxicity

3) The immunoreactions of angiogenin, bFGF, VEGF and MMP-10 were much weaker in the TK1-2 treated tumor tissues than the control tumor tissues, while no difference was found in the expression of PCNA and MMP-9

4) TK1-2 could be effectively used as a new anti-cancer agent