Introduction:
Protein C inhibitor (PCI) was initially found in human plasma as an inhibitor of activated protein C (APC), the main protease of the anticoagulant protein C pathway [1]. PCI has a reactive site (354Arg-355Ser), which is important for inhibition of serine proteases; therefore, PCI is categorized as a member of the serine protease inhibitor (SERPIN) family [2]. Subsequent studies revealed that PCI inhibits other serine proteases of the blood coagulation system including thrombin [3], factor Xa [3], factor XIIa [4], plasma kallikrein [4], and thrombin-thrombomodulin complex [5], proteases of the fibrinolysis system including tissue plasminogen activator (tPA) [6] and urinary plasminogen activator (uPA) [7], and also proteases of the fertilization system such as prostate-specific antigen (PSA) [8] and sperm acrosin [9]. The main source of human plasma PCI may be the liver because plasma PCI levels are markedly decreased in patients with liver disease [10]. Human PCI mRNA is also detected in kidneys [11] and reproductive organs [12]. Recently, we found that the PCI antigen and mRNA levels are significantly lower in renal cell carcinoma tissues (RCC) than in non-tumoral renal tissues. Subsequently, we demonstrated that normal renal proximal tubular epithelial cells (RPTEC), but not RCC or RCC cell lines (Caki-1), express PCI [13]. In the present study, we evaluated the effect of PCI on tumor growth and metastasis of the human MDA-231 breast cancer cells. The effect of PCI on angiogenesis was also evaluated.

Methods:
Human PCI-expressing MDA-231 cells (MDA-PCI) and mock-expressing MDA-231 cells (MDA-Mock) were prepared by transfecting pRC/CMV containing human PCI cDNA or pRC/CMV without PCI cDNA insert. Recombinant PCI was prepared using baculovirus expression system. The in vitro invasion assay was performed using Matrigel basement membrane matrix and a modified Boyden chamber system. The in vivo tumor growth was monitored by measuring the tumor volume twice a week after intracutaneous injection of each group of MDA-231 cells (5 × 10⁵ cells/mouse) in severe combined immunodeficiency (SCID) mice. The in vivo effect on lung and bone metastasis was evaluated by counting the number of lung metastatic foci after intravenous injection of each group of MDA-231 cells (106 cells/mouse) and radiological examination of metastatic tissues. The number of lung metastatic nodules and bone osteolytic areas produced by MDA-PCI cell lines was significantly less than those produced by MDA-Mock cell lines. Data obtained from all in vitro and in vivo angiogenesis assays suggest that PCI inhibits angiogenesis.

Discussion:
Recently, we demonstrated that PCI expression is significantly decreased in RCC, as compared to non-tumoral renal proximal tubular epithelial cells, suggesting that PCI may affect tumor growth, angiogenesis, and metastasis. Based on these backgrounds, in the present study, we evaluated the effect of PCI on angiogenesis and metastasis of the breast cancer cell lines, MDA-231 cells. The effect of PCI on angiogenesis was also evaluated. The invasiveness, tumor growth, and metastasis of MDA-231 cells were effectively inhibited by PCI in vitro and in vivo. This is known that uPA plays a critical role in the growth of cancer. As a potent inhibitor of uPA, the PCI inhibition of uPA may explain the inhibitory activity of PCI on angiogenesis. This study also showed for the first time that PCI is an anti-angiogenic factor. However, it is not known whether the protease inhibitory activity of PCI is required for its anti-tumoural activity. It is now under investigation whether the reactive site of PCI is responsible for anti-angiogenic activity.

References:

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