In Vivo Efficacy of Human Apolipoprotein(a) Kringle in Liver Cancer

Angiogenesis is the process of the formation of new capillaries from pre-existing blood vessels and is regulated by a local balance between angiogenic stimulators and inhibitors. In the activated endothelium, angiogenic growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) predominate, whereas vascular quiescence is achieved by the dominance of inhibitory factors like angiotatin, endostatin, and thrombospondin. In case of tumor, the formation of these new capillaries is essential for their growth and metastasis. Without blood vessels, tumors can not grow beyond a critical size (2^3 mm^3) or metastasize to another organ. Consequently, tumor vascularization is a vital process for the progression of a neoplasm from a small, localized tumor to an enlarging tumor with the ability to metastasize, and this make cancer a clinically relevant target for anti-angiogenesis therapy.

Hepatocellular carcinoma (HCC) is characterized by a propensity for vascular invasion and a high metastatic potential. The intraheaptic or extraheptic metastasis is the main mechanism of early recurrence after resection of small HCCs. It has been reported that various number of angiogenic factors including growth factors, proinflammatory cytokines, enzymes, and viral proteins are associated with angiogenesis in HCC. Among the growth factors, VEGF is one of the most potent angiogenic factors involved in neovascularization. Although the clinical significance of angiogenesis in HCC is not clearly demonstrated compared with other common human cancers, there are several reports on the potential correlation between the expression of VEGF and the progression of HCC. In addition, the early postoperative recurrence in the liver remnant or distant sites is a common phenomenon after resection of HCC, which should be considered as a result of metastasis. In the event of the hepatic metastasis progression, it is believed that there are two distinctive stages as angiogenesis independent prevascular stage and an angiogenesis dependent stage. At the first angiogenesis independent prevascular stage, the circulating endothelial cell adhesion to endothelium, which facilitates metastatic cell implantation and growth when micrometastasis are smaller than 400 μm in diameter. The transition from the prevascular stage enables the cancer cell to proliferate along an angiogenesis dependent stage, which lead to the clinically relevant macrometastatic growth. As a result, the transition from preangiogenic to angiogenic micrometastasis is critical in the progression of liver metastasis.

The high degree of neovascularization observed in HCC as well as the angiogenic transition to proliferation stages makes this tumor type an interesting target for antiangiogenic approaches. Antiangiogenic approaches aim at the suppression of tumor growth via the inhibition of tumor vascularization. Since introduction of this concept in 1971, several attempts have been made in various cancer applications. Recently, the antiangiogenic drug bevacizumab, a recombinant humanized monoclonal antibody directed against vascular endothelial growth factor (VEGF), in combination with conventional chemotherapy, demonstrated significant improvement on the
survival of patients with metastatic colorectal cancer. With this success, the numerous antiangiogenic molecules are under clinical investigations including several phase II studies on HCC. Especially in the case of bevacizumab, the current ongoing studies are the phase II randomized study of bevacizumab in patients with unresectable HCC receiving chemoembolization and the phase II study in patients with unresectable nonmetastatic HCC without main portal vein invasion. Among the various antiangiogenic molecules, the inhibitors targeting directly the endothelial cell can provide the broader spectrums compared with the single target agents such as neutralizing antibody against VEGF, bevacizumab, since the cancer cells can produce the multitude of proangiogenic factors and also create the microenvironments favorable for the angiogenesis.

In this study, we examined the antiangiogenic activities of apolipoprotein(a) (apo(a)) kringle domain in the experimental animal models for the hepatic metastasis and the tumor growth. The kringle domain is a protein structure that consists of approximately 80 amino acids with conserved rigid triple disulfide bonds and that appears to be an independent folding unit. Kringle domains have been found in many proteins, including prothrombin, plasminogen, urokinase, hepatocyte growth factor, and apolipoprotein(a) [apo(a)]. These proteins have a surprisingly diverse array of functions as they are growth factors, proteases, or coagulation factors. There has been an increasing body of evidence showing that kringle domains can act as inhibitors of angiogenesis. Angiostatin, which consists of the first four kringle domains of plasminogen, inhibits endothelial cell migration and proliferation. It also inhibits primary tumor growth as well as angiogenesis-dependent growth of metastases in mice. In addition, kringle 2 of prothrombin, the kringles of hepatocyte growth factor, and the kringle domains of tissue-type and urokinase-type plasminogen activator have been demonstrated to inhibit endothelial cell proliferation and/or migration. Unlike other kringle-containing proteins, Apo(a) consists of 15 to 50 tandemly repeated kringle domains that closely resemble plasminogen kringle 4 (KIV), followed by sequences that are homologous to the kringle 5 (KV) and protease domains of plasminogen. Although the physiological and biochemical role(s) of the apo(a) kringle domains are not clearly understood yet, we were able to demonstrate that the apo(a) kringle domains can inhibit angiogenesis in vitro and suppress tumor growth in vivo.

Among apolipoprotein(a) (apo(a)) kringle domains, apo(a) kringle V is unique and shows a significant sequence homology with plasminogen kringle 5, that has been reported to be a potent inhibitor of endothelial cell proliferation and migration. To determine the potential activities on the hepatic metastasis, we prepared the recombinant protein encoding apo(a) kringle V and produced in yeast Pichia pastoris and examined its in vivo activities. The effects of LK8 on metastasis were determined in an experimental model of hepatic metastasis established by injecting LS174T human colorectal cancer cell into the spleens of Balb/c (nu/nu) mice. Systemic administration of rhLK8 significantly inhibited hepatic metastasis of LS174T human colorectal cancer cells, as determined by the number of tumor nodules on liver surface, by the hepatic replacement area, and by the survival benefits.

The long term effects of LK8 were also investigated using the AAV mediated gene transfer for the suppression of hepatic tumor growth. AAV vectors are considered to be very useful tool for the application of the gene therapy since AAV is not self replicable and non pathogenic while transducible in several cell types including non-dividing cells. By the single administration of AAV-LK8, the growth of HCC xenograft was significantly suppressed. In conclusion, these observations suggest that rhLK8 may be an effective angiogenesis inhibitor both in vitro and in vivo and a promising candidate for the treatment of hepatic metastasis.
References

논문, 최근 특허


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