論 文 の 内 容 の 要 旨

論文題目　Preparation and Characterization of a Targetable Gene Carrier for the Antiangiogenic Therapy of Cancer

**（血管新生阻害療法によるがんの遺伝子治療を志向した**

**アクティブターゲット型遺伝子キャリアの創製および機能評価）**

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Gene therapy is gaining popularity as a promising strategy to treat many intractable diseases. Especially, with the understanding of the genetic basis of cancer, an entirely new approach to the treatment of cancer using gene transfer techniques has emerged. The understanding that tumor growth and metastasis are angiogenesis dependent processes increased the interest in targeting tumor vasculature in anticancer therapy. A gene therapy-mediated approach for the delivery of antiangiogenic agents has several advantages, including the potential for sustained expression.

Gene delivery is a multiple step approach which depends on the ability of a gene carrier to overcome several biological barriers and safely deliver the transgene to the target cells. Polymeric gene delivery systems, i.e., polyplexes, have been emerged as a safe alternative to the viral vectors to realize the potential of nucleic acids in clinics. Polyplex micelle formed through electrostatic interactions of PEG-polycation block copolymer and plasmid DNA represents a promising strategy for systemic nonviral gene delivery.

In this study, poly(ethylene glycol)-poly(L-lysine) (PEG-Plys) block copolymer was applied as a basic platform for formation of a polyplex micelle and was further optimized by increased molecular weight of PEG to enhance shielding effect and thus micelle stability in blood; disulfide crosslinked core which stabilizes micelle structure in the extracellular entity, while facilitating smooth release of the plasmid DNA in the intracellular reductive environment; and introduction of cyclic RGD ligand to the micelle surface for specific targeting of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin

receptors, which are overexpressed on tumor angiogenic endothelial cells. The effects of these implementations on gene expression *in vitro*, blood circulation ability and tumor growth inhibition effect by plasmid DNA encoding an antiangiogenic agent *in vivo* were investigated.

Long PEG chain and moderate crosslinked density were found to increase micelle stability in blood while maintaining high transfection efficiency *in vitro* and revealing tumor growth inhibition effect *in vivo*. Cyclic RGD ligand on the micelle surface further increased its transfection efficiency in cells overexpressing $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin receptors, as a result of receptor-mediated endocytosis and modified intracellular trafficking. Moreover, increased tumor-to-blood ratio was found for the RGD modified micelles.

Finally, the 15% disulfide crosslinked micelle with cyclic RGD ((c(RGDfK)-PEG-P(Lys-SH15)) loading plasmid DNA encoding the soluble form of vascular endothelial growth factor (VEGF) receptor-1 (soluble fms-like tyrosine kinase-1: sFlt-1), as the VEGF inhibitor, was tested for its antiangiogenic effect in BxPC3 pancreatic adenocarcinoma tumor bearing mice. BxPC3 xenograft was selected as a model of hypovascular tumor with thick fibrotic tissue, on which standard chemotherapy has only limited effect due to the inaccessibility of the therapeutic agents to the cancer cells. Systemic administration of thus obtained micelle achieved significant inhibition of tumor growth up to day 18 and its antiangiogenic ability was confirmed by decreased vascular density of the tumor tissue compared to the control. Significant therapeutic activity of the c(RGDfK)-PEG-P(Lys-SH15) micelle was achieved by combined effect of increased tumor accumulation, interaction with endothelial cells and enhanced transfection efficiency as a result of receptor specific endocytosis.

In summary, the feasibility of the antiangiogenic gene therapy against an intractable fibrotic tumor was demonstrated by the micelle evaluated in this study. These results suggest that RGD targeted crosslinked polyplex micelles can be effective plasmid DNA carriers for antiangiogenic gene therapy.