学位論文内容の要旨

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学 位 論 文 題 名

Development of a novel active targeting system for siRNA delivery to liver sinusoidal endothelial cell in vivo
（肝臓類洞内皮細胞への siRNA 送達を目的とした革新的な能動的標的化システムの構築）

Different types of liver cell as well as liver sinusoidal endothelial cells (LSECs) are related with various types of hepatic diseases. A selective gene delivery system would be an ideal approach for achieving a subsequent efficient therapy for different types of liver diseases. Liposomes (LP) are suitable nanocarriers that have the capacity to deliver drug particles to various target cells in vitro or diseased tissues in vivo. Based on these considerations, we selected the ApoB-100 segment RLTRKRGLK (3359-3367) for use as a novel ligand in designing a selective targeting system for LSEC. We designed two peptide named as RLTR and KLGR for this purpose.

RLTR or KLGR peptide modified pegylated liposomes (PEG-LPs) were prepared by lipid film hydration method by adding the required amount of RLTR-PEG to the Egg phosphatidylcholine (EPC)/Cholesterol (7:3) lipid solution. Rhodamine-DOPE was incorporated to label the lipid component. The size and zeta potential peptide modified LP ranges from 115 nm to 132 nm and 12 mV to 27 mV respectively. In vivo accumulation experiment showed that both the RLTR-PEG-LPs and KLGR-PEG-LPs were accumulated along with the liver blood vessels. Next, accumulation of both labeled RLTR-PEG-LPs and KLGR-PEG-LP along with the liver blood vessels were drastically inhibited by a pre-treatment with unlabeled RLTR-PEG-LPs or KLGR-PEG-LPs. This surprising accumulation of this carrier system through blood vessels caused us to think that this nano particle might be an ideal system for targeting the LSECs. There are very few reports of targeting LSECs using cationic or neutral nanocarriers. Those systems failed to achieve high accumulation in LSECs. In this study we seized the opportunity to develop this nanocarier to target LSECs. For delivery of endothelial cell specific Tie2 siRNA we used KLGR peptide modified YSK05-MEND. 0.5 mol% of stearylated KLGR at YSK05-MEND showed best knockdown effect as it caused about 80% knocking down of Tie2 gene expression compared to non treat. The interesting fact about this knocking down is though the FACS analysis data shows that uptake of modified YSK05-MEND increases with the increase of mol% of STR-KLGR on YSK05-MEND surface, the knockdown efficiency decreases. Endosomal escape efficiency measurement proofs that possible reason behind this phenomenon might be the decreasing endosomal escape efficiency of YSK05-MEND with the increasing mol% of STR-KLGR on MEND surface. It is also observed that a

lower density of STR-KLGR at the MEND surface follows macropinocytosis but at high peptide density at the MEND surface they follows clathrin-mediated endocytosis which is related with lysosomal degradation. It indicates that shifting of uptake pathway from lower density of peptide to higher density at the MEND surface is responsible for difference in knocking down of gene expression.

0.5 mol% STR-KLGR modified YSK05-MEND can successfully deliver siRNA to LSEC *in vivo*, This nanocarrier can become an useful tool for targeting LSEC