Development of novel engineering technology for liposomal siRNA

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Recent expectation regarding the role of nucleic acids as future therapeutic agents has been growing. In particular small interfering RNA (siRNA), which induces sequence-dependent gene silencing, has been widely studied. We previously developed liposomes modified with polycation as carriers of siRNA and succeeded in showing their potent gene knockdown efficiency. In the present study, we investigated the possibilities of novel engineering technology to prepare a polycation liposomal formulation of siRNA. Our technology enabled simple preparation of liposomal siRNA that is expected to be useful compared with existing technology.

Lipids were dissolved in organic solvent. To form liposomes, additional DEPC-treated water containing siRNA or not was added into the organic solution. Optimal organic solvent, volume of water, and composition of liposomes were determined. Next, the knockdown efficiencies of the polycation liposomal siRNA were determined by using human umbilical vein endothelial cells (HUVECs). Liposomes were modified with Ala-Pro-Arg-Pro-Gly (APRPG) as a targeting peptide toward angiogenic endothelial cells on their surface (APRPG-PEG Lip). Biodistribution of the liposomal siRNA injected i.v. was determined by *in vivo* fluorescence imaging system.

As a result, in the condition that used 2-propanol, most uniform liposomes were prepared. In addition, liposomalization was possible with various lipids. Particle size of liposomes was able to be regulated by changing the condition of preparation. Liposomal siRNA prepared by novel method showed high knockdown efficiency compared with that by conventional method, indicating that the liposomes were equipped with siRNA and functional peptide with simple method. Alexa750-labeled siRNA encapsulated in APRPG-PEG Lip was accumulated in the solid tumor *in vivo*.

In the present study, we established the simple technology to prepare liposomal siRNA. This technology would be applicable for a variety of liposomes and systemic administration. Our data suggested that liposomal siRNAs prepared by our technology would be useful for developing siRNA medicines.