論文の内容の要旨

Abstract of Dissertation

Design and Characterization of Stearoyl Polycation/siRNA Complexes toward Lung Metastatic Cancer

(肺転移がんへのsiRNAデリバリーを目指したステアロイル基導入ポリカチオン複合体の調製と機能評価)

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RNA interference (RNAi) is a powerful regulatory mechanism of the target mRNA degradation induced by the complementary RNA strand. Because of the selectivity of the targeted mRNA degradation as well as the potential of silencing almost all the endogenous genes, small interfering RNA (siRNA) has been highlighted as an attractive choice for future cancer therapeutics. However, the *in vivo* therapeutic application of siRNA needs to fulfill some requirements to exert RNAi in the target cells. Naked siRNA should be protected from degradation by nucleases and remained in the bloodstream from clearance by the reticuloendothelial system (RES). The negative charge of the siRNA inhibits its interaction with negatively charged plasma membrane for the internalization into cells. Even though some portions of siRNAs could be internalized into cells, the internalized siRNA would be degraded in lysosome. Hence, the carrier system to protect siRNA from the external circumstances and to escape endosome efficiently before the lysosomal degradation would be required for effective siRNA therapeutics.

The material selection is important to design an efficient siRNA delivery carrier enabling to satisfy the requirements. Previously our laboratory reported that the cationic polyaspartamide derivative, poly{*N*-[*N*-(2-aminoethyl)-2-aminoethyl]aspartamide} (PAsp(DET)), possessed pH-sensitive endosome destabilizing activity. PAsp(DET) can destabilize the cellular membrane only at the endosomal pH and, thus, obtain a high endosomal escape ability. PAsp(DET)-based polyplexes with plasmid DNA have shown to have remarkable transfection efficiency without marked cytotoxicity in various cultured cells including a primary cell. However siRNA complex formed with PAsp(DET) did not have RNAi because the siRNA complex is unstable in cell culture condition and bloodstream and quickly dissociated into siRNA and the polymer.

To overcome this instability, stearic acid as a hydrophobic moiety was conjugated to the side chain of PAsp(DET) at a limited introduction ratio. Stearoyl introduction successfully contributed the formation of larger siRNA complex with higher association number in ***Chapter 2***. Stearoyl PAsp(DET)/siRNA complexes tolerates the cell culture condition better than does unmodified PAsp(DET)/siRNA complex, allowing for improved cellular internalization. Stearoyl PAsp(DET)

showed 50% - 60% endogenous VEGF and BCL-2 gene suppression in Panc-1 cells, implying that the polymer had potential application for cancer therapy. This impressive endogenous gene suppression was explained by the enhanced cellular uptake obtained from stearoyl introduction, and also by more efficient endosomal escape ability of PAsp(DET) backbone structure.

Stearoyl moiety was also introduced into PEG-*b*-PAsp(DET) and PEG-SS-PAsp(DET) for systemic administration in ***Chapter 3***. Stearoyl PEG-SS-PAsp(DET)/siRNA complex was administrated intravenously in B16F10-Luc lung metastatic tumor and showed 50% luciferase gene suppression. The siRNA delivery of the polymer accumulated higher in the lung than stearoyl PEG-*b*-PAsp(DET), which could be the main reason of better luciferase gene suppression in the lung tumor. In addition, stearoyl PEG-SS-PAsp(DET)/siRNA complex had longer blood circulation measured by *in vivo* confocal microscopy, suggesting that the polymer may also be an effective carrier to a subcutaneous tumor model. From precise analysis of images in the confocal observation, some of stearoyl PEG-SS-PAsp(DET)/siRNA complex exerted aggregation at initial circulation period, resembling the behavior of stearoyl PAsp(DET)/siRNA complex even though the majority of the siRNA complex was not aggregated and uniformly flowed. This aggregation tendency of stearoyl PEG-SS-PAsp(DET) can be probably explained by rapid response of glutathione toward the disulfide bond of stearoyl PEG-SS-PAsp(DET)/siRNA complex in the bloodstream or natural exchange reaction of disulfide bond during synthesis procedure and micelle formation.

A study was extended to therapeutic siRNA delivery in ***Chapter 4***. EGFR gene was selected as a target gene to inhibit B16F10-Luc lung metastatic tumor. The most efficient sequence of EGFR siRNA was predicted by siRNA design tools and the sense and antisense strand were modified by 2´-OMe to eliminate the activation of innate immune response in the cells. Stearoyl PEG-SS-PAsp(DET)/siRNA complex was administered intravenously into mouse bearing the lung metastatic tumor but unexpectedly showed no inhibition of the tumor, probably due to inefficient delivery of siRNA by the polymer or inappropriate selection of the target gene. Finally, the polymer was investigated its delivery ability to BxPC3 subcutaneous tumor and showed that some amount of siRNA successfully accumulated into the tumor in the preliminary experiment. This study shows that therapeutic siRNA delivery by stearoyl PEG-SS-PAsp(DET) was not successful yet. However, the polymer still has potential for the therapeutic siRNA delivery in other types of tumor and should be investigated as next research.