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論 文 要 旨

THESIS SUMMARY

専攻： Department of	化学環境学 専攻	申請学位（専攻分野）： Academic Degree Requested	博士 （Engineering） Doctor of
学生氏名： Student's Name	Huang Chih Hao	指導教員（主）： Academic Advisor(main)	西山伸宏
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

In this thesis, I reported the development of a novel molecular design of siRNA (small interfering RNA)-conjugated polymer system that responds to intracellular redox potential. The developed siRNA-conjugated polymer system containing 2-nitrobenzenesulfonamide linkage achieved enhanced extracellular stability, compared to conventional siRNA-conjugated polymer system containing disulfide linkage, as well as cleavability in response to intracellular environment with high specificity, leading to effective induction of siRNA bioactivity (RNA interference, RNAi) in the cell.

In Chapter 1, I suggested a new siRNA-conjugated polymer system containing 2-nitrobenzenesulfonamide group as a chemical linker. So far, many efforts have been dedicated to construction of appropriate methodology toward effective induction of siRNA bioactivity at the targeted tumor tissue, including siRNA carrier formation and chemical modification of siRNA. Among them, siRNA-conjugated polymer system potentially provides siRNA with new functionalities for improved therapeutic properties. In this regard, chemical linker is necessary for siRNA-conjugated polymer system for effective gene silencing; the vicinal presence of the conjugated polymer hinders recruitment of siRNA into RNAi pathway, due to the steric hindrance effect. In general, disulfide group has been often utilized as the conventional chemical linker for effective siRNA release in the cell. However, its extracellular stability remains to be improved (e.g., the half-life of disulfide linker in the blood is several hours). To this end, in this chapter, I suggested a new siRNA-conjugated polymer system having 2-nitrobenzenesulfonamide group as a chemical linker, for improved extracellular stability and selective siRNA release in the cell. 2-Nitrobenzenesulfonamide group can be cleaved in response to the exclusive presence of the combination of glutathione (GSH) and glutathione-*S*-transferase (GST) in the cell, toward the cleavability with high selectivity in the cell.

In Chapter 2, I described the synthetic part of the new siRNA-conjugated polymer system. In design, PEG was selected as a polymer for conjugation with siRNA (PEG-siRNA conjugate containing 2-nitrobenzenesulfonamide group, PEG-sul-siRNA), because PEG release in the cell leads to effective induction of siRNA bioactivity, confirming the cleavage of the linker in the cell. For the comparison, PEG-siRNA conjugate containing conventional disulfide group (PEG-disulfide-siRNA) and non-cleavable carboxylic amide group

(PEG-car-siRNA) were also synthesized, in order to demonstrate the functionality of 2-nitrobenzenesulfonamide group for siRNA-conjugated polymer system. PEG-siRNA conjugates were synthesized via copper-free click chemistry for effective production, and were purified by ion-exchange chromatography. The successful synthesis of PEG-siRNA conjugates was confirmed by agarose gel electrophoresis.

In Chapter 3, I described the stability and cleavability of PEG-siRNA conjugates in mimicked intracellular/extracellular conditions. In mimicked extracellular condition, the suppressed siRNA release for PEG-sul-siRNA system, compared to PEG-disulfide-siRNA system, was observed. In addition, PEG-sul-siRNA enables siRNA release selectively in the mimicked intracellular condition, which is driven by the combination of GSH and GST. These results strongly suggested the utility of 2-nitrobenzenesulfonamide group as a chemical linker for siRNA-conjugated polymer system with highly selective siRNA releasability in the cell. Moreover, it is worth to note that the stability against light exposure was also achieved in my design of siRNA-conjugated polymer system, compared to conventional disulfide system, potentially leading to the easy handling for usage and storage. Altogether, I successfully demonstrated that installment of 2-nitrobenzenesulfonamide group into siRNA-conjugated polymer system can overcome the issue of extracellular stability in conventional disulfide system.

In Chapter 4, I described the evidence that PEG-sul-siRNA successfully released siRNA in the cell for cultured cell system. When the chemical linker was cleaved and PEG was released, siRNA is exposed to RNAi pathway, leading to effective induction of gene silencing. In this regard, enhanced gene silencing effect for PEG-sul-siRNA system was observed, compared to PEG-car-siRNA system, confirming the cleavage of 2-nitrobenzenesulfonamide group in the cell. In addition, the optimized PEG conjugation site of siRNA was determined to be 5’ terminus of the sense strand; the higher gene silencing ability, when PEG was conjugated to 5’ terminus of the sense strand, compared to the systems with PEG conjugation to the other sites, was achieved, possibly due to the less steric hindrance by the remained group of the linker fragment around 5’ terminus of the antisense strand, toward the effective gene silencing. I successfully finished the new molecular design of siRNA-conjugated polymer system for this study.

Consequently, the new design of siRNA-polymer conjugate containing redox-sensitive 2-nitrobenzenesulfonamide group was developed in this study. I strongly believe that the developed molecular design will contribute not only to the development of siRNA-based therapeutics, but also to other bioresponsive materials, toward further application in the biomedical field.