論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Tumoral Acidic pH-responsive Polycarboxybetaine-coated Lipid Nanoparticle for Effective siRNA Delivery
著者(和文)	SUNGYi-Jung
Author(English)	Yijung Sung
出典(和文)	学位:博士(学術), 学位授与機関:東京工業大学, 報告番号:甲第12301号, 授与年月日:2022年12月31日, 学位の種別:課程博士, 審査員:西山 伸宏,三浦 裕,小畠 英理,曽根 正人,栁田 保子,小倉 俊一郎
Citation(English)	Degree:Doctor (Academic), Conferring organization: Tokyo Institute of Technology, Report number:甲第12301号, Conferred date:2022/12/31, Degree Type:Course doctor, Examiner:,,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工学 ライフエンジニアリング	系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of	(Philosophy)
学生氏名： Student's Name	Yi-Jung Sung		指導教員（主）： Academic Supervisor(main)	西山伸宏	
			指導教員（副）： Academic Supervisor(sub)	三浦裕	

要旨（英文 800 語程度）
Thesis Summary (approx.800 English Words)

Small interference RNA (siRNA) has been developing for tremendous disease treatment including cancer therapy. In particular, siRNA delivery is challenging because it is susceptible to degradation within cytosol owing to its physiochemical properties. Therefore, lipid-based nanoparticles such as lipid nanoparticles (LNPs) are commonly used as carriers for the systemic delivery of siRNAs due to their low toxicity, biocompatibility as well as the ability to encapsulate highly negatively charged siRNA.

The limitation of delivery of siRNA encapsulated LNPs to tumor site is mainly nonspecific protein adsorption by phagocytic cells from mononuclear phagocyte system (MPS), which leads to high accumulation of LNPs in liver and spleen and rapid clearance from blood circulation. It was reported that positively charged nanoparticles were easily adsorbed by serum protein despite the fact that cationic particles were able to effectively interact with cellular membrane during endocytosis process.

The external surface of LNPs plays an important role in differentiating between a specific cancerous environment and a physiological environment. The ability to recognize tumor-specific conditions and deliver the therapeutic siRNA to targeted tumor site is essential for an ideal LNP system in gene cancer therapy. In general, such LNPs consists of a pH-responsive lipid to identify the tumor-specific microenvironment, and a polyethylene glycol (PEG) lipid for stealth property with inhibited uptake by phagocytic cells from MPS, which encapsulates siRNA and delivers nucleic acid to the target site. So far, these LNPs have insufficient ability to deliver siRNAs to tissues or organs other than the liver.

In this study, we constructed a cancerous pH-responsive LNP to encapsulate siRNA *via* surface modifications allowing the particle to maintain neutrally charged at a physiological pH of 7.4, and switch to a positive charge in response to the acidic pH (6.5) of tumor tissues in its vicinity *via* the protonation of amino group. It was expected that the cationic surface of LNPs would target to anionic surface of cancer cells with high efficiency through the modification of “ethylenediamine-based polycaboxybetaine” molecule.

We designed an ethylenediamine-based polycaboxybetaine lipid for surface modification through conjugating this polycaboxybetaine to 1,2-Distearoyl-sn-glycero-3-phosphorylethanolamine (DSPE)-based lipid. LNPs grafted with this DSPE- ethylenediamine-based polycaboxybetaine possessed an average particle diameter of 130 nm, neutrally charged zeta potential of 0-5 mV at pH 7.4 and positively charged potential of >15 mV at pH 6.5,which was suitable for penetrating into tumor through enhanced permeability and retention (EPR) effect and avoiding non-specific protein adsorption from MPS. It was expected to improve the current limitation of siRNA encapsulated LNP, especially in tumor targeting.

Our research showed that ethylenediamine-based polycaboxybetaine modified LNP successfully enhanced cellular uptake, endosomal escape and *in vitro* gene silencing activity when cells were incubated with LNP at pH 6.5, which resulted from the switch to positively charged surface owing to the protonation of amino group of zwitterionic polycaboxybetaine moiety in response to tumoral acidic pH. Besides, the pH-dependent protonation within the endosomal lumen might further lead to osmotic swelling of endosome and subsequent membrane disruption which improve the effective siRNA release.

We demonstrated the specific tumor accumulation behavior and extended blood circulation through administration of this zwitterionic polycaboxybetaine-modified LNP in subcutaneous CT26 and SKOV3-luc tumor bearing models. Moreover, *in vivo* gene knockdown of both mRNA and protein level indicated the siRNA delivery ability even in protein enriched blood fluids. Furthermore, pH-responsive property and slow clearance of polycaboxybetaine-modified LNP led to tumor growth inhibition with periodic intravenous administration.

The ethylenediamine-based polycaboxybetaine zwitterion was used as a “smart shell” of LNPs, which expressed the ability to switch to positive charge in response to tumoral acidic pH. Thus, this polyzwitterion-modified LNP could seamlessly circulate throughout the blood stream at physiological pH, while facilitating cellular uptake of siRNA in tumor tissues and transporting required nucleic acids to target tumor site. In addition, this siRNA-LNP system effectively targeted to two distinct cancer cells, which result in tumor growth suppression. In summary, our research demonstrates the potential of pH-responsive ethylenediamine-based polycarboxybetaine as a promising surface modification material for LNPs, which has the ability to improve enhance the efficiency as well as targeted delivery of nanomedicines such as siRNA medicines. Our polycaboxybetaine-modified LNP offers a promising opportunity as a nucleic acid delivery vector in the treatment of cancers and other inflammatory diseases that possess an acidic microenvironment.