審 査 の 結 果 の 要 旨

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Advances in development of nanomedicines in recent years have highlighted the researches on multidrug delivery system. Among them, polymeric micelles composed of self-assembled core-shell structure have attracted enormous attention not only from a fundamental viewpoint as formulation of drug delivery, but also because they can encapsulate molecules of various properties in the core structure for targeting delivery. It is expected to be applied for cancer treatment. Particularly micelles cooperatively encapsulate both cytotoxicity agent and cancer stem cell (CSCs) inhibitor. Based on these features, this study explored the strategy of encompassing STS into the core of Epi-loaded polymeric micelles (Epi/m), for intracellular synchronization of therapeutic effect. This polymeric micelle system was optimized to maximize loading ratio aimed for synergistic activity, release rate and safety. The ability of STS/Epi/m to treat both naïve and Epi-pretreated orthotopic breast tumors were studied, and the antitumor effect of STS/Epi/m on recalcitrant tumors of pancreatic and kidney tumor were evaluated. The outline of this paper will be described below for each chapter.

In the First Chapter, I outlined cancer development and treatment, as well as the significance of eradicating CSCs for eliciting prolonged survival. Thus, the development of STS/EPI/m was explained, particularly regarding the advantages for co-delivering both conventional anticancer agents and CSC-inhibitors in a single platform. The purpose of this research and the importance in related fields is presented.

In Chapter 2, a series of dual drug co-loaded micelles were prepared in methanol and characterized as candidate micelles for further study. Among these micelles, STS and EPI co-loaded micelles presented excellent profile with high loading efficiency compared with other two-drug loaded micelles.

In Chapter 3, STS/EPI/m were prepared by mixing STS with PEG-*b*-oly(aspartate-hydrazide-epirubicin) copolymer in methanol and N,N-dimethylformamide for optimization to overcome batch to batch variability of micelles. To explore the suitable drug ratio in EPI and STS co-encapsulating micelles, various initial drug ratios of EPI to STS were used for micelles preparation and the maximum drug ratio of EPI to STS inside the micelles is around 1:1. The diameter of the micelles increased with the addition of STS from 50 nm without STS to 80 nm for micelles having a 1:1 STS/EPI ratio. For studying the activity of STS/EPI/m in biological studies, micelles with 5 Epi per 1 STS were selected, which show comparable size

to EPI/m, *i.e.* 50-nm. The release profile was aslo explored and the data demonstrated the concomitant released of the drugs from the micelles.

In Chapter 4, the antitumor activity of STS/EPI/m was explored against breast cancer. The *in vitro* and *in vivo* experiment conducted for evaluation antitumor activity of STS/EPI/m against both naïve and EPI-pretreated relapsed breast tumors. The anti-CSCs ability of this micelles was also studied by analyzing the CSCs subpopulation alteration after treatments with STS/EPI/m. The results demonstrated that STS/EPI/m exerted superior antitumor activity against naïve breast tumors, and effectively reduced the growth of the relapsed, extendeding overall survival significantly. This enhancement in the antitumor effected was associated with the ability of the micelles to overcome drug resistance mechanisms.

In Chapter 5, the potential of STS/EPI/m against recalcitrant tumors, that is cancers with less than 5-year of life expectancy, including pancreatic cancer and kidney cancer, was further investigated. Thus, the micelles were challenged in an orthotopic model of kidney cancer made by implantation of RenCa cells in the kidney of Balb-c mice. The STS/Epi/m significantly prolonged mice survival. Moreover, in a model of pancreatic cancer, which is known to pose severe barriers for the accumulation of drug carriers, the STS/EPI/m significantly reduced the tumor growth rate. These results indicate a promising activity profile for STS/EPI/m, and support the development of therapeutic strategies co-delivering chemotherapeutics and anti-CSC agents for improving the responses of intractable cancers.

In Chapter 6, the significance of the findings is explained, particularly from the viewpoint of the extension of this research to other drugs and tumors.

Overall, in this study, a polymeric micelle system capable of cooperative delivery of a cytotoxic agent and a CSCs inhibitor was successfully developed. The design of these micelles to synchronize the release of both drugs inside the cells was important for safely eradicating both differentiated cancer cells and CSCs. Thus, the STS/Epi/m provide a new strategy for developing nanomedicines with synergistic activity. This system represents an important advancement for the field of bioengineering, particularly, for nanomedicines against cancer, indicating the potential of manipulating drug interactions within nanomedicines compartments as an effective modality for designing therapies eliminating both cancer cells and CSC sub-populations in tumors. Thus, the concept could be extrapolated to other anticancer agents showing synergistic activity, leading to unprecedented antitumor effects. The STS/Epi/m system is now being evaluated in the preclinical stages by Kowa, Ltd., and Nanocarrier, Ltd., aiming to proceed to its further clinical development. Thus, if successful, the STS/Epi/m would represent the first-in-its-type to be evaluated in humans. Given the main role of CSC in the malignant tumor phenotypes, it is my expectation that the STS/EPI/m could provide an effective solution to patients with intractable tumors.

According to the reviews, this thesis is eligible for applying a diploma of PhD (engineering).