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題目(和文)	複数の環状RGDペプチドを担持したポリマーコンジュゲート型汎用性腫瘍標的リガンド分子の開発と光増感剤デリバリーへの応用
Title(English)	Development of polymer conjugates having multiple cyclic RGD peptides as versatile ligand molecules to target malignant tumors and its application in delivery of photosensitizers and nucleic acids
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論　文　要　旨（英文）　（800語程度）

Dissertation Summary (approx. 800 words in English)

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（要　旨）
(Summary)

PDT has been receiving greater attention in recent years and has now become an essential field in medical research. But few PSs can be chosen as candidates for clinical application, because most PSs have some drawbacks including hydrophobicity, low photostability, and poor tumor-specificity, which may cause untoward damage to non-cancerous cells and limit the application of PDT. Antibody-PS conjugate was one method to offer the tumor targeting ability to PS. In spite of the outstanding specificity, large size and too strong affinity of antibodies sometimes limit their penetration into deep portions of tumors especially in poorly vascularized tumors, and inhomogeneous antigen expression in a tumor tissue leads to the heterogeneous distribution of the antibodies, overshadowing successful PDT.

In this study, to improve the efficacy of PDT, I develop a cyclic RGD peptide (cRGD) functioned polymer conjugate, targeting tumor-associated vasculature of PS to overcome tumor heterogeneity. The cRGD peptide binds explicitly to $\alpha_v\beta_3$ integrin, which is known to be overexpressed on activated endothelial cells of growing vessels and also on melanoma, glioma, lung, ovarian and breast cancer cells. However, the applicability of the monomeric cRGD peptide has been limited by relatively low affinity compared with an antibody and final rapid washout from the tumor. One strategy for overcoming these limitations is the application of multiple cRGD peptides to improve the affinity to the $\alpha_v\beta_3$ integrin. But the widely used dendrimer was challenging to be functioned by more than 4 cRGD peptides, because of difficulty in synthesis or poor hydrophilicity caused by the massive number of cRGD peptides. Therefore, I designed the linear polymer containing 15 cyclic RGD peptides to achieve the tumor targeting delivery, homogenous intratumoral distribution, tumor-associated vasculature targeting, and enhanced antitumor efficacy.

After ring-opening polymerization of N-carboxy anhydride (NCA), deprotection reaction, side chain conjugation reaction between carboxyl groups of PGlu and amine groups of cRGD, and introduction of PS to the polymer terminus, the PS-polymer conjugates were synthesized. Through the side chain conjugation reaction, the target number of cRGD peptides was conjugated to the azide-PEG-PGlu with narrow molecular weight distribution. The same method was used to synthesize the control material. PS was conjugated to the polymer terminus and purified by HPLC. After these steps, PS-polymer conjugates showed monodisperse molecular weight distribution, proving the stability of

the conjugates.

Cellular uptake of the PS-polymer conjugates was analyzed by IVIS and confocal microscope to investigate the interaction between multiple cRGD and integrin. The 700DX-PEG-cRGD, 700DX-PEG-PGlu-cRGD5, and 700DX-PEG-PGlu-cRGD15 revealed significantly high cellular uptake depending on the number of cRGD peptides, while no significant difference could be obtained between free 700DX and 700DX-PEG-PGlu-cRAD15. In addition, PSs were internalized into the cells via an endocytic process. The high cellular uptake efficiency of 700DX-PEG-PGlu-cRGD15 compared 700DX-PEG-cRGD, free 700DX, and 700DX-PEG-PGlu-cRAD15 might be attributed to the high affinity to integrin. The high affinity also enhanced the tumor targeting ability, tumor retention time and antitumor effect. In the study of cellular uptake, I found the PS-polymer conjugates localized in the endosomes, suggesting it had the potential to be used as the agent for PCI. To confirm this hypothesis, cells treated with PS-polymer conjugates were exposed to light. In the cell treated without irradiation, all the PSs were localized in endosomes. After exposure to the light, the PS and lysotracker could not be found in the cell treated with 700DX-PEG-PGlu-cRGD10 and 700DX-PEG-PGlu-cRGD15, suggesting broken of endosomes happened in the cells treated with them. The cells incubated with free 700DX and 700DX-PEG-cRGD5 showed no differences before and after irradiation. By this ability, PS-polymer conjugates could enhance the transfection efficacy heavily, or reduce the toxicity of PEI/siRNA complex by decreasing the necessary amount.

Overall, I developed the multiple cRGD-functionalized polymers with a high affinity to tumor cells overexpressing $\alpha_v\beta_3$ integrin. After conjugated them with PS, I found PS-polymer conjugates exhibited enhanced cellular uptake, tumor target ability and prolonged tumor retention. Beside the PDT application, the PS-polymer conjugates also had potential in PCI application.

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