論　文　の　内　容　の　要　旨

A Study on the Transfection with Polyplexes against In Vitro Multicellular Tumor Spheroid Model and In Vivo Solid Tumor

（高分子複合体を用いた in vitro 多細胞癌スフェロイドモデル及び in vivo 固形腫瘍に対する遺伝子導入に関する研究）

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A variety of non-viral polymeric gene vectors have received much attention in the past decade (1) for the delivery of genetic materials to the targeted cells in an effective and safe manner. Especially, the polyplexes formed by the electrostatic interaction between plasmid DNA (pDNA) and polycations have been designed to condense pDNA, protect pDNA from rapid nucleolytic degradation, and facilitate its cellular uptake in order to achieve effective gene delivery. One of the advantages of polyplex systems is the possibility of various structural modifications to improve the stability and transfection efficiency of the polyplexes. Among such modifications, PEGylation [modification with poly(ethylene glycol)(PEG)] of polycations is a promising way to realize systemic gene delivery due to the improved stability of polyplexes in biological media (2). A typical PEGylated polyplex is a core-shell type polyplex (polyplex micelle). Polyplex micelles have been demonstrated to show high colloidal stability under biological media and substantial transfection activity against various cells even after preincubation with serum proteins.

The chemical structures of polycations in block copolymers substantially affect the capability of polyplexes as efficient gene vectors. In this regard, the development of highly transfectable but remarkably low cytotoxic PEG-*block*-polycation copolymers was recently reported (3), which are PEG-*b*-poly(*N*-substituted asparagine) copolymers having the *N*-(2-aminoethyl)-2-aminoethyl group in the side chain (PEG-*b*-P[Asp(DET)]) (Figure). Polyplex micelles from PEG-*b*-P[Asp(DET)] showed efficient and non-toxic transfection which motivated further clarification of the effects of PEGylation and the chemical structures of polyasparagine-based polyplexes on their transfection and cytotoxic behaviors. For this purpose, the comparative study were carried out with two types of polyasparagine-based polycations having a subtle difference in the number of methylene units in the side chain: *N*-(2-aminoethyl)-2-aminoethyl group (P[Asp(DET)]) and *N*-(3-aminopropyl)-3-aminopropyl group (P[Asp(DPT)]). Furthermore, to explore the effect of PEGylation on polyplex behavior, two types of PEG-*b*-cationic polyasparagines, PEG-*b*-P[Asp(DET)] and PEG-*b*-P[Asp(DPT)], were prepared (Figure) for the construction of polyplex micelles. As an evaluation model, multicellular tumor spheroid (MCTS) is focused on this study because it is known to be very useful three-dimensional in vitro tumor model, representing

morphological and functional features of in vivo avascular solid tumors, and because they are characterized by prolonged viable spans with actively proliferating outer cell layers (4).

The protonation behaviors which affect the transfection activities through buffering capacity are evaluated in Chapter 2. P[Asp(DET)] and PEG-*b*-P[Asp(DET)] showed pH sensitive protonational behaviors and were expected to show buffering capacities in endosome to increase their transfection efficiencies. P[Asp(DET)] and P[Asp(DPT)] polyplexes were prepared to elucidate the effect of chemical structures on the transfection. Also, their PEGylated block copolymers, PEG-*b*-P[Asp(DET)] and PEG-*b*-P[Asp(DPT)] formed each polyplex micelle. For the evaluation model, human hepatoma HuH-7 MCTS successfully reproduced heterogeneous tumoral microenvironments and elongated life span, which support its usefulness as an evaluation system.

The transfection activity and cytotoxicity of polyplexes and polyplex micelles were evaluated with MCTS as well as conventional monolayer culture cells in Chapter 3. Worth noting is that the prolonged viable span of MCTS allowed long-term evaluation of more than 10 days of the expression of transfected genes. The high sensitivity of HuH-7 MCTS against polyplex induced cytotoxicity resulted in the destruction of their structures as in the cases of linear/branch polyethylenimine and P[Asp(DPT)] polyplexes. P[Asp(DET)] polyplex and PEG-*b*-P[Asp(DET)] polyplex micelle showed high transfection efficiencies probably induced by their specific protonational behaviors. The different transfection activities between P[Asp(DET)] and P[Asp(DPT)] structure-based polyplexes indicate the significant role of chemical structures in the design of polymeric vectors. PEGylation of polyplexes affected various transfection behaviors of polyplexes. PEGylated polyplex micelles showed lower cytotoxicity at the same N/P ratios to their non-PEGylated polyplexes. Also, they showed delayed transfection which may be related to high stability of polyplex micelles required for effective in vivo use.

In Chapter 4, the possibility of gene delivery for overcoming the transport barriers in heterogeneous tumoral environments was examined with newly developed polyplex systems against HuH-7 in vitro. The heterogeneous environments of solid tumors can slow down the movement of molecules including oxygen and nutrients. Thus, the resultant hypoxia is recognized as a barrier for the delivery of therapeutic materials. PEG-*b*-P[Asp(DET)] polyplex micelles showed higher penetrability towards the quiescent center of MCTS than P[Asp(DET)] polyplexes in HuH-7 cell line. To confirm the result of penetration, the hypoxia-selective plasmid was constructed. The selectivity of hypoxia-inducible expression system was confirmed by the transfection of hypoxic tumor cells in the cores of MCTS. Polyplex micelles showed transgene expression in hypoxic inner region of HuH-7 MCTS. PEG-*b*-P[Asp(DET)] polyplex micelles showed different localization of transfected gene expression from P[Asp(DET)] polyplexes. PEGylation affected the physicochemical properties of polyplexes, which may induce the improved percolation towards heterogeneous cellular environment of solid tumors.

Encouraged at the results of in vitro MCTS, the penetration and transfection were evaluated with P [Asp(DET)] polyplex and PEG-*b*-P[Asp(DET)] polyplex micelle against human pancreatic

adenocarcinoma BxPC3 MCTS and in vivo solid tumor in Chapter 5. The results were well agreed with the penetration and the distribution of transfection observed in MCTS models. PEG-*b*-P[Asp(DET)] polyplex micelle proved its high penetrability and effective transfection in BxPC3 solid tumor.

It is summarized that the well-designed polyplex micelles increase the transfection efficacy and the penetrability, and reduce cytotoxicity, as evaluated through in vitro MCTS model and in vivo solid tumor. Considering the barriers in tumoral environments for the therapeutic transports, the designed PEGylated polyplex in this study is expected to achieve successful treatment based on its low cytotoxicity, durable transfection as well as improved penetrability**.**

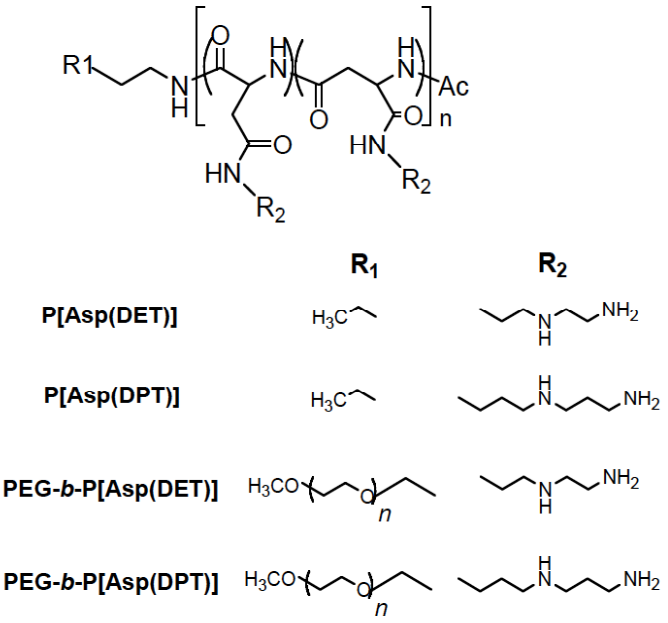

Figure. Chemical structures of polycations

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