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論　文　内　容　要　旨

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学位論文題目	A trial for the construction of a novel drug-delivery system against HER2-positive cancer cells using anti-HER2 recombinant antibody (抗HER2組換え抗体を用いたHER2陽性ガン細胞に対する新規薬剤送達システムの作製の試み)		

内容要旨

【Background & Object】

In our laboratory, various functional proteins derived from bacteria have been used for preparation of the tools applicable for drug-delivery system (DDS). For example, application of sortase A (SrtA), a transpeptidase produced by Gram-positive bacteria, has been investigated to develop novel DDS composed of drug-carriers labeled by various targeting modules on their surface. Recombinant fragment antibodies toward cancer cells produced both from bacterial expression system (scFv) and from mammal cell expression system (Fab) are considered to be potential targeting modules of DDS for cancer-therapeutics. In the present study, to obtain appropriate targeting modules for DDS (DDS-TM) applicable in SrtA transpeptidation, recombinant scFvs and Fab against human epidermal growth factor receptor 2 (HER2) were prepared with SrtA-recognition motif (LPETGG) at their C-terminal. In addition, the liposomes intended to be used as DDS carrier were prepared with SrtA-acceptor motif (penta-glycine) and their characteristics were investigated. And, the development of novel DDS against HER2-positive cancer cells by SrtA transpeptidation of these SrtA substrates was studied.

【Materials & Methods】

As first, both recombinant scFvs and Fab derived from Trastuzumab, a humanized monoclonal antibody against HER2, were prepared in this study. Briefly, the scFvs were designed to possess SrtA recognition motif and His-tag at their C-terminal, which were then prepared using *Escherichia coli* expression system. In addition, Fab against HER2 was also prepared using mammalian expression system in CHO cell. Recombinant scFvs and Fab were purified by Ni-affinity and Protein L-affinity chromatography, respectively. The specificity of the recognition against HER2 was evaluated by indirect-immunofluorescence staining on HER2-positive cancer cell lines.

Subsequently, liposomes containing lipopeptide with SrtA-acceptor motif were prepared by standard thin film hydration method and purified by gel filtration. The lipopeptide was incorporated into the liposomes by two methods: a two-step preparation method that have been used from our previous studies, and a simplified one-step preparation method. Hydrodynamic size of the prepared liposomes was measured by the dynamic light scattering, content of lipopeptide incorporated in liposomes was determined by *o*-phthalaldehyde (OPA) protein/peptide assay, and integrity of liposomes was also evaluated.

In order to investigate the transfer of targeting module on liposomes by the transpeptidation activity of SrtA, a model targeting-module derived from mutant green fluorescent protein (mGFP) with LPETGG motif on their C-terminal was adopted. Furthermore, surface labeling of liposome with Fab was also conducted and the labeling result was confirmed by the fluorescent intensity measurement and immunoblotting. The specific delivery of the Trastuzumab-derived Fab-labeled liposome to the HER2-positive cancer cells was observed using microscopy.

【 Results & Discussion】

From the result of the preparation of recombinant antibodies, lower yields of scFvs were observed because of the formation of inclusion body in *E. coli*. On the other hand, Fab produced from CHO cell was successfully prepared with both high yield and purity, which also showed higher stability. Among these recombinant antibodies, Fab showed superior reactivity toward the HER2-positive cancer cells. Therefore, a recombinant antibody derived from Trastuzumab, Fab with SrtA-recognition motif produced from CHO cell expression system was considered to be a promising DDS-TM against HER2-positive cancer cells.

Judging from the result of dynamic light scattering, the average size of lipopeptide-incorporated liposomes was larger than that of liposomes without lipopeptide. This result indicates that lipopeptide-incorporated liposomes were successfully prepared and the SrtA acceptor-sequence (penta-glycine) was equipped onto liposomes. The prepared lipopeptide-incorporated liposomes showed enough integrity at physiological pH condition for 4 days more. Between the two methods for preparing lipopeptide-incorporated liposome, one-step preparation method was finally adopted for further study because the liposomes prepared by this method were labeled with higher amount of a model ligand (mGFP) by SrtA transpeptidation. Furthermore, as the results of fluorescent intensity measurement and immunoblotting, lipopeptide-incorporated liposomes were successfully labeled with Trastuzumab -derived Fab, and this Fab-labeled liposome showed enhanced delivery of fluorescent dye (uranine)-containing liposome to HER2-positive cells. Thus, it is expected that the uranine delivered to HER2-positive cancer cells can get into the target cells by some cellular function such as endocytosis. These results demonstrate that a model DDS, which is composed of recombinant Fab against cancer cells as the DDS-TM and SrtA acceptor-peptide-incorporated liposomes as the DDS-carrier prepared by SrtA transpeptidation, possesses the potential to function as the DDS against target cancer cells.

【 Conclusion】

Trastuzumab-derived Fab-labeled liposomes were successfully prepared using the SrtA transpeptidation in this study for a novel model DDS against HER2-positive cancer cells. It is considered that this concept for preparing DDS by SrtA transpeptidation has potential to create diverse DDSs against different target cells in an easier way and the product DDSs are applicable in cancer therapeutics against variety of cancer cells including HER2-positive cancers.

【 Keywords】

single-chain fragment variable (scFv), fragment antigen binding (Fab), sortase A, drug-delivery system (DDS), cancer therapy.