学位論文要旨

Interaction mechanisms of small unilamellar vesicle and monoclonal antibody targeting to oxidized LDL receptor protein LOX-1

（酸化 LDL 受容体タンパク質 LOX-1 を標的にする小型単層ベシクル
およびモノクローナル抗体の相互作用メカニズム）

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Lectin-like oxidized low density lipoprotein (OxLDL) receptor-1 (LOX-1) is a major OxLDL receptor membrane glycoprotein, and is associated with atherogenesis and malignant tumorigenesis. Cellular uptake of OxLDL via LOX-1 promotes above diseases by activating its signaling pathways. An accumulation of many previous studies has demonstrated that LOX-1 is a promising drug target for the treatment and prevention of those diseases, however, there are no LOX-1 selective inhibitors or drug delivery system (DDS) carriers that can be used clinically. In this doctoral dissertation, two different types of artificial ligands specifically targeting to LOX-1 were focused on, and their interaction mechanisms with LOX-1 were studied. One is anionic liposome (small unilamellar vesicle), and the other is monoclonal antibody (mAb). These are promising agents for LOX-1 targeting therapy.

In chapter II, the feasibility of nano-liposomes as LOX-1 selective DDS carrier was studied based on a pharmaceutical science approach. Small unilamellar vesicles (SUVs) composed of negatively charged phospholipid DOPG (DOPG-SUV) or neutrally charged phospholipid DOPC (DOPC-SUV) were prepared in different sizes (70 or 124 nm in diameter). And their affinities toward LOX-1 clusters immobilized on self-assembled monolayer (SAM) sensor chips were evaluated by using surface plasmon resonance (SPR) measurements. As a result, anionic DOPG-SUV sized with 30 nm pore diameter filter (DOPG-SUV30) showed about 20 times stronger affinity to LOX-1 clusters than acetylated LDL (AcLDL; a chemically stable surrogate reference substance equivalent to OxLDL). DOPG-SUV30 had a negative surface charge comparable to AcLDL (-42 mV), and its size (70 nm) was about twice of AcLDL. This affinity enhancement was due to the multivalent interaction of more LOX-1s with DOPG-SUV30 particle than with AcLDL. Furthermore, a competitive cellular uptake assay using LOX-1 expressing cells also demonstrated that DOPG-SUV30 was internalized more preferentially into living cells than AcLDL, even in the presence of a 150 fold excess amount of AcLDL. DOPG-SUV100 (-42 mV, 124 nm) showed almost the same LOX-1 affinity as that of DOPG-SUV30. These results indicated that the affinity with

LOX-1 clusters was not further enhanced even if the particle size exceeds 70 nm. This was probably due to the fact that the more bulky DOPG-SUV100 had a larger size exclusion effect to unbound particles on LOX-1 clusters plane. It means that there is an optimum particle size (70 nm) in ligand binding onto LOX-1 clusters surface. In case of DOPC-SUV30 (-8.4 mV, 68 nm), the affinity was lost due to the neutral surface charge. As for the safety, DOPG-SUV30 did not show a cell teratogenicity and VCAM-1 induction, which is a biomarker of cellular dysfunction triggered by LOX-1 signaling, indicating a low risk of safety for *in vivo* use. Taken together, this study demonstrated that DOPG-SUV30 can function as a DDS carrier targeting to LOX-1 expressing pathological sites of atherosclerosis and cancer at least for *in vitro* use.

In chapter III, a chicken-derived anti-LOX-1 mAb, named as HUC52 was focused on. It binds to human LOX-1 extracellular C-type lectin-like domain (CTLD) and inhibits uptake of OxLDL. It has two cysteine residues (C99 and C107) capable of forming disulfide bond in the third complementarity-determining region of heavy chain (CDR-H3). Since it is rare in human or mouse-derived mAbs, HUC52 may form a special CDR-H3 conformation. Three-dimensional structural model of the Fab domain and its interaction mechanism with CTLD dimer were estimated by using computer simulation techniques. The CDR-H3 shaped a β hairpin like structure by being bridged with a disulfide bond. Docking simulation indicated that HUC52 mask OxLDL binding interface on CTLD. The regions of Y101-Y105 (CDR-H3), S52-Y57 (CDR-H2), and D31 (CDR-H1) of HUC52 heavy chain interacted with their epitopes. In particular, S52, Y101, S103 and Y105 formed hydrogen bonds with CTLD. Twenty-five residues on CTLD dimer were estimated as epitopes. Of these, six residues (S160, Q192, S198, S199, F200 and R248) emerged as key epitopes for the cross-reactivity of HUC52 to LOX-1 orthologs. This simulated Fab-CTLD dimer complex model could explain logically the cross-reactivity of HUC52 to LOX-1 orthologs. In case of lacking of the disulfide, CDR-H3 shaped a random coil structure, and the binding of Y105 on CDR-H3 to CTLD were lost completely. These results suggested that HUC52 need the disulfide in CDR-H3 and involvement of Y105 for the specific and high affinity CTLD binding.

Above the two studies carried out in this doctoral dissertation have revealed that nano-liposome (DOPG-SUV30) and monoclonal antibody (HUC52) each binds specifically to LOX-1 CTLD and inhibits OxLDL uptake by entirely different mechanisms. I believe these findings contribute to the development of LOX-1 selective DDS carriers and biopharmaceuticals that will be used for LOX-1 targeting therapy in the future.