Liposome-based immunity-inducing systems for cancer immunotherapy

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Keywords: Liposome / Cancer immunotherapy / Cross-presentation / Adjuvant / Tumor microenvironment / Cellular immunity

Introduction

Recent advancements in biotechnology and deeper understanding of the molecular basis of immunology have led to novel strategies for treating infectious diseases and cancer. Especially, success of immune checkpoint inhibitors such as ipilimumab and nivolumab in cancer treatment clearly provides scientific and medical evidence underscoring the effectiveness of immunotherapy (Hodi et al., 2010; Topalian et al., 2014). However, it has also been reported that immune checkpoint inhibitors showed therapeutic effects to a part of cancer patients only slightly (Tumeh et al., 2014). In these patients, cancer-specific cytotoxic T lymphocytes (CTLs) that can attack tumor cells directly are rarely observed. Furthermore, the induction of CTLs with specificity for neoantigen, which is derived from mutated tumor cell proteins, is important to achieve therapeutic effects in cancer patients (Hugo et al., 2016; Rizvi et al., 2015; Tumeh et al., 2014). Therefore, cancellation of immunosuppression in tumor microenvironments and adoption of a strategy to activate tumor-specific CTLs are crucially important to improve immunotherapeutic effects and to apply immunotherapy to patients for whom immune checkpoint inhibitors show no therapeutic effects.

Antigen-presenting cells (APCs) such as dendritic cells and macrophages are regarded as a target for immunotherapy because these cells start and activate antigen-specific immune responses (Banchereau and Steinman, 1998; Mellman and Steinman, 2001). Exogenous antigens are taken up by APCs and are degraded in endosome/lysosomes. They are subsequently degraded antigenic peptides bound to major histocompatibility complex (MHC) class II molecules. These antigenic peptide/MHC class II complexes are presented to CD4-positive T cells, which engenders helper T cells-based humoral immune responses. In contrast, endogenous antigenic proteins existing in cytosol of APCs are processed in proteasome and are then

carried onto MHC class I molecules. These antigenic peptide/MHC class I complexes are presented to CD8-positive T cells, which engenders CTL-based cellular immune responses. A part of the exogenous antigen is also carried onto MHC class I molecules via transfer from endosome to cytosol or in early endosomes. This presentation process of exogenous antigen is known as “cross-presentation” (Joffre et al., 2012). Therefore, the delivery of antigen into APCs in the body, the control of intracellular distribution of antigen in these cells for induction of antigen-specific CTLs is crucially important to achieve cancer immunotherapy. In addition, APCs should be activated (matured) and moved to lymph nodes for antigen presentation to T cells. Therefore, various functions are required for effective antigen carriers to deliver antigen to APCs in a specific manner to promote the activation of APCs and to release antigen at suitable sites or intracellular compartments. To date, antigen carriers of various kinds such as polymeric particles, micelles, lipid-based particles, nanogels, organic–inorganic hybrid materials, and carbon nanomaterials have been studied to overcome various barriers for immune induction. Among them, liposomes are regarded as good candidates because of their safety, size controllability, and capability for easy functionalization (Schwendener, 2014). This review describes the design of liposome-based antigen carriers to induce cross-presentation and antigen-specific immune responses. First, the strategies to achieve cross-presentation using liposomes modified with fusogenic proteins, peptide and synthetic polymers or specific receptor-targeting liposomes were discussed. Subsequently, the importance of adjuvant molecules in antigen carriers to activate APCs was described. Finally, recent advancements in the combination strategy of antigen carriers with cancellation system of tumor immunosuppression were introduced.

Design of liposomes as antigen carriers

Cross-presentation

Promotion of cross-presentation is important for the induction of exogenous antigen-specific cellular immune response, which is crucially important to eliminate virus-infected cells or tumor cells. Although precise mechanisms of cross-presentation remain unclear, the subset of dendritic cells, the internalization mechanism and intracellular distribution of antigen strongly affect the efficiency of cross-presentation (Fehres et al., 2014; Gutiérrez-Martínez et al., 2015; Joffre et al., 2012). Transfer of antigen into cytosol (known as “cytosolic pathway”) is regarded as the main pathway of cross-presentation (Joffre et al., 2012). Antigen delivered into cytosol is processed in proteasome and is carried onto MHC class I molecules in endoplasmic reticulum, as endogenous antigens are (Fig. 1). To achieve cross-presentation by “cytosolic pathway”,

cytoplasmic delivery of antigen is crucially important. For this purpose, pH-sensitive liposomes have been widely used because of their pH-responsive content release properties and destabilization ability of endosomal membrane. One strategy for obtaining pH-sensitive liposomes is conjugation of pH-sensitive materials to antigen-loaded liposomes (Fig. 2). Incorporation of viral fusogenic proteins to liposomes is an effective strategy for providing cytoplasmic delivery performance to liposomes. Sendai virus fusogenic protein-incorporated liposomes induced direct fusion with plasma membrane and delivered antigenic protein into cytosol, which led to induction of antigen-specific immune response, cancer immunotherapeutic effect and neutralizing antibody responses against HIV (Kunisawa et al., 2001; Yoshikawa et al., 2006; Sakaue et al., 2003). Influenza-virus-derived fusogenic protein (Hemagglutinin)-loaded liposomes (Virosome) have also been used for the cytoplasmic delivery of antigen (Bungener et al., 2002). Hemagglutinin changes their conformation at acidic pH and exposes hydrophobic residues. These residues are inserted to the target membrane, which induces the adjacence of target membrane and viral membrane and their fusion (Bullough et al., 1994). Virosome efficiently delivered antigenic proteins into cytosol by membrane fusion behavior with endosomes and induced cellular immune responses to eradicate tumor or influenza virus-infected cells (Bungener et al., 2002; Huckriede, et al., 2005).

Learning from these naturally occurring membrane fusogenic proteins, synthetic fusogenic molecules have been studied. Liposomes modified with cell-penetrating peptides such as octaarginine (R8) and fusogenic peptides (such as GALA, KALA) were reported as efficient antigen delivery carriers for the induction of cross-presentation (Nakamura et al., 2008 and 2014; Shaheen et al., 2011). Furthermore, arginine derived from R8 acted as a substrate for inducible nitric oxide synthase (iNOS) and produced NO/$ONOO^-$ increased the activity of proteasome, which promoted cross-presentation (Nakamura et al., 2014). Synthetic polymers having pH-responsive membrane disruptive ability were also studied intensively. A typical example of pH-responsive polymer is poly(carboxylic acid). Poly(ethyl acrylic acid) (PAA) showed no interaction with lipid membrane under neutral pH conditions, but membrane solubilization occurred under acidic pH conditions because of mixed micelle formation with lipids and protonated PAA molecules (Murthy et al., 1999). Carboxyl group-introduced poly(glycidol)s were also reported as pH-responsive polymers. Succinylated poly(glycidol)-modified liposomes induced membrane fusion after protonation of their carboxyl groups (Kono et al., 1994 and 1997). Ether group in the main chain of poly(glycidol) might suppress the penetration of polymers into a deep site

of the lipid membrane, which might inhibit lipid solubilization like PAA and might induce membrane fusion. The pH-responsive region of carboxylated poly(glycidol)s can be controlled by changing the spacer groups next to carboxyl groups (Sakaguchi et al., 2008). 3-methyl glutarylated poly(glycidol) (MGluPG) showed high membrane fusion activity at weakly acidic pH corresponding to endosomal pH. MGluPG-modified liposomes delivered model antigenic proteins (ovalbumin, OVA) into cytosol of dendritic cells via membrane fusion with endosomal membrane, which induced cross-presentation of OVA (Yuba et al., 2010 and 2013a). In addition, modification of carboxylated poly(glycidol)s increased the cellular association of liposomes, suggesting that carboxylates on the liposome surface were recognized by scavenger receptors on dendritic cells (Yuba et al., 2008, 2010 and 2013a). Because recognition by scavenger receptors is known to induce cross-presentation (Albert et al., 1998), not only cytoplasmic delivery of antigen but also cellular uptake pathway might contribute to the efficient induction of cross-presentation by MGluPG-modified liposomes. Promotion of cross-presentation by MGluPG-modified liposomes also achieved antigen-specific Th1/Th2 response in chicken or dogs, which decreased the number of *Salmonella Enteritidis* in the caecum of chicken or prevented *Porphyromonas gingivalis* infection in oral cavity of dogs (Watarai et al., 2014; Shimizu et al., 2017). pH-sensitive polymer-lipids having MGluPG analogues in polar head groups were developed for efficient fixation of pH-sensitive polymer onto liposomal membrane (Yuba et al., 2013b). These polymer-lipid-incorporated liposomes also induced cross-presentation not only in murine dendritic cells but also in human monocyte-derived dendritic cells using antigenic long peptides identified from human cancer patients (Hirayama et al., 2016; Sayem et al., 2016).

Another pathway for cross-presentation is known as the “vacuolar pathway” (Joffre et al., 2012). By the vacuolar pathway, antigen localized in early endosome or other mildly acidic compartments directly binds to MHC class I molecules during recycling of MHC class I molecules (Fig. 1). Reportedly, specific receptors-mediated endocytosis such as Fcγ receptor (FcR), C-type lectin receptors (CLR), scavenger receptors (SR), and heat shock protein receptors relate to cross-presentation via the vacuolar pathway (Fehres et al., 2014; Gutiérrez-Martínez et al., 2015; Joffre et al., 2012). In fact, Fcγ receptor-mediated internalization of liposomes or glycan-conjugated liposomes promoted MHC class I-restricted presentation and cellular immune responses (Fehres et al., 2015; Machy et al., 2000). Belizaire and Unanue examined the relation between intracellular distribution of liposomes and cross-presentation (Belizaire and Unanue, 2009). pH-sensitive liposomes composed of dioleoylphosphatidylethanolamine (DOPE)

and cholesteryl hemisuccinate (CHEMS) selectively released their contents in early endosomes of peritoneal macrophages, whereas pH-insensitive liposomes delivered into late endosome/lysosome. DOPE/CHEMS liposomes induced antigen presentation via both MHC class I and II molecules, whereas pH-insensitive liposomes induced only MHC class II-mediated presentation. Cross-presentation by DOPE/CHEMS liposomes inhibited by chloroquine, suggesting that antigen release from these liposomes was suppressed by inhibition of endosomal acidification (Belizaire and Unanue, 2009). These results reflect the importance of intracellular antigen release control for cross-presentation via the vacuolar pathway.

Activation of antigen presenting cells

Incorporation of adjuvant molecules to liposomal antigen delivery system is an effective strategy for activation of APCs and enhancement of immune responses (Fig. 2). Lipid adjuvant monophosphoryl lipid A (MPLA) is a clinically used adjuvant molecule as an additive of HPV vaccine or other liposomal vaccine formulation (Mata-Haro et al., 2007). Introduction of MPLA to liposomes strongly promotes immune responses via Toll like receptor 4 (TLR4) signaling in APCs. Instead of bacteria-derived MPLA, synthetic adjuvant molecules of various types have been studied to improve immunity-inducing effect of liposomal vaccine. A typical example for synthetic adjuvant is a cationic lipid such as 1, 2-dioleoyl-3-trimethylammonium-propane (DOTAP) and DiC14-amidine (Lonez et al., 2012; Watson et al., 2012; Yan et al., 2007). These cationic lipids can activate APCs via interaction with TLR4 or production of reactive oxygen species (ROS) (Lonez et al., 2012; Watson et al., 2012; Yan et al., 2007). Actually, DOTAP-introduced liposomal vaccine induced antigen-specific immune responses for HPV-infected cells or tumor cells (Chen and Huang, 2008; Chen et al., 2008). Introduction of 3, 5-didodecyloxybenzamidine (TRX) to liposomes modified with MGluPG analogues (3-methyl glutarylated hyperbranched poly(glycidol)s, MGlu-HPG) improved their immunity-inducing effects (Yoshizaki et al., 2014). Furthermore, other anionic adjuvant molecules can be introduced to liposome vaccine formulation having cationic lipids via electrostatic interaction. For example, CpG-ODN, which is TLR9 agonist, was introduced to TRX-incorporated MGlu-HPG liposomes. Introduction of multiple adjuvant molecules further activated antigen-specific cellular immunity and induced strong therapeutic effects in tumor-bearing mice (Yoshizaki et al., 2017).

APCs have C-type lectin receptors to recognize specific polysaccharides in pathogens (Figdor et al., 2002). Therefore, the use of polysaccharides and their

derivatives is an effective approach to increase cellular association of liposome and to activate immunocompetent cells simultaneously. Various hydrophobic moiety-introduced polysaccharide derivatives have been synthesized for the surface modification of liposomes as vaccine carriers (Sihorkar and Vyas, 2001). β-glucan and α-mannan are known to activate immune cells via recognition by specific receptors Dectin-1 and Dectin-2, respectively (Kataoka et al., 2002; McGreal et al., 2006; Sukhithasri et al., 2013). pH-responsive group-introduced β-glucan and α-mannan derivatives were newly developed as multifunctional polysaccharides having both immune activation property and pH-sensitivity (Yuba et al., 2017a). It is particularly interesting that introduction of 3-methyl glutaryl ester groups to curdlan or mannan enhanced their adjuvant effects and these polysaccharide-modified liposomes delivered antigenic proteins into cytosol of dendritic cells. Subcutaneous administration of these liposomes to tumor-bearing mice induced strong immunotherapeutic effects compared with dextran derivatives (Yuba et al., 2017a). Selection of polysaccharide and introduction of functional moieties would provide more effective adjuvant and immunity-inducing systems.

Cancellation of immunosuppression

Reportedly, cancer immunity in cancer patients is suppressed strongly by "Cancer immunoediting" (Shankaran et al., 2001). Therefore, cancellation of immunosuppression in tumor microenvironments is important to achieve cancer immunotherapeutic effects (Fig. 2). Myeloid-derived suppressor cells (MDSC) or regulatory T cell (Treg) strongly involve tumor immunosuppression. These cells secrete immunosuppressive cytokines such as IL-10 or TGF-β and engender inactivation of immunocompetent cells and activation of Treg. To overcome immunosuppression in the tumor, liposome-based drug delivery systems of various kinds have been reported. Combination delivery of IL-2 and inhibitor of TGF-β type I receptor using poly(ethylene glycol)-modified liposomes encapsulating nanogel increase CD8-positive T cells and NK cells in tumor and cancer therapeutic effect was also improved strongly (Park et al., 2012). Combination of PEG-modified liposome embedded the inhibitor of TGF-β type I receptor with pH-sensitive dextran-modified liposomes encapsulated antigenic proteins also strongly increased their immunotherapeutic effects by increased infiltration of CD8-positive T cells into tumor tissues (Yuba et al., 2017b). These reports indicate the importance of regulation of TGF-β signaling in tumors for liposome-based immunity-inducing system. TGF-β signaling in tumors can be canceled by delivery of TGF-β siRNA using lipid-based nanoparticles (Xu et al., 2014). Antigenic peptide

delivery using mannose-modified lipid-calcium phosphate nanoparticles containing CpG-ODN suppressed the melanoma growth strongly after cancellation of TGF-β signaling (Xu et al., 2014). Recently, these lipid-calcium phosphate nanoparticles were combined with polymeric micelles containing sunitinib, an inhibitor for tyrosine kinase. These combination systems improved the infiltration of CTL to tumor and decreased MDSC, Treg, tumor-associated fibroblasts and collagen contents in tumor microenvironment, resulting in strong antitumor effect to advanced melanoma models (Huo et al., 2017). Consequently, the combination of antigen delivery system and inhibitor of various signaling pathways is expected to provide effective immunity-inducing systems. Tumor tissues are constructed by complicated immunosuppressive environment composed not only of tumor cells but also of various immunosuppressive cells, fibroblasts and stroma. Deeper understanding of immunosuppressive environment in tumor is necessary. These findings are expected to lead to the design of suitable drug delivery systems (DDSs) for target cells or target molecules.

Conclusion

Here, the recent developments on liposome-based carriers to realize cancer immunotherapy were introduced. Selective uptake by APCs, activation of APCs and promotion of cross-presentation can induce antigen-specific cellular immunity. In addition, combination with a cancelling system of the tumor immunosuppressive environment exhibited strong antitumor effects in tumor models. Improvement of these DDS functions and intentional assembly of these DDS systems are expected to provide novel immunity-inducing systems to achieve highly effective cancer immunotherapy.

Conflict of interest

The authors declare that no competing interest, financial or otherwise, exists in relation to this study.

Acknowledgement

This work was supported by JSPS KAKENHI Grant Number JP15H03024.

Figure Captions

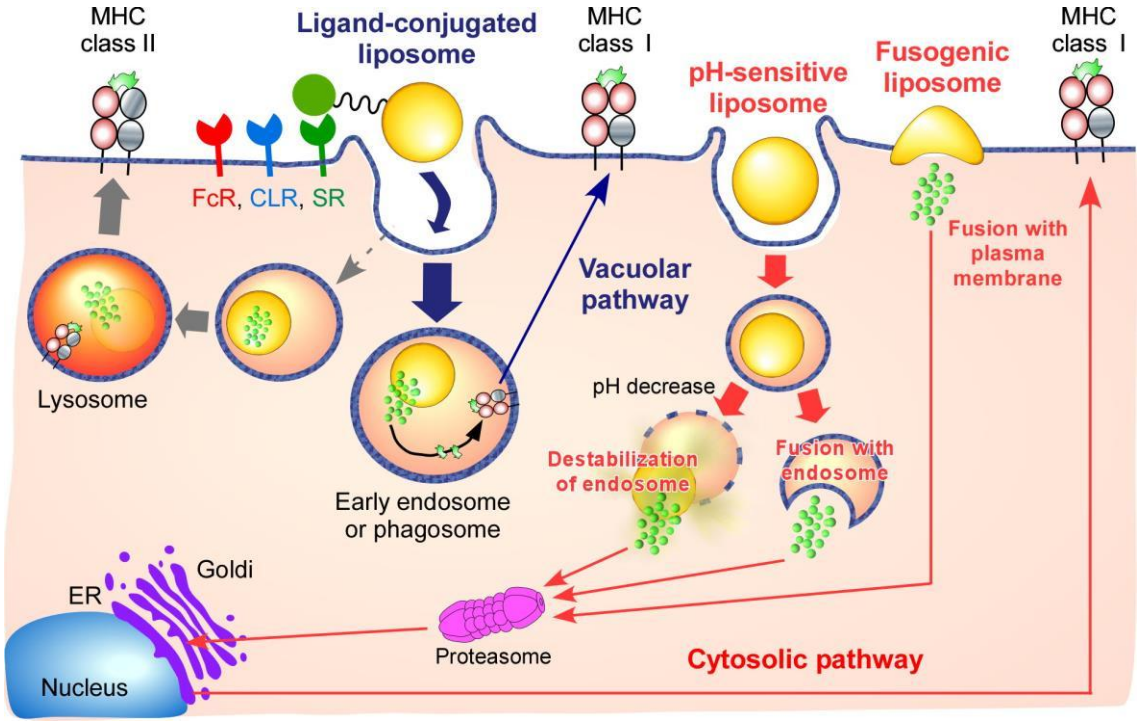

Figure 1. Strategy to promote cross-presentation using liposome-based antigen carriers.

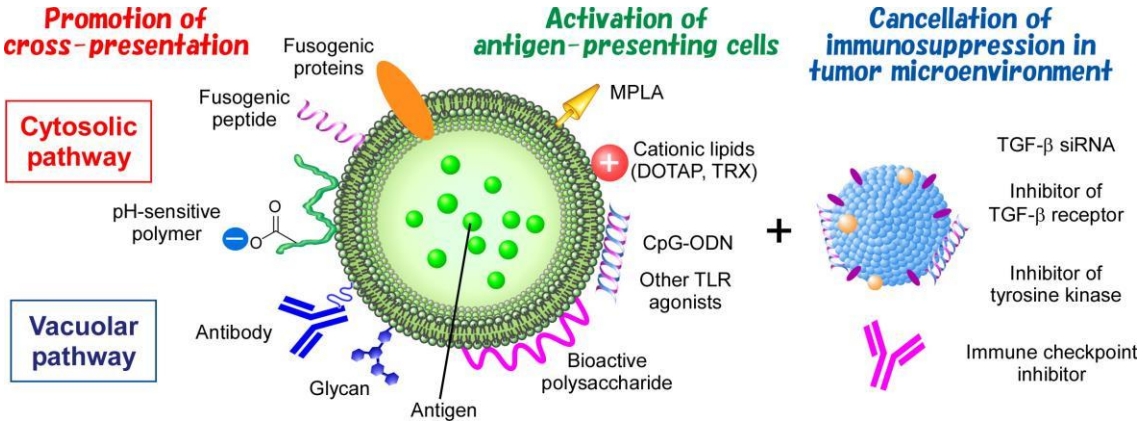

Figure 2. Design of liposome-based antigen carriers for cancer immunotherapy.

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