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Review

Liposome-based immunity-inducing systems for cancer immunotherapy

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ABSTRACT

Cancer immunotherapy has gained much attention for next-generation cancer treatment. To conduct cancer immunotherapy, efficient antigen delivery systems must be able to deliver an antigen selectively to antigen-presenting cells, release it at suitable sites for induction of cross-presentation, and simultaneously induce activation of immunocompetent cells. Liposomes are a candidate for use as such multifunctional antigen delivery carriers because of their capability for easy functionalization. This review describes the rational design of liposome-based antigen delivery systems. Surface modification of liposomes by pH-responsive or fusogenic materials can achieve cytoplasmic delivery of antigen, leading to cross-presentation of exogenous antigen via a "cytosolic pathway." In contrast, targeting surface receptors on antigen presenting cells or the selective release of antigen in early endosome induced "vacuolar pathway"-mediated cross-presentation. Introduction of adjuvant molecules such as Toll like receptor agonists, synthetic cationic lipids or bioactive polysaccharides to liposomes improved their immunity-inducing ability. Combination with cancelling systems of immunosuppression in tumor microenvironment enhanced antitumor immunity of antigen delivery systems. Further understanding of immunity-inducing mechanism and molecular basis of tumor immunosuppressive environments and purposeful design of liposome-based antigen delivery systems can provide effective immunity-inducing systems for cancer immunotherapy.

1. 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 peptide and are 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 migrate 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

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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.

2. Design of liposomes as antigen carriers

2.1. 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, 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, 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, 2013a). In addition,

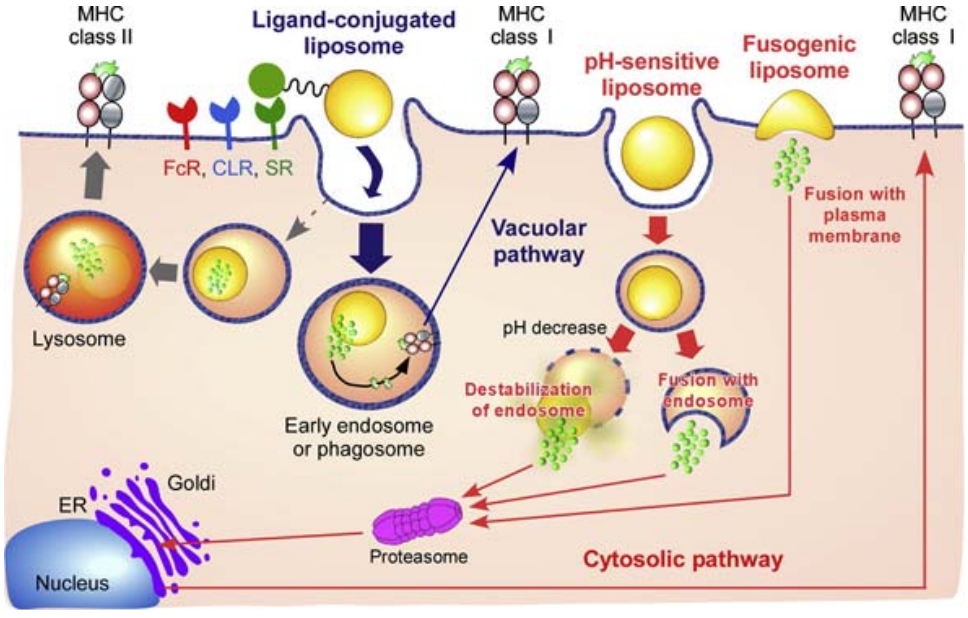

Fig. 1. Strategy to promote cross-presentation using liposome-based antigen carriers.