Roles of Virus-like particles in particularcancer vaccines

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Abstract. Cancer is one of the major threats to modern human health and cancer vaccines have been developed during the last few decades for particular cancers. Due to the lack of viral genetic materials and highly specific recognizable antigens, Virus-like particles (VLPs) successfully substituted chemotherapy to have a therapeutic effect to the tumor cells. VLPs employed different platforms ranging form yeasts, bacteriophage, mammalian cells and insect cells to make variants with different functions. Via APC cross-presentations, VLPs can effectively affect plenty of immune cells and thus triggering anti-viral response. In HCC treatment, HBsAg-derived VLPs and MrNV VLPs show therapeutic effects. For skin cancer, CuMBTT VLPs play a major role in treating in preventing B16F10 melanomas. MS2 VLPs have demonstrated great efficiency in treating Xct-related breast cancer while the GPI- anchored form of VLPs have shown efficacy in the HER2-triggered breast cancer.

Keywords: Virus-like particles; Cancer vaccines; Breast cancer; HCC; Skin cancer.

1. Introduction

Cancers are killing millions of people in human history and still posing a huge challenge to current medical treatment. As stated by the National Cancer Institute of the United States, previously, most vaccines in the global market are designed for the infectious diseases while cancer vaccines are uncommonly mentioned. As stated by the National Cancer Institute of the United States, the cancer vaccines belong to a type of biological response modifiers that stimulate the host immune system to re-establish the functionalized parts to fight against diverse kinds of diseases [1]. There are various types of vaccines which can be chosen at the first time but the requirements of having high immunogenicity and presentation efficiency of tumor antigens limit the choices. Plenty of entities have been employed as tumor antigen delivery platforms while only virus-like particles (VLPs) remain the most reliable tool after several experimental trials.

VLP refers to the particles that can self-assemble due to the protein expressions of viral capsids, cores and envelopess or particles with a single layer prepared from the virus with multiple layers of envelopes. The origin of VLPs render them capacities of resembling or mimicking the structure and symmetry of the prior virus to trigger similar immune recognition and response in the patients while never aggressively influence the host and replicate since they have no viral genome and replicases [2]. This review aims to discuss the basic and development of VLP vaccines and its applications in treating cancers.

2. Design of VLPs

VLPs are expressed viral structual proteins that can self-assemble to form nanoparticles with diameter of 20 nm to 200 nm. Due to a lack of viral genome, the safety of VLPs is ensured as they can only imitate the normal statics of the infectious virus while not capable of replication and thus resulting in infections [3]. There are VLPs using various expression platforms ranging from yeast (Pichia pastoris), mammalian cells (HepG2 cells), and insect cells, bacteria (E.coli) and cell free systems [4, 5].

In addition, plenty of research tend to focus on creating new platforms using bacteriophages and plant-based nanomedicines. Cowpea mosaic virus (CPMV), potato virus X (PVX), tabacco mosaic virus (TMV) and physalis mottole virus (PhMV) have been tested and applied into clinical trials to develop a possible delivery platform that can incorporate the therapeutic cargo such as DNA, RNA

and other chemical agents to effectively influence the target. Different therapeutic aims of VLPs determine which expression system should be employed considering different host environment, costs and their expression levels. The abilities of applying post-tralational modifications should also be taken into account to properly fold, generate and specially modify the targeted proteins. Despite the fact that the most virus may self-assemble to pack its genetic material utilizing a supramolecular process, some virus like red clover necrotic mosaic virus(RCNMV), brome mosaic virus and Hibiscus chlorotic virus are designed to contain the cargo via the reassembly process under conditions ranging from appropriate buffer and ionic conditions. The buffer and ionic strength can control whether the virus need to assemble or disassemble in order to further influence the exchange of contained cargo inside and outside of the virus. What is more, the electrostatic interation between the cargo and the VLP should be considered for the successful package of VLPs by imitating viral environments [6-8].

3. Immune response to VLPs

VLPs can be recognized as exogenous antigens and presented by the antigen presenting cells (APCs) to the host advanced immune system to generate specialized immunoglobulins. Via the major histocompatibility classes (MHC I and MHC II), cross-presentation between B or T lymophocytes to T helper cells (or other APCs such as macrophages) connecting with VLPs occurs to elicit proper cellular and humoral immune responses [9, 10]. In addition, VLPs also have the encapsulation functions to enclose the antigens and act as a delivery particle to present antigens to T lymphocytes and produce immunological reactions. A range of cells like T helper cells, B cells and cytotoxic T cells can be potently activated by dendritic cells-related antigen presentation process with VLPs. In particular, most of the cancer vaccines rely on the cytotoxic T cell response conducting by MHC-1. However, there are remaining challenges for the cancer vaccine development, for example: shortened time of memory response and relatively weaker immunological response when VLPs cannot be consistently applied to the host to stimulate the immune system [11].

As cytotoxic lymphocytes play the most significant significant role in eliciting anti-tumor immunity by APCs' cross presentations, the general cancer vaccine can bind to T cells preventing cancer cells bind to them and initiate tumor growth. During the above process, IL-2, IFN-γ, TNF-α molecules are secreted as the pro-inflammatory cytokines by T helper cells to assist elimination of tumor cells by cytotoxic T cells [12]. The coat proteins expressed by VLPs can normally present multivalent epitors to the B cell receptors which result in stronger stimulation of naïve B cells and long lived humoral immune response. In addition, some of the other VLPs contain PAMPs which is the pathogen-associated molecular pattern of the corresponding viruses in order to boost the innate immune response. Furthermore, VLPs can also create endogenous adjuvant effect especially the one comprised of bacteriophage. The coat proteins from bacteriophage like PP7 can surround ssRNAs that are responsible for the activation of toll-like receptors 7and 8 to elicit innate immune response because the coat proteins are on the surface of VLPs after it's self-assembly [13-15].

4. VLP application in cancer vaccine

4.1. HBV&HCV

Hepatocellular carcinoma (HCC) is one of the most common types of malignant tumor ranked as the third leading cause of cancer-induced mortality. The relative survival rate within 5 years for people with liver cancer is around 25% globally. HBV and HCV, which account for the major etiology of HCC, induce 65% cases of liver cancers in the U.S... Although current HBV preventive vaccines can effectively inhibit hepatocellular carcinomas, low accessibility to vaccines in the undeveloped countries and shortage of eradicative therapeutic vaccine to acute EBV restrictthe elimination of HBV. In addition, the scarcity of widely available HCV preventive vaccine has limited the control and treatments of HCV-induced liver cancer. To efficaciously restrain the prevalence of viral-induced liver cancer, HBV and HCV-specific cancer preventive vaccines are crucial and multifarious types of

therapeutic vaccines are desirable to make personalized treating plans for patients with pre-existing liver cancer.

The first licensed HBV-VLP vaccine could trace back to 1981, Bulmberg discovered a type of Australian antigens, which are now recognized as Hepatitis B surface antigen (HBsAg) in the serum of HBV-infected patient. Later on in 1986, a newly improved recombinant version of HBsAG was generated using the yeast host cells instead of human cells to decrease biosafety considerations. HBsAg monomers could assemble into VLPs by themselves and trigger proper immune response to cause productions of neutralizing antibodies without the risks of getting viral infections as no viral genome was involved in the construction of VLPs. The resulting purified HBsAg-VLPs were shown to have around 22 nm of diameters. However, the vaccination with a single small S antigen of HBsAg had undesired effects of achieving appropriate protection from immune system due to longer responding time from immune system and unsuccessful seroprotections. Recent studies suggest a polyomavirus VP1 can act as a promising epitope carrier to generate chimeric VLPs which display a neutralizing antibody versus HBV surface antigen HBsAg. The polyomavirus-derived pseudotype VLP can employ two types of capsid protein fused with anti-HBsAg molecules to have particular neutralizing capacities especially when harbouring with the Fc-scFv-VP2 molecule. It is recognized as the first example of virus-specific VLP that have viral neutralizing abilities to potentially treat the chronic HBV infections [16]. In addition, macrobrachium rosenbergii nodavirus (MrNV) VLPs were investigated to express an immunodominant region which control the conduction of humoral immune response when having HBV antigenic reactions. Immunization of MrNV VLPs in the murine models triggered higher amount of antibodies than the available HBV-VLP vaccine in the market (Engerix-B) including the ‘a’ determinant-specific antibodies responding to the utilized so-called ‘a’ HBV antigenic determinant. Excluding Engerix-B, other FDA-approved VLP vaccines for HBV also used S antigens as the targeted epitopes ranging from Recombivax HB, Heplisav-B in the yeast system to provide sufficient production of protective antibodies.

Except HBV, HCV is also prevalent worldwide and it currently infects around 200 million of individuals. With regard to HCV, chronic liver diseases are developed in nearly 75% cases when patients get acute HCV infections leading to relatively rarely happened cirrhosis in liver that cause viral-infected hepatocellular carcinoma [17]. Due to the high cost of anti-retroviral therapy, HCV vaccines are required to ease the global burden of HCV infections. In 2007, HBsAg-derived VLPs have been demonstrated to present the heterologous antigenic epitope hypervariable region1 from HCV. The successful vaccination trial in mice with chimeric HBV VLPs displaying HVP1 showed increased production of HVR1-specific antibodies in the immunized mice serum which further illustrate the potential preventive effects of the vaccine [18]. The expected countered influence of immonogenicity between HBsAg molecule and HVR1 peptide was not appeared in the trial showing probabilities of creation of multivalent hepatitis vaccine to decrease the viral load in the future. In the animal models like mouse, baboon and chimpanzee, the immunogenicity of HCV VLPs expressing glycoproteins E1 and E2 were evaluated through the E1 and E2 specific humoral and cell-mediated immune responses. The results showed that the HCV VLPs were capable of generating protecting antibodies to the immunized animals especially in the HCV-susceptible chaimpanzees. Another enveloped pseudotyped VLP with HCV E1 and E2 antigens of different origins also aroused good reactivities of both antigen-specific antibodies in mouse and macaque [19].

4.2. Skin cancer

Severe skin cancer is known as melanoma, an antigenic and immunogenic disease arised from melanin-producing cells-melanocytes. Generally, a vaccine specific to melanoma should aim to expand the repertoire of spontaneous cytotoxic T cells produced at the local site of tumors and through body blood circulation for long-term immune surveillance [20].

To prevent B16F10 melanoma, VLP-based vaccine like recomvinant cucumber-mosaic VLP (CuMV) was employed to fuse with the universal tetanus toxoid (TT) epitope TT830-TT843 which

can act as epitopes for detections of T-helper cells in the interior surface of VLP to prevent interactions with the existing TT-specific antibodies in the body [21].

Molecule AF488 was utilized to label and track CuMVTT in the drainage dynamics studies. The VLP was observed by the accumulations of fluorescent molecules in regional lymph nodes showing effective drainage in short periods. After that, adjuvant micron-sized microcrystalline tyrosine was added with CuMVTTp33-VLP which was the CuMBTT VLP expressing H-2Db restricted p33 peptide from LCMV antigens to increase the VLP-releasing time from 4 days to 9 days while boosted more depot formed when MCT was applied. Importantly, anti-viral cytokines IFN-γ and TNF-α was induced to be released when CuMVTTp33 VLP was applied with MCT. Higher litres of p33-specific T cells were also observed in the immunized mice having B16F10 melanoma containing p33 protein. In the meantime, the tumor progessions were considerably inhibited indicating strong anti-metastasis effect of this VLP.

4.3. Breast cancer

For women between 20 and 50, breast cancer is recognized as one of the most intimidating cancer type due to consistent recurrence and resistance to the classical therapies [22]. Despite the fact that the development of cytotoxic anti-cancer chemotherapy has effectively triggered the reduction in tumor sizes, tumor progression will slow down the therapy efficiency and cause discontinuation of the targeted medicine. (Cancer vaccines: on the threshold of success) Considering drawbacks of conventional vaccine, VLPs were gradually analyzed and invented in different forms as breast cancer therapies because of the capabilities of generating stronger immune response with faster antigen recognition speed and successful mimicry of real virus structures. Principally, the VLP vaccines can stronger immune response with lower risks of getting infections due to a lack of real viral genetic materials compared to other subunit vaccines.

There is a considerable amount of research evidence pointing that aggressive forms of breast cancer actually arise from breast cancer stem cells (BCSC) that can self-renew and differentiate into diverse subtypes of carcinogenic cells for later spreading and metastasis. Cancer chemotherapy and conventional radioactive therapy tend to have lower effects to eradicating tumors due to the continuous relapse of cancer cells with increasing resistance from BCSC. (Cancer stem cells (CSCs) in drug resistance and their therapeutic implications in cancer treatment). BCSCs have strategies to evade attacks from oxidative free-radicles and other chemical molecules from immune systems by employing and expressing cystine-glutamate antiporter protein xCT [23, 24].

In the tumor cells, xCT has variable abilities to bind with different sets of molecules like epidermal growth factor receptor (EGFR) and CD44. Production of antioxidant glutathione will be upregulated if the xCT protein bind with CD44 so that cancer cells are avoided from oxidative stress. Additionally, the xCT can also adjust and influence the glutamine and glucose metabolism to stay away from ferroptosis. By repressing protecting functions of xCT to tumor cells, the reproductivity and functionality of CSCs can be controlled in vitro. In the meantime, pulmonary metastases and tumor invasion will be suppressed in vivo upon application of xCT-specific VLPs. Boli et al. introduced this VLP-related immunotherapy using MS2 VLP(AX09-0M6) having surface antigen loops containing six extracellular xCT domains. Application of AX09-0M6 VLPs showed higher titre of IgG2a antibody against xCT protein. Through observing changes of reactive oxygen species generations and BCSC functionalities in response to AX09-OM6, the binding structures between IgG isolated form AX09-OM6 immunized mice and tumorspheres developed from BCSCs were proved to inhibit xCT activities. Furthermore, in order to examine the inhibitory effect of AX09-0M6 to tumor growth, subcutaneous tail vein injection of HER2+ TUBO-derived into AX09-OM6 treated mice and control normal female mice groups were utilized and the result indicated a considerable inhibition to the formation of pulmonary nodes. Tumor metastases were also tested using mice with an already-formed subcutaneous 4T1 tumor. The AX09-0M6 administration in mice effectively reduce the pulmonary metastasis of the tumor and the experiment successfully proved that the VLP that block xCT function can inhibit tumor metastasis progression [25-30].

In regard to other antigens presented by breast cancer-related VLPs, human epidermal growth factor receptor-2(HER2) can also be seen as a possible therapeutic target. HER2 is a growth-promoting protein that can help control the growth and repair of the breast cells. Recently, Palladini et al. developed a HER2-VLP vaccine containing a multivalent display platform for self-antigens like HER2 protein which is a viable way of overcoming immune cell tolerance particularly the B cells. As plenty studies about VLP vaccines have introduced many of their therapeutic benefits because of the induced antoantibodies by VLP [31], this group created a modular VLP-based antigen-presenting platform using multivalent HER2 extracellular domain (ECD) which utilized a split-protein conjugation system called Spycatcher-Spytag system. The HER2 extracellular domain was genetically fused to the Spycatcher and the VLP subunit based on AP205 bacteriophage was modified with Spytags. After separate expressions and purifications, the ECD was mixed with VLP to ensure a high-density directional coupling of large antigens to the surface of icosahedral VLPs. The multiple presentations of HER2 ECD generated both the functions of helper-T cells and antigen multivalency to induce stronger polyclonal HER2-specific antibody response. Additionally, this VLP vaccine was developed based on the Drosophila S2 insect cells that their paucimannose insect glycan molecules were able to boost the antigen-presenting capacities of APCs and dendritic cells. It is notable that this AP205 VLP vaccine can also effectively inhibit the growth of mammary carcinoma in human HER2 transgenic mice indicating strong immune response against HER2.

In other studies, the HER2 antigen was modified into a glycosylphosphatidylinositol-anchored form to achieve expression of tumor associated antigens for induction of strong antitumor immune response by a quick protein transfer process. The GPI-achored form of VLP vaccinated mice showed positive results of Th2- and Th1-elicited antibody production against HER2 antigen without any chemical and genetic modifications indicating easier development of antigen-specific therapeutic vaccines. On the other hand, the soluble form GPI-HER2 vaccine resulted in the production of weak Th2 response. In another study using Sweet Bac technology, Grabher et al. employed the recombinant baculovirus(rBV) containing genes coding for full-length HER2 and HIV-1 Gag matrix protein for the productions of another type of HER2-displaying VLPs. Spo doptera frugiperda Sf9 insect glycosylated cells expressing HER2 antigen were cultured to generate the chimeric VLPs. In order to assess the efficacy of this HER2-specific VLP, BALB mice were immunized with both insect cell glycosylated HER2-displaying VLP and mammalian-like glycosylated HER2-displaying VLPs with two different types of adjuvants and the HER2-triggered tumor sized was observed during the experiment. Higher litres of HER2-specifc antibodies and stronger effector T cell functions were elicited in mice vaccinated with insect cell glycosylated HER2 VLP in comparison with the mammalian-like glycosylated HER2-displaying VLPs [32-35].

Recent study utilized another virus called Physalis mottle virus (PhMV)-like particles equipped with HER2-derived fragment CH401 epitope by using copper-free click chemistry. When subcutaneous injections of PhMV-VLP were applied, a HER2 specific immune response was elicited in the immunized mice showing higher inhibition rate of tumor and higher survival rate of mice [36]. Other VLP-based breast cancer vaccines could target other molecules in the carinogenesis pathway ranging from insulin-like growth factor-1 receptor (IGF-1R), polymorphic epithelial mucin (MUC1), and interleukin-33 and small interferin RNA (siRNA). IGF-1R, which is a TAA overexpressed in 50% of breast cancer cases, plays a significant role in development and metastasis of tumor [37]. Salazar-Gonzalez et al. developed a VLP vaccine based on viral protein VP2 in human parvovirus B19V, IGF-1R epitopes containing a B-cell epitope and a T-cell epitope in different combinations like VP2-P8(B-cell epitope), VP2-249(T-cell epitope) and VLP that were self-assemblyed with both VP2-P8 and VP2-249. These 3 chimeric types of VLPs conducted sufficient cellular and humoral immune responses indicating high efficacy of the VLP cancer vaccine. Furthermore, the compacity of delating and inhibiting breast tumor growth were measured in the murine model and its ability of treating breast cancer was then confirmed [38].

5. Conclusion

In recent years, the potential of immunotherapy against cancers has aroused interests of many researchers because the chemotherapy is not appropriate to be used as treatments in some circumstances. VLP, which can self-assemble to efficiently deliver the cargo with low risks of getting viral infections, has been analyzed and tested. Compared with other nanoparticles, VLPs are able to incorporate different polyvalent antigens form different cancer-related proteins in order to induce strong type-specific immunological response. Many preclinical studies have shown that the VLP vaccines wih high safety profiles can also introduce strong immunogenicity to the host, making them exceptional candidates for diverse cancer treatments.

Overall, the selection of tumor antigens and specilised interior and exterior design of VLP particles can substantially influence the efficacy of VLP-based cancer vaccines. With the intention of producing an effective and specific VLP-based vaccine, genetic factors from different individuals should be considered as tumor antigens may be varied from person to person due to mutations [39, 40]. These previous promising results suggested that the VLP-based vaccine coulb be utilized as both preventive and therapeutic cancer vaccines in the future.

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