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A lipid-based LMP2-mRNA vaccine to treat nasopharyngeal carcinoma

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Abstract. This paper focuses on developing a lipid-based vaccine targeting nasopharyngeal carcinoma (NPC), a highly severe and invasive epithelial malignancy associated with the Epstein-Barr virus (EBV). The researchers selected EBV latent membrane protein 2 (LMP2) as the preferred antigen for the vaccine. They synthesized full-length LMP2 using an in vitro transcription method and encapsulated it into cationic liposomes based on (2,3-dioleacyl propyl) trimethylammonium chloride (DOTAP) to create the mRNA vaccine (LPX-mLMP2). Cell assays in the study demonstrated that antigen-presenting cells efficiently took up LPX-mLMP2 and expressed LMP2. This led to the formation of peptide-major histocompatibility complexes (pMHC) for presentation. Moreover, the proliferation of antigen-specific T cells at the tumor site indicated the promising potential for mRNA vaccines in combating virus-induced cancers, such as NPC. The researchers concluded that the newly developed mRNA vaccine encoding the antigen offered advantages in the context of NPC and highlighted the attractiveness of mRNA vaccines as candidates for cancer immunotherapy.

Keywords: LMP2-mRNA, Nasopharyngeal carcinoma, Epstein–Barr virus.

1. Introduction

Nasopharyngeal carcinoma (NPC) is the most frequently occurring cancerous tumor in the head and neck region and is primarily found in East and Southeast Asia. Global statistics from 2020 indicate 133,354 newly diagnosed cases and 80,008 deaths attributed to NPC [1]. The primary treatment approaches for NPC involve radiation therapy and chemotherapy. These treatments have shown promising outcomes for NPC patients in the early and intermediate stages of the disease. However, local recurrence and distant metastasis often lead to treatment failure and reduced survival rates.

The Epstein-Barr virus (EBV) is a commonly found pathogen, and it is estimated that more than 90% of adults worldwide have been infected with EBV [2]. EBV infection is strongly linked to Nasopharyngeal Carcinoma (NPC) and is responsible for most cases. Certain latent EBV antigens possess strong immunogenic properties and can stimulate the activation of cytotoxic T lymphocytes (CTLs) that target specific antigens. These CTLs have become the focal point of immunotherapies

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designed to combat EBV-associated malignancies. Therefore, there is promise in utilizing therapeutic EBV vaccine strategies to treat NPC. Some of these strategies have already undergone clinical trials, such as dendritic cell (DC)-based vaccines [3] and recombinant viral vector-based vaccines [4, 5]. DC-based EBV vaccines have demonstrated effectiveness and safety in individuals who respond to them. However, the widespread implementation of these vaccines may face challenges due to the high cost of creating personalized vaccines for each patient. On the other hand, recombinant viral vector-based vaccines have shown the ability to enhance EBV-specific CD8+ and CD4+ T-cell responses in patients with NPC. Nevertheless, the safety aspect, particularly the potential risk of viral gene integration into the host genome, requires further evaluation. Therefore, developing new vaccines that offer increased immunogenicity and safety levels is essential.

2. Methods

2.1. Material

Set up two Petri dishes of RPMI-1640 and DMEM to form the control group. At the same time, add 10% FBS, 1% penicillin and 1% streptomycin to the two culture dishes. Put it into the cell incubator to maintain the temperature at 37 degrees and control the carbon dioxide to 5% [6].

Vaccine liposome LPS uses thin-film dispersion. Firstly, add equiproportional lipid substance and cholesterol molar solution and 2ml of dichloromethane into a distillation flask, distilled by a rotary evaporator, and then perform hydration and homogenization. Finally, uniform LPs are obtained.

Based on the amino acid sequence provided by the National Center for Biotechnology, mLMP2 was synthesized from the linearized DNA template by in vitro transcription. Pack mRNA into LPs at a 3:1 N/P ratio.

To assess the status of LPs and LPX, the average particle size and zeta potential of LPs were measured using a Malvern laser force analyzer, and all samples were tested two or three times below 25 degrees. Observing the appearance through the perspective of e-sports. Store LPX-mLMPS at 4 degrees to improve stability, and detect leakage and infection efficiency.

Mix a part of LPX-mLMP2 with 20% serum at a volume of 1:1, then let it stand at 37 degrees for 2 hours, and then put it into a centrifuge to control it at 4 degrees. The obtained liquid was removed from the supernatant, and mixed with normal saline, and LPX-mLMPS was dispersed in normal saline as a control.

Another aliquot of LPX-mLMPS was also mixed with serum and incubated at 2, 5, 10, 30 minutes and 1, 2 hours, respectively. The stability of each experimental group was compared.

2.2. Measurement

Assay of uptake cells using mCy5. Dendritic cells from commercial mice were seeded on gel well plates, cultured for 24 hours, and allowed to transfect LPX-mGFP. Finally, flow cytometry was used to collect and study the images.

TC-1 method was used to measure and verify antigen expression. TC-1 cells were seeded on 6-well plates, and cells were collected 24 hours later and washed twice with PBS.

2.3. Experiment

Dilute LPX-DiD with normal saline and inject it into mice. Six hours later, the mice were sacrificed and the individual organs were isolated. Then the IVIS spectrum living image system was used to detect the LPX expression.

Randomly divide the tumor-mouse into four groups. Comparison of saline, mLMP2, LPX-mir, or LPX-mLMPS treatment effects. (Mice were sacrificed five days later and individual organs dissected).

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3. Results

3.1. Storage and Serum Stability of LPX.

The particle sizes and electrokinetic potential in colloidal dispersions, a measure of effective electric charge in nanoparticles [6, 7], of LPX-mLMP2 were shown as remain unchanged in the results. With the use of electrostatic interactions investigating serum proteins, the particle size of LPX-mLMP2 is shown as increased while the zeta potential decreased. On the other hand, an electropherogram is used in the investigation of the stability of LPX-mLMP2. This technique is to separate protein molecules based on their size and charge by applying current through a medium [8]. It is shown that LPX helped in the mRNA serum stability with incubation lasting for 2 minutes, 5 minutes and an hour. In terms of the transfection activity, as shown in the result, it has a 10% drop overall, due to the degradation of mRNA and the serum protein absorption, leading to a decrease in uptake inside cells. To conclude, LPs show a mRNA protective effect in vivo.

3.2. LPX Uptake in Cells

Using mCy5, the absorption of LPX by cells was investigated. The amount of LPX-mCy5 that was taken in increased and peaked at 4 hours. From the mechanistic analysis, caveolin-mediated endocytosis, a clathrin-mediated endocytic process involving the caveolae (bulb-shaped plasma membrane with a length of 50-60 nm) [9] and clathrin-mediated endocytosis, as shown in figure 1, the most common endocytic event in mammals [10] which plays a significant role in vesicular transport [9], were implicated in the cellular uptake of LPX-mCy5, since filipin and chloropromazine could considerably impede this process at the significance level at which the p-values equal to 0.05 and 0.001.

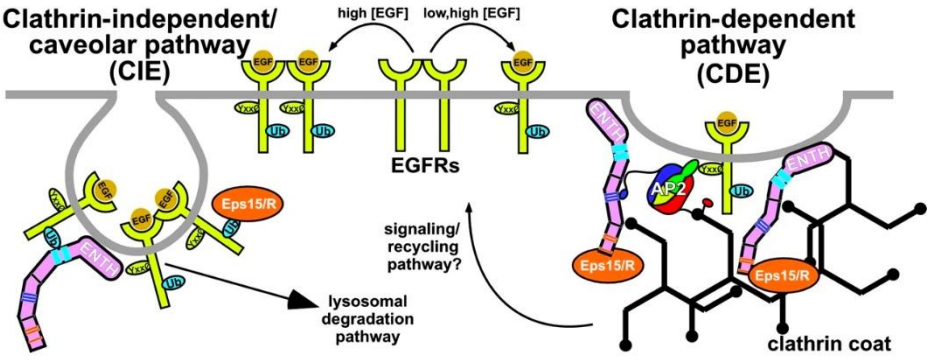

Figure 1. Diagram showing the different endocytosis mentioned in the text above [11]

3.3. Displaying and Presenting the Antigens

The transfection rates of blank LPs and mLMP2 were compared to those of the control. LPX-mLMP2 and Lipo-mLMP2 demonstrated transfection efficiencies that were greater than those of naked mLMP2According to this study, it was concluded that cationic carriers could enhance mLMP2 expression. However, lipo is more toxic than LPs and cannot be used in vivo. As naked mRNA is susceptible to destruction in the presence of nucleases, it is found that the production of the antigen is a critical stage in the immunization process.

3.4. Biodistribution and the in Vivo Translation of LPX.

It was prepared by injecting LPX- mLMP2/DID intravenously, through the vein, into mice to evaluate its biodistribution. The tissues were separated and examined after 6 hours. LPX-mLMP2/DiD was collected in some organs, including the liver, spleen and lungs. This could be because the nanoparticles' passive targeting abilities made them simply targets for phagocytosis, in which cells and particles are ingested and engulfed by phagocytes [12], by the mononuclear macrophage system. By using bioluminescence imaging, LPX-mLUC was used to examine translation efficiency in vivo.

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Bioluminescence imaging has been developed over the last decade for molecular imaging of small laboratory animals, enabling the study of biological processes that are ongoing in long-lived animals [13]. This is comparable to the cationic lipids, which are the most commonly used for the transfection of genes, also known to have less mLUC expression in the liver than in the lung and spleen [14], DOTMA and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE). The spleen is crucial for immunological control as it has the largest number of DCs. Therefore, DCs expressed in spleens were conducive to provoking an adaptive immune response in vivo.

4. Discussion

The blood samples were taken five days after administering the third immunization to mice with LPX-mLMP2. liver function test biochemical evaluations. It was concluded from the study that LPX-LMP2 had little hepatotoxicity and nephrotoxicity since all of the groups had values that were similar to those of the control group.

The tissues were separated, weighed, and applied with the hematoxylin and eosin staining method after immunization. Organ indexes showed no significant alterations, showing that the organs had not suffered harm as a result of the immunization. Also, the findings of the H&E staining revealed that the primary organs did not exhibit any evident histopathologic changes. LPX-mLMP2 was shown to be secure in vivo by these studies [6].

However, the cost of this technology is too high as this is a kind of biotechnology, and it always has a high cost in terms of the expensive operating costs in the biotechnology companies, including research, development, and testing that takes years to complete [14].

5. Conclusion

The cationic liposomes with mLMP2 encapsulation may productively express and display LMP2 antigen in vivo. After immunization, the tumor location shows an increase in antigen-specific T lymphocytes, the principal defense against NPC. Therefore, mRNA vaccination is an effective virus-related cancer immunotherapy. However, as the technology is still developing, it may not be generalized in the community within a short period of time.

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