Macromolecular structures based on virus-like particles as carriers for potential vaccines based on stabilized mRNA

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Vaccines based on virus-like particles (VLPs) have been available and in use for almost 40 years. They do not contain viral genetic material and therefore are not infectious. Consequently, they provide a safer alternative to traditional live-attenuated or inactivated vaccine platforms. VLPs are typically composed of one or several structural proteins, allowing them to mimic the wild-type virus and stimulate the immune system. The first such vaccine was developed against hepatitis B virus (HBV) and was approved for use in 1986. It was based on the HBV small surface antigen (sHBsAg) produced in yeast. Since then, numerous studies have been conducted focusing, among other aspects, on the introduction of heterologous epitopes into sHBsAg or on fusion of antigens with those from other pathogens. These approaches aimed to determine whether such modifications of the sHBsAg protein affect its ability to self-assemble into particles or its immunogenicity.

As a new approach caused by SARS-CoV-2 pandemic, vaccines based on messenger RNA (mRNA) have emerged as a novel and attractive platform for antigen presentation. Their application is not limited to the prevention of viral diseases — they can encode bacterial, parasitic, or tumor-associated antigens. Due to the short half-life of mRNA molecules, lipid-based carriers are typically employed to ensure efficient delivery into host cells and to protect the mRNA from degradation. One of the advantages of mRNA-based vaccines is that they can be rapidly adapted to the currently circulating pathogen variants; therefore, mRNA represents one of the most promising vaccine candidates against rapidly mutating RNA viruses, e.g. for influenza virus. The effectiveness of currently used vaccines against the influenza A virus is limited due to the high antigenic variability of the virus, underscoring the need to investigate novel vaccine platforms.

In this doctoral dissertation, I am proposing a bivalent vaccine composed of virus-like particles (based on sHBsAg) and mRNA encoding the influenza A nucleoprotein (NP). The primary objective was to develop an efficient method for producing and purifying recombinant sHBsAg from insect cells. To simplify the purification process compared with the previously described protocols, a Twin-Strep tag was introduced to the gene construct (TS-sHBsAg) to facilitate protein purification by affinity chromatography. Using this strategy, a large amount of highly purified protein was obtained in a single step and subsequently used in downstream experiments. Next, modified TS-sHBsAg variants were constructed by introducing motifs (derived from the nucleic-acid binding domain of HBV capsid protein) designed to enable mRNA binding. Their production and purification were evaluated, followed by an assessment of whether these modifications affected the ability of sHBsAg to self-assemble; all but one variant successfully formed virus-like particles. In addition, efforts were made to identify optimal conditions for VLPs disassembly and subsequent reassembly. However, because these attempts were unsuccessful, this line of research was discontinued.

The next objective was to design a template for vaccine mRNA, which was subsequently used to produce stabilized mRNA encoding the influenza A nucleoprotein (mRNA-NP). This antigen was selected based on previous research demonstrating that the nucleoprotein is highly conserved across influenza A strains and capable of eliciting a robust immune response, making it a promising target for broad-spectrum influenza vaccination. During the course of the research, the ability of the proposed TS-sHBsAg variants to bind mRNA was also evaluated. Following these results, the most promising variants were selected for mice immunization studies.

The final stage of the research described in this dissertation involved mouse immunization with different combinations of VLPs and mRNA (with or without AddaVax adjuvant), followed by analysis of both humoral and cellular immune responses. Both the sHBsAg-based VLPs and the mRNA encoding nucleoprotein proved to be immunogenic. Since packaging of mRNA into the VLPs was unsuccessful, vaccine formulations containing VLPs together with lipid nanoparticles carrying mRNA-NP were used. The most promising candidate comprising both VLPs and mRNA was one of the modified TS-sHBsAg variants combined with lipid nanoparticles containing mRNA-NP.

Taken together, the obtained results confirm that the combination of VLPs and mRNA can serve as a new and attractive vaccine platform. In the course of this work, a new method for the purification of recombinant sHBsAg from insect cells was developed using affinity chromatography and the functionality of the produced proteins was confirmed. Additionally, the constructed mRNA template also proved to be effective. Overall, these results contribute to the search of universal, multi-pathogen vaccine strategies and establish a promising platform for further development of this experimental approach.