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Next generation of mRNA and lncRNAs based-vaccines platforms: Highlighted for HPV in head and neck squamous cell carcinoma, cervical and colorectal cancers and oncogenic viruses HBV/HCV in hepatocellular carcinoma

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Abstract

More than 200 HPV genotypes and several HPV types associated with particular diseases as oral lesions (Hecks disease, oropharyngeal carcinoma, laryngeal papillomas), anogenital warts (Bowenoid papulosis, Buschike–Lowenstein tumor), Epidermodysplasia verruciformis (plane warts, Pityriasis– like plaques, squamous cell carcinomas of sun–exposed skin) have been described. Several advances in the genomics era and the development of new technological tools for next-generation sequencing have been showed that approximately 98% of RNAs are not translated into proteins. This study aims to succinctly describe long non-coding RNAs (lncRNAs) and their correlation with the hallmark of cancers since they comprise a class of RNAs that do not code for proteins. Oncogenic mutations occur with higher prevalence in non-coding regions, and the correlation of lncRNAs is extremely important for better understanding the biology of cancer cells as well as elucidating the molecular mechanisms underlying cancer. Thus, these may be promising tumor biomarkers and potential therapeutic targets exemplified in hepatocellular carcinoma, cervical and colorectal cancer induced by oncogenic viruses. Future studies are need to delve deeper into the cellular signaling pathways involved in biological processes. This theme addresses innovative issues on the ONU 2030 agenda from the perspective of global one health.

Keywords: mRNA; lncRNA; HPV; HBV; HCV

1. Introduction

More than 200 HPV genotypes and several HPV types associated with particular diseases as oral lesions (Hecks disease, oropharyngeal carcinoma, laryngeal papillomas), anogenital warts (Bowenoid papulosis, Buschike–Lowenstein tumor), Epidermodysplasia verruciformis (plane warts, Pityriasis– like plaques, squamous cell carcinomas of sun–exposed skin) have been described [1].

Papillomaviruses (PVs) have closed circular double-stranded DNA genome (8kb) presenting icosahedral capsid structural late proteins - L1 (major protein self-assembled into VLP) - L2 (structural proteins minor capsid also induced high-titer neutralizing antibodies) [1].

According to the central dogma of molecular biology, RNAs are responsible for mediating the transfer of genetic information from the nucleus (genes) to the cytoplasm through proteins. Several advances in the genomics era and the

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development of new technological tools for next-generation sequencing have revealed that approximately 98% of RNAs are not translated into proteins. An overview of the various classic technological platforms (inactive viruses, attenuated viruses, subunit protein vaccines and virus-like particle - VLP) vaccines; and new generation of vaccine platforms (replicating and non-replicating viral vectors, nucleic acid vaccines – DNA and RNA and antigen-presenting cells) have been described [2].

2. Messenger RNA (mRNA)

Among the numerous RNAs, messenger RNA (mRNA) stands out, as "messenger" molecule that allows genetic information stored in the cell nucleus DNA to be transported to the ribosomes for protein synthesis. Two scientists – Hungarian biochemist Katalin Karikó and American doctor Drew Weissman – had been beginning research the *in vitro* synthetic mRNA platform in the 1990s, when they worked together at the Pennsylvania University. Recently these researchers were awarded the Nobel Prize in Medicine in 2023 for their innovative discoveries in the development of mRNA vaccines, key role in combating the covid-19 pandemic.

Some biopharmaceutical companies from Pfizer and its partner BioNTech and the Moderna, mRNA-1273 vaccine have successfully used the new technological platform. Significant obstacles in the development of vaccines based on mRNA have been recorded after experimental trials involving inoculated laboratory animals, that showed inflammatory reactions with the production of cytokines in which the immune system recognized the mRNA produced in vitro as an antigen. Thus, with the need to modify the nitrogenous bases of mRNA proven by clinical trials, scientists replaced the nitrogenous base uridine with a similar molecule called pseudouridine. Therefore, the mRNA was absorbed by cells without provoking an unwanted inflammatory response, increasing the production of the target protein of interest.

mRNA vaccines and new mRNA-based medicines are the potential of the future. Some laboratories have been already carried out immunization tests against all types of coronavirus and for the prevention of viral and non-viral infectious diseases. This biotechnological strategy may also be applicable to autoimmune diseases and degenerative pathologies such as Alzheimer's. Biopharmaceutical companies are developing mRNA drugs with applicability for different types of solid tumors, melanoma and HPV, for example. Furthermore, there is the development of mRNA vaccines in phase 2 clinical trials for cancer of different types: (i) melanoma; (ii) colorectal cancer; (iii) head and neck carcinoma; (iv) prostate cancer; (v) lung cancer.

3. Long non-coding RNAs (lncRNAs)

Long non-coding RNAs (lncRNAs) comprise a class of RNAs that do not code for proteins, but are essential in the regulation of diverse cellular processes. Furthermore, they share genetic similarity with mRNAs and are made up of around 200 nucleotides. In this context, they have tissue-specific expression patterns and lower expression levels than mRNAs. These are classified as poorly conserved, measure approximately 100kb in size and correspond to 27% of human genes.

These are transcribed from intergenic, exonic or intronic regions of protein-coding genes by RNA polymerase II. As mechanisms of action, lncRNAs maintain genomic integrity in addition to playing an important role in biological processes such as cell differentiation and development, transcriptional and post-transcriptional regulation, splicing and translational regulation of genes [3].

4. Tumor Biomarkers

Oncogenic mutations occur with higher prevalence in non-coding regions, and the correlation of lncRNAs is extremely important for better understanding the biology of cancer cells as well as elucidating the molecular mechanisms underlying cancer. Currently in medical clinical research, most tumor biomarkers and therapeutic targets are proteins. However, only 1.5 to 2% of the human genome is translated into proteins. Following this trend, the detection of lncRNAs can assist in cell signaling pathways as they are highly tissue specific. These may be promising tumor biomarkers since the expression of lncRNAs in cancer correlates with overall survival, metastasis, stage or tumor grade with regard to prognosis [4].

5. Therapeutic targets and Oncogenic Virus

To date, researchers are investigating the possibility that some circulating lncRNAs have oncosuppressive properties *in vivo* as a preventive method for inducing new cancers. To illustrate, we highlight hepatocarcinomas that can be induced by hepatitis B and C viruses (HBV/HCV), and the papillomavirus (HPV) responsible for cervical cancer and head and neck cancer.

5.1. *Hepatitis B virus* (HBV)

Viral hepatitis is increasingly present in infections diagnosed around the world. Thousands of people are infected by these often silent diseases that only manifest themselves when clinical signs are very advanced. Hepatitis B virus (HBV) is one of the main causative agents of long-term chronic infection [5].

HBV is classified in the *Hepadnaviridae* family divided into two genera: *Orthohepadnavirus* and *Avihepadnavirus.* HBV genotypes have been described in nine types based on a divergence of more than 8% (A – J, type I is a subtype of type C) and based on a divergence of more than 4% genotypes A – D, F and I [1,5].

It is estimated that around 350 million people worldwide are carriers of the hepatitis B virus (HBV) and approximately 2 million are infected individuals in Brazil. Infection produces two types of viral particles: whole genome and spherical particles containing infectious particles (42 nm), as well as non-infectious spherical or filamentous particles (22 nm) and subviral particles composed exclusively of HBsAg. An expression system (as used in papilloma vaccines, the first licensed recombinant vaccine produced from yeast expression) was used for isolation of Hepatitis B surface antigen from human plasma of chronic HBV patients (Heptavax– B, Merck & Co), and was launched in 1981 [1,5].

For the past 30 years, HBsAg has been used as a commercial vaccine against hepatitis B. The best way to prevent HBV between the ages of 18 and 59 is to get the vaccine. There are two types of successful virus-like particle (VLP)-based vaccines involving hepatitis B virus surface antigen (HBsAg) and core antigen (HBcAg) expressed in *Escherichia coli*. Immunogenicity conditions can be tested in mice with alternative routes of administration of the HBV vaccine and trials of new formulations. The development of recombinant vaccines composed exclusively of HBsAg (Engerix–B, SmithKline and Recombivax, Merck & Co) was possible with the advancement of genetic engineering.

Therefore, it is of high importance to monitor surveillance markers (hepatitis B core-related antigen (HBcrAg) and Mac-2 binding protein glycan isomer (M2BPGi) and tumor markers (alpha fetoprotein - AFP), protein induced by absence of vitamin K or antagonist-II; (PIVKA-II), alpha-fetoprotein lens culinaris agglutinin reactive fraction (AFP-L3), Dickkopf-1 (DKK-1) to HBV infection and progression of liver damage as chronic hepatitis, cirrhosis, hepatocellular carcinoma (HCC), even chronic hepatitis recurrent over the years. There may be some risks of complications involved in the natural history stages of the disease [6,7].

Nowadays, mRNA-based vaccine platforms is a novel therapeutic approach for Hepatitis B virus-associated hepatocellular carcinoma (HCC) [9], tumor-associated antigen (TAA) as AFP (alpha-fetoprotein) and GPC3 (glypican 3) associated hepatocellular carcinoma [8].

5.2. *Hepatitis C virus* (HCV)

Hepatitis C virus (HCV) belongs to the order *Nidovirales*, family *Flaviridae*, genus *Hepacivirus*. HCV is an enveloped virus with a single-strand positive polarized RNA genome of approximately 9,400 nucleotides. HCV disease causes end-stage liver disease as well as chronic hepatitis. It is a global public health problem that affects more than 70% of the population and it is estimated that 170 million people suffer from chronic hepatitis lesions. This virus leads to severe fibrosis and cirrhosis, liver failure or hepatocellular carcinoma [1,5,7,9]. Genetic variations particularly within the hypervariable region-1 (HVR-1) and low titers of anti-E2 antibodies or interference of non-neutralizing antibodies with the function of neutralizing antibodies may be associated with non-protection against HCV. These findings suggest the detection of anti-E2 peptide immunoglobulin in patients with hepatocellular carcinoma and/or chronic HVC as potential therapeutic and/or prophylactic vaccines against viral infection since most antiviral therapies fail and the anti-HVC vaccine is currently not available [10].

5.3. *Human papillomavirus* (HPV)

Oncogenic viruses are responsible for the development of tumors such as *human papillomavirus* (HPV) that infect the anogenital epithelium causing cervical, anal and penile cancer. There are viral groups based on their oncogenic activity such as high-risk, low-risk and undetermined risk types. Several biotechnological assays are based on the production of

recombinant products originated by the interaction of a viral genome with the genome of a host cell such as bacteria, yeast, cell cultures and insects. Recently, virus-like particles (VLP) have been used regardless of the viral nature, genome, since some viral particles have the ability to self-assemble. There are some VLP vaccines approved as HPV vaccines [1,10,11].

More than 200 HPV genotypes and several types of HPVs associated with specific diseases, such as oral lesions (Hecks disease, oropharyngeal carcinoma, laryngeal papillomas), anogenital warts (Bowenoid papulosis, Buschike–Lowenstein tumor), Epidermodysplasia verruciformis (flat warts, pityriasis – plaque-like, squamous cell carcinomas of sun-exposed skin) have been described. Vaccination against HPV is the best preventive strategy against cervical cancer, cervical intraepithelial neoplasia and genital warts [1]. Prevalence of high-risk HPVs genotypes and predictors factors as demographic, behavioral and biological variables for cervical cancer in unimmunized Brazilian women without cytological abnormalities and sexually active had been investigated throught of the cross-sectional epidemiology study [10].

Papillomaviruses constitute a family of epitheliotropic and mucosotropic viruses that have a double-stranded circular DNA genome. The expression of genes and the role of proteins involved in DNA damage repair pathways in cell lines such as primary human keratinocytes (PHK) and HPV-positive (SiHa – HPV-16 and HeLa – HPV-18) and HPV-negative (C33A) in human cervical carcinoma cell lines, as well as immortalized keratinocyte cell lines (HaCaT, in tumor control) have been described as possible prognostic markers of cervical cancer [1,10]. Some studies have investigated the ability of the cytokine to inhibit the *in vitro* proliferation of normal and HPV-infected keratinocytes, as well as the expression of the E6 and E7 oncogenes. Cytokines include growth factor (TGF-β), tumor necrosis factor (TNF) and type I interferons (IFN -α and IFN-β), which are produced by epithelial cells. The cytokine TGF-β has proven to be an inducer and inhibitor of the growth of tumor cells not infected by HPV 16 and 18. This effect appears to be associated with the inhibition of the expression of oncogenesis E6 and E7. IFN-α inhibits the transcription of the E6 and E7 genes in HPV-18-infected HeLa cells and also inhibits the expression of the HPV-16 E7 protein. In contrast, few studies have investigated cellular mechanisms and morphological changes in the host cell [10,11].

Experimental studies in mice and non-human primates showed strong immunogenicity and anti-tumor effects demonstrating messenger RNA-HPV therapeutic vaccine as prophylactic vaccine for HPV [8]. Therefore, another platforms DNA-based vaccines, recombinant proteins, nanoparticles, synthetic peptides, viral and non-viral vectors may be novel therapeutic targets and prophylactic HPV vaccination [12] (figure 1).

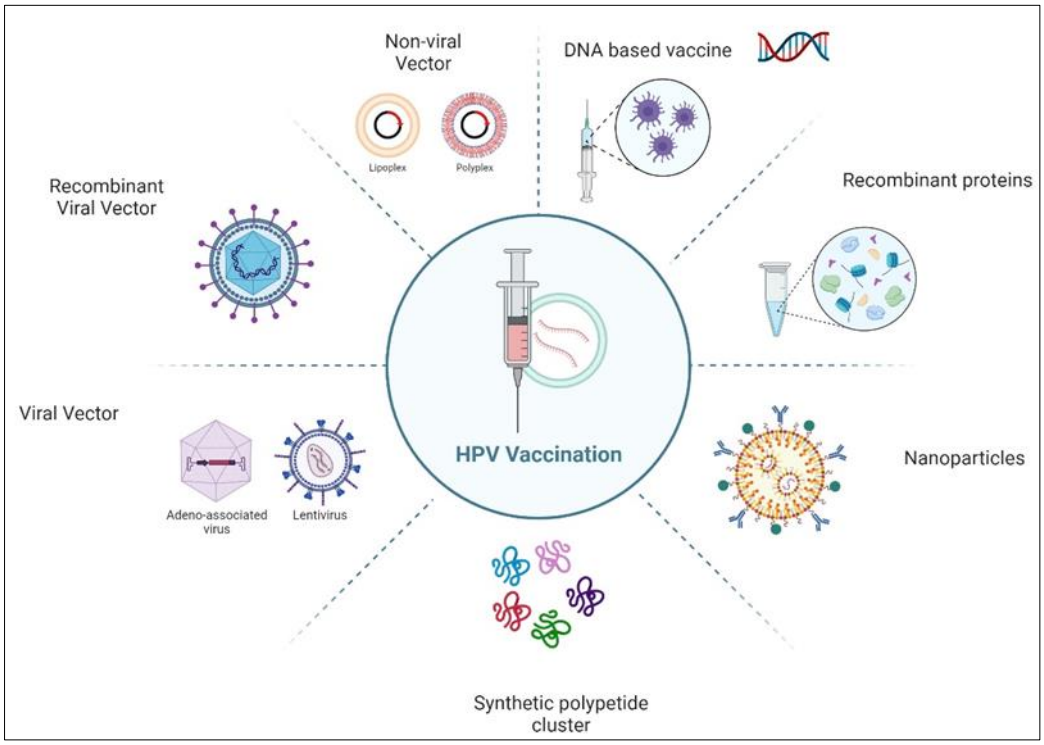

Figure 1 Schematic representation of novel therapeutic targets and prophylactic HPV vaccination

Tumor microenvironment including upregulation of PD-1/PD-L1 immune checkpoint inhibitor (CPI) detected in HPV-antigen expressing TC-1 tumors associated with E7 RNA-LPX immunization mediates complete remission of progressing HPV16-positive tumors and establishes protective CD8+ T cells memory [13].

Chimeric protein derived from the fusion of the HPV-16 E7 oncoprotein and the herpes simplex virus type 1 glycoprotein D (gDE7) mRNA vaccines induced activation of E7-specific memory $CD8^+$ T cells. Self-amplifying or non-replicating mRNA-linked lipid nanoparticle (LNP)-encapsulated was be able to preventing HPV in mice experimental assays using only single dose immunization mRNA vaccine [14] (Figure 2).

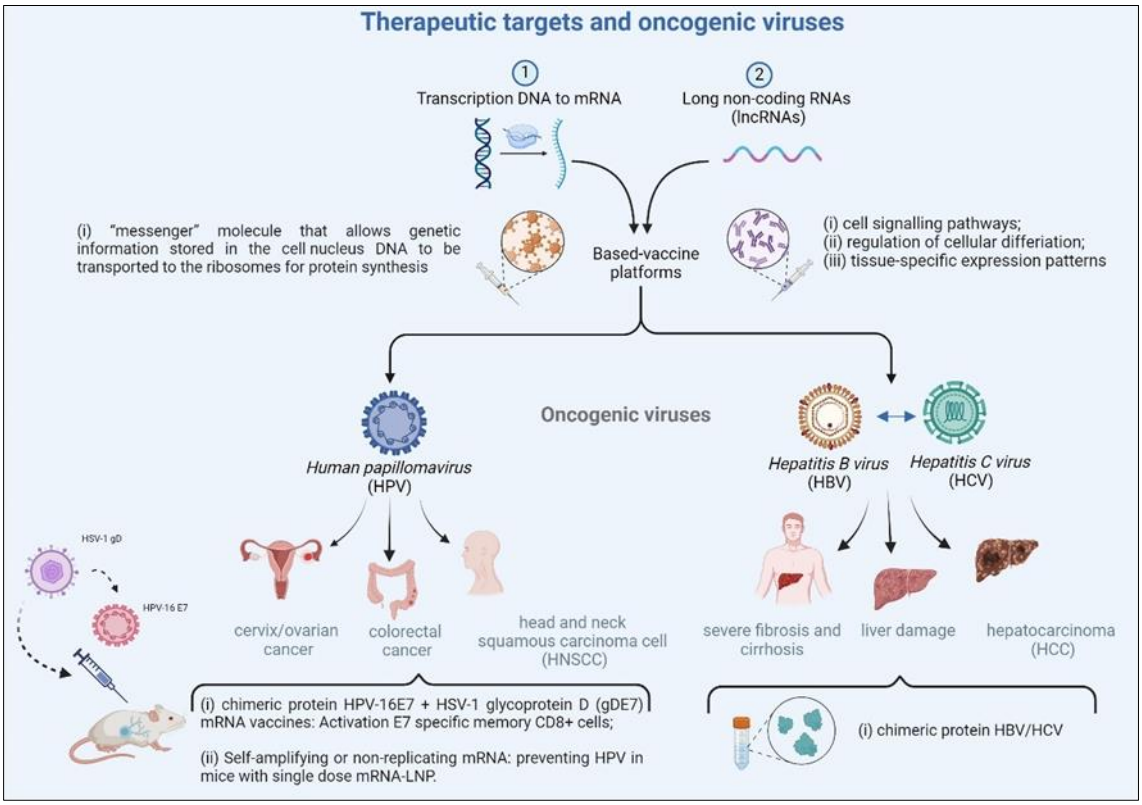

Figure 2 Therapeutic targets and oncogenic viruses: HPV and HBV/HCV

6. Conclusion

Several potential benefits of mRNA and lncRNA-based vaccine may be mitigating risks in HPV related diseases as cancer anus-genital, head and neck squamous cell carcinoma, colorectal tumors and hepatocellular carcinoma. There is a real need for in-depth investigation of cases considered positive with viral load due to viral hepatitis as well as the correlation between papillomavirus and other specific viruses. The improvement of the heterologous antigen vaccine model based on the hepatitis B virus protein containing HCV antigen as a chimeric vaccine developed by the author has generated advances in the area of biomedical engineering. New research on tumor biomarkers must be better investigated to correlate the natural history of oncogenic viruses and mitigate the cases of hepatocellular carcinoma (HCC) and cervical and colorectal cancer in terms of genomic and epidemiological surveillance of solid tumors in clinical trials. Moreover, mRNA and lncRNA-based vaccine and their specific molecular interactions addressed to steps of upstream since cell line thawing, pilot and industrial-scale scale-up until downstream to purification of the substance from chromatograph columns to provide an advance in personalized medicine.

Compliance with ethical standards

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Disclosure of conflict of interest

No potential conflict of interest was reported by the author.

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