Vaccine therapies for cancer: challenges and strategies

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Abstract. Vaccine refers to the biological products that can make the body immune to specific diseases after inoculation and refers to biological products made with various pathogenic microorganisms for preventive vaccination. Vaccine is an effective means of disease prevention, especially for the control of epidemic diseases and infectious diseases play a vital role. Vaccines are mainly used to attenuate and inactivate pathogenic microorganisms, which can reduce their harm while retaining the ability of pathogens to stimulate organisms to produce immune responses. By injecting the vaccine, the organism can produce a specific immune response to the pathogenic microorganism, and the corresponding immune response can be quickly generated when it is exposed to the pathogenic microorganism again. Vaccination is a simple, safe and effective way to train a person's immune system to produce antibodies that can protect people from harmful diseases before they are even exposed to them. Vaccination uses the body's natural defense mechanisms to build resistance to specific infections and boost a person's immune system. In addition to preventing disease, vaccines are increasingly being used to treat cancer. Cancer vaccines, as part of cancer immunotherapy, can recognize specific proteins on cancer cells, stimulate the immune system, kill existing cancer cells and prevent cancer from continuing to develop. This review summarizes the types, clinical applications and development of cancer vaccines, and discusses the advantages and disadvantages of cancer vaccines as well as the opportunities and challenges for their future development.

Keywords: Cancer; vaccine; therapy; challenge.

1. Introduction

Cancer refers to malignant tumors originating in epithelial tissue and traditionally the term "cancer" refers to all malignancies. Cancer has following characteristics such as off-normal cell differentiation and unlimited proliferation, uncontrolled growth, infiltration and metastasis. The occurrence of tumor is a complex multi-factor and multi-step process, which can be divided into carcinogenic, carcinogenic and evolutionary, and smoking, infection, occupational exposure, environmental pollution, unreasonable diet and genetic factors are important reasons for the occurrence of cancers. More and more residents are influenced or even be killed by cancers. According to the figures from the NCC, the overall incidence from 2000 to 2018 had increased. Meanwhile, however, the mortality rate of cancer patients had been reduced. The age-standardized incidence rate (ASIR) for all cancers is increasing by about 1.4% per year and the age-standardized mortality rate (ASMR) is decreasing by about 1.3% per year. The NCC also says there will be 4,824,700 [1] new cancer cases and 2,574,200 new cancer death in China in the year of 2022. Among the males, the incidence of lung cancer tops the list, followed by the colorectal cancer, the liver cancer. Meanwhile, the lung cancer happens most for female cancer patients as well, followed by the breast cancer and the thyroid cancer. For the death rate, the lung cancer causes the most deaths in both male patients and female patients. For male patients, the liver cancer and the stomach cancer cause the 2nd death rate and the 3rd death rate while that for female patients are colorectal cancer and the liver cancer.

Vaccines are biological products made of various pathogenic microorganisms for vaccination. There are two types of vaccines: live and dead. Commonly used live vaccines are BCG vaccine, polio vaccine, measles vaccine, plague vaccine and so on. Commonly used dead vaccines are pertussis vaccine, typhoid vaccine, meningitis vaccine, cholera vaccine and so on. The main principle of

vaccine is to make pathogenic microorganisms, such as bacteria, Rickettsia, viruses and their metabolites, artificially attenuated, inactivated, or genetically modified methods, into automatic immune preparations for the prevention of infectious diseases. The vaccine retains the characteristic of the pathogen stimulating the animal immune system. When the animal is exposed to the pathogen that is not harmful, the immune system will produce certain protective substances, such as active physiological substances and special antibodies. When the animal is exposed to the pathogen again, the animal's immune system will follow its original memory to create more protective substances to prevent the pathogen from harming.

This review introduces the mechanism of cancer vaccines and the disadvantages of each type of cancer vaccine and discusses the strategies of address the problems of developing cancer vaccines.

2. Current types of cancer vaccines

With the continuous development of vaccine technology, the types of vaccines becomes more diverse. Currently, four types of vaccines have been widely recognized for cancer treatment, including DNA vaccines, RNA vaccines, polypeptide vaccines and cellular vaccine. The main difference between these vaccines is the form in which the antigen is presented [2]. The development of the vaccines are introduced as Table 1.

Table 1. Development of cancer vaccines.

Time	Live attenuated	Killed organisms	Purified protiens or polysaccharides
18th century	Small pox (1798)		
19th century	Rabies (1885)	Thyroid (1896) Cholera (1896) Plague (1897)	
Early 20th century	Tuberculosis (1927) Yellow fever (1935)	Pertussis (1955) Inflfuenza (1936) Rickettsia (1938)	Diphtheria toxoid(1923) Tetanus toxoid (1926)
20th century	Polio (oral) (1963) Measles (1963) Mumps (1967) Rubella (1969) Adenovirus (1980) Typhoid (1989) Varicella (1995) Rotavirus rassortants (1999) Cholera (attenuated) (1994) Cold-adapted influenza (1999)	Polio (injected) (1955) Rabies (cell cultured) (1980) Japanese encephalitis (mouse brain) (1992) Tick-bome encephalitis(1981) Hepatitis A (1996) Cholera (WC-rBS) (1991) Meningococcal conjugate (group C) (1999)	Anthrax secreted proteins (1970) Meningococcus polysaccharide (1974) Pneumococcus polysaccharide (1977) Haemophilus influenzae type B polysaccharide (1985) H. influenzae type b conjugate(1987) Typoid(Vi)polysaccharide(1994) Acelllular pertussis(1996) Hepatitis B(plasma derived) (1981)
21st century	Rotavirus (2006) Zoster (2006)	Japanese encephalitis (2009)(vero cell) Cholera (WC only) (2009)	Pneumococcal conjugates (2000) Meningcoccal conjugates (2005) Pneumococcal conjugates (2010)

2.1. DNA vaccines

DNA vaccine and RNA vaccine are both genetic vaccines, also known as nucleic acid vaccines. TAA is a protein that is abnormally expressed by cancer cells and is the target of most cancer vaccines [3]. The vectors encoding TAA can be viruses and plasmids [2]. DNA vaccine was first proposed in 1990. Wolff's team directly injected plasmid DNA into mouse muscles, and found that after injection,

the corresponding protective protein antigen could be expressed in eukaryotic cells, stimulating the body to produce long-term specific humoral and cellular immune responses, and inducing the production of cytotoxic T lymphocytes (CTL) [4]. DNA vaccine consists of antigen coding gene and plasmid vector.

Different from the traditional vaccine mechanism, DNA vaccine directly injects recombinant eukaryotic expression vector encoding a certain protein antigen into the body, so that foreign genes can express the generated antigen in the body and activate the body's immune system, thus inducing specific humoral and cellular immune responses. When the virus comes again, B cells and T cells have a memory immune response to it. It can be eliminated quickly.

DNA vaccines can be used in the treatment of influenza, and DNA vaccines have been approved for use in cancer, mainly for bladder tumors, melanoma [5], glioblastoma, liver cancer and pancreatic cancer. In addition, vaccines for breast and ovarian cancer are also under experimental study [6].

2.2. RNA vaccines

There are many types of RNA vaccines. The common RNA vaccines are messenger RNA (mRNA) vaccines, circular RNA (circRNA) vaccines, and self-amplifying RNA (saRNA) vaccines.

2.2.1. mRNA vaccines

Messenger RNA (mRNA) is a single-stranded ribonucleic acid that is transcribed from a strand of DNA as a template. mRNA carries genetic information that can direct the production of a target protein or immunogen and activate the immune response in the body. mRNA is mainly divided into two types, namely non-replicating mRNA and self-amplifying RNA. Non-replicating mRNA vaccines encode antigens that are required for immunogenic responses, including the 5 'and 3' untranslated region (UTRs) and open reading frame (ORF). Compared to non-replicating mRNAs, self-amplified mRNAs have an additional coding region in the ORF that enables continuous intracellular RNA amplification, thereby amplifying antigen expression [7].

The mechanism of mRNA vaccine is to introduce the mRNA containing the encoding antigen protein into the human body, which can be directly translated, skip the replication and transcription process before translation, and form the corresponding antigen protein, so as to induce the body to produce specific immune response and achieve the role of immunization prevention.

Compared with traditional vaccines, mRNA vaccines have higher development efficiency, convenience and speed. Save time and economic costs; it has the advantages of stimulating adaptive immune response through cytokines and automorphism. Compared with DNA vaccines, which can be expressed in the nucleus, mRNA vaccines are only expressed in the cytoplasm, which can express the target protein efficiently with low rejection. In addition, mRNA vaccines are easier to metabolize, reducing the burden on the host [8]. However, it is also limited by instability, innate immunogenicity and inefficiency of delivery in vivo.

The mRNA vaccine, which is mainly in trials, targets melanoma, prostate, lung, solid and gastrointestinal cancers. These clinical trials have evaluated the clinical efficacy of mRNA vaccines, but the trials have not been fully completed, and no mRNA vaccines against cancer have been approved for marketing [9].

2.2.2. Circular RNA vaccines

Circular RNA is a closed-loop RNA molecule that lacks the 3 'end and 5' end and is divided into natural circRNAs and synthetic circRNAs. It has a unique covalent ring structure that protects it from exonuclease degradation [10]. Circular RNAs are specific to certain cell types as well as developmental and disease stages and have the ability to regulate gene expression. Dysregulation of its expression has also been linked to a variety of diseases, including cancer, making circRNA a potential therapeutic tool [11]. This vaccine is currently used for the treatment and prevention of COVID-19 and refractory melanoma malignancies. It is stable, easy to store, has fewer side effects and can extend the immune response time, which has great potential [12].

circRNA can be used as a tumor marker to aid in tumor prediction and treatment. Despite the breakthrough in circRNA immune checkpoint inhibitor therapy, a circRNA vaccine still has a long way to go [13].

2.3. Polypeptide vaccines

The formation of tumor antigen (TA) and the overexpression of proteins in the process of tumor development, tumor antigen can be degraded into peptides in dendritic cells and constitute the expression product of histocompatibility complex (MHC) human leukocyte antigen (HLA), thereby activating T cells for immune response. The 8-12aa peptide of the TA coding sequence is used to prepare peptide vaccines [14].Currently, tumor antigens used in peptide vaccine development are mainly tumor-associated antigen (TAA) and tumor-specific antigen (TSA). TAA expresses antigens derived from its own proteins in tumor cells, and TSA is associated with antigens resulting from non-synonymous mutations or other genetic alterations [15]. Peptide vaccine has the advantages of safety, good tolerability and good immunogenicity. Although peptide vaccine has made some achievements in clinical practice, its development still faces many challenges.

In clinical practice, the therapeutic effect of peptide vaccine against melanoma, breast cancer, esophageal cancer, lung cancer, leukemia, pancreatic cancer and head and neck squamous cell carcinoma has been tested and achieved phased results. And a peptide vaccine for prostate cancer has been approved for sale in the United States [16].

2.4. Cellular vaccines

Cellular vaccines are usually prepared from cells or components within cells and can be divided into whole tumor cell vaccines and dendritic cell vaccines. Whole tumor cell vaccine is the direct preparation of inactivated tumor cells or tumor cell extracts into vaccines. Dendritic cell vaccines directly introduce tumor-associated antigens into dendritic cells and then inject them back into the patient, where they are presented to induce a specific immune response [17]. These two types of cell vaccines sometimes have poor effects in clinical trials, which can be solved by increasing the immunogenicity of tumor cell vaccines and combining with adjuvants.

3. Current challenges and strategies of cancer vaccine development

3.1. RNA vaccine

Although giant leaps has made in the region of RNA vaccine recently, there are still lots of work to do in order to produce mature RNA vaccines. Three main challenges will be listed in the following statement.

Firstly, when mRNA vaccines induce the cellular immune response, the tumor microenvironment are simultaneously inhibiting their effect. This inhibition can cause severe side effects and is even able to prevent T cells from killing the tumor cells and cause the exhaustion of the T cells. Therefore, the combination of RNA vaccine and the immune checkpoint inhibitors should be one of the main projects for future research. That's the reason why the trial for the combined therapy of the melanoma vaccine BNT 111 and the immune checkpoint inhibitors has become an important project for researchers [18-20].

Secondly, producing RNA vaccines still proves to be a problem for some developing countries. While developed countries have already master how to produce a mass of RNA vaccines, the industrial chains of RNA vaccines are immature in many developing countries. For instance, the development of chromatographic filters, hollow fiber columns and many other high-value instruments takes quite a long period of time and the production of RNA vaccines involves many subjects such as material science, fluid dynamics, which many developing countries has not master many of the key techniques in these areas yet. These prove that the industrial development of RNA vaccine producing ability in many countries still needs lots of improvement. On top of that developing countries is faced with the patent issue of many devices, which slows down the pace for mRNA mass

production. In order to address the problem, developing countries like China has paid lots of attention on developing the alternative original materials for Bsa I, DNase I and many other essential materials [21].

Thirdly, the preservation conditions for RNA vaccines are very severe. Since there are hydroxyl group on the ribose, it is very fragile and can be degraded easily. Therefore, it costs lots of money to preserve these vaccines. For instance, the current Covid-19 vaccine should be preserved under the condition of -80℃, the carrier protectants such as lipid nanoparticles can only be stored for 3 months. Therefore, the development of vaccine delivery system and protective agents should also be developed while focusing on the development of new RNA vaccine so that the vaccines can be preserved for a longer period of time [22].

Fourthly, more clinical trial figures are still needed. It is reported that some mRNA vaccine might cause some rare and fatal thrombosis. The effect of the combined therapy for the RNA vaccine and the chemotherapy still needs long-term trial as well. In addition, researchers have not reach a consensus on the best injection route. Therefore, more trials on different injection approaches remains to be conducted [21, 22].

Meanwhile, many other solutions have been come up recently. Researchers have been focused on the design and optimization for the coding sequence such as modify the nucleotide of methylcytident (m5C), 1-methylpseudouridineand the noncoding region such as improve the 5'- and 3'-UTR elements [17].

3.2. DNA vaccine

The previous researches that DNA vaccine have disadvantages as follows. Limited protein immunogens(the immunogens are not useful for non-protein based antigens such as bacterial polysaccharides),inducing antibody production against DNA, the possibility of inducing immunologic tolerance by antigens expressed inside host body, atypical processing of bacterial and parasite proteins and the possibility of causing cell to become cancerous after inserting foreign DNA into the host genome [18].

To enhance the quality of DNA vaccines, the selection of antigens, vector, delivery route, dose, timing adjuvants are becoming essentially important points for the improvement. The key point while designing the DNA vaccine is to opt for appropriate target antigens. The genes from the pathogen and the form of gene must be chosen for the patients. When the selection of the desired gene is completed, the patient can finally proceed for its modification for the achievement of its immunogenicity of the DNA vaccine.

The expression of the antigen vectors is also an important factor for improving the DNA vaccines. Most DNA vaccine studies use plasmids carrying promoters which can constitutively yield high levels of protein in most mammalian tissues. More changes can also be made to increase the protein production in transfected host cells. To solve these problems, the codon optimization can be an effective method.

What's more, more clinical figures are still needed for further development. The future clinical trials will focus on five main aspects:1)increase the immunogenicity of these vaccines,2)try and assess new antigens and the combination antigens,3)explore the combination approaches with other cancer vaccines or tumor therapies.

3.3. Proteins or synthetic peptides of cancer antigens

Researchers have made distinct achievements on the protein vaccines which can be used for infectious diseases. However, the protein and peptide vaccines have the problems such as many immune activator is needed, many times of vaccinate is needed which would cause more cost. Moreover, the proteins and peptide vaccines that focus on cancers are very little. Only some recombinant protein vaccines have developed around cancer indications have been developed up to now. For example, only three proteins targeting hormone-resistant prostate cancer, esophageal cancer and pancreatic cancer have been developed by Japanese researchers so far. OSE-2101 was one of

very few protein vaccines for treating non-small cell lung cancer rolled out by French scientists [19]. What's more, these very few products are still in the phase III clinical trial and there hasn't been any novel protein or peptide cancer vaccines since 2021. Therefore, the protein or peptide cancer vaccines still need technical breakthroughs and more clinical figures for further research.

4. Conclusions

With the emergence of cancer vaccine, it brings a powerful treatment for cancer. The tumor vaccines can be divided in to four categories: the DNA vaccine, the RNA vaccine, the peptide and protein vaccine and the cellular vaccine. The RNA vaccine directly target the antigens on the tumor cells and express proteins to kill them. The proteins and peptide proteins activate T cells to target the tumor antigens. The whole tumor cell vaccine is the direct preparation of inactivated tumor cells or tumor cell extracts into vaccines. Compared with traditional therapies, cancer vaccines has the advantages of preventing the tumor cells from growing and dividing, targeting the tumor antigens specifically, having the potential wide range of applicability and having less side effects than traditional therapies. However, many problems are needed to be solved such as immune escape during the treatment, the resistance of the drug, unable to mass product and the lack of clinical figures. Luckily, the researchers are focusing on the tackling above mentioned problems and more matured clinical trial are being conducted. Therefore, the cancer vaccine will finally be a important treat method for cancer patients in the future days.

Author Contributions

All the authors contributed equally and their names were listed in alphabetical order.

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