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Current Trends in Vaccine and Vaccinology

Plant Based Therapeutics

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Abstract

Infectious diseases are common cause of death throughout the world. The tool for recombinant proteins production is achieved by the advent of recombinant DNA technologies and these recombinant proteins are used as therapeutic agents to cure the infectious diseases. Many expression systems for the pharmaceuticals production have been developed through which various types of immunoglobulins, enzymes and edible vaccines are synthesized that are used as prophylactic or as therapeutics. Development of preventive and therapeutic strategies applied to new emerging pathogens by two new coronaviruses such as severe acute respiratory syndrome coronavirus and Middle East respiratory syndrome coronavirus emerged in humans. The four diagnostic methods for SARS are available such as viral detection by the reverse transcription polymerase chain reaction (RT PCR), enzyme linked immunosorbent assay of the nucleocapsid proteins, virus induced antibodies by the immunofluorescence assay and inoculation of specimen of patients in cell culture. Plant based vaccines also important in animal models. The expression of heterologous proteins is increased by novel techniques such as magnifection and optimization.

Keywords

Infectious diseases; Vaccine; Immunoglobulins; Stable expression system; Transient expression system.

Introduction

The infectious diseases are commonly caused by disease-causing microorganisms and it may lead to the death. There are a number of factors that has a significant role these includes, e.g. global warming, deficiency of healthcare facilities, and costly preventive treatments. Data from the last 10 years shows that increase in the frequency of diseases also predict more rise in the future [1]. This encourages the pharmaceutical and the biotechnological industries to make use of living systems for heterologous expression of compounds. Biopharmaceutical and edible vaccines derived from the plants have several advantages as it involves simple and convenient approach, provide high yield of proteins, have lower storage and production cost, the removal of the pathogen contamination, the less processing required, as well as the safe delivery of the oral vaccines[2]. Moreover plants produce active form of complex protein such as glycosylated protein[3].

In plants the expression of the biopharmaceutical proteins is based on both the stable and transient expression systems. Stable transfer of the foreign gene is targeted either to the chloroplast or the nucleus.

In 1989, immunoglobulins derived from transgenic tobacco plant were assembled as functional antibodies [4]. Several studies confirmed that plants can be use to produce edible vaccines. Mucosal vaccination that is capable to induce antigen specific immune response both in the systemic and the mucosal compartments obtained from rice [5,6]. Similarly edible vaccines produce from transgenic carrot to prevent the diarrheal diseases and also from soybean[7,8].

Gaucher's disease is the widespread lysosomal storage disorder caused by the mutations in gene that encodes glcerucoebrosidase (GCD) which is a lysosomal enzyme and catalyses the hydrolysis of glucocerebroside (GlcCer) also called glucosylceramide, leading to the accumulation of GlcCer in the lysosomes of the macrophages. These storage cells called Gaucher cells are found in the spleen, liver and bone marrow.

Gaucher's disease is treated by the replacement of defective glucocerebrosidase by normal GCD, procedure called Enzyme Replacement Therapy i.e. ERT [9].

For production of an active glucocerebrosidase, glycosylation is crucial with the presence of both the high-mannose (Man) and the complex oligosaccharide chains in GCD.

Gaucher's disease is treated with the recombinant GCD that expressed in the mammalian Chinese hamster ovary (CHO) cells, this recombinant GCD is called Cerezyme. Plant cell expression system that is based on the transgenic carrot cells grown in the suspension

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culture is capable for the production of the glycoproteins. It is demonstrated that GCD expression in the carrot cells (prGCD), along with its targeting to storage vacuole, result in generation of a protein with the terminal Man structures in vivo, thus eliminating the need for the postproduction enzymatic modification in vitro. Plant cell-expressed GCD has no adverse reactions which indicate the safety of the drug[10].

Several problems are associated with nuclear transformation including gene silencing [11], low yields i.e. 1 % of total soluble protein, a high risk of the transgene contamination etc. Alternative strategy is the use of chloroplasts transformation for the industrial production of pharmaceutical compounds like antibodies, growth factors, cytokines, and antigens, enzymes and hormones [12,13].

In plants the transient expression of the foreign genes does not need the integration of the transgene into the host genome, and nor it does follow the central dogma for expression. Transient expression is achieved by either plant viruses or by the agroinfiltration[14], It also saves the time that is needed for the generation of transgenic plants, and gives higher protein yield because of the absence of the chromosomal integration and result in position effects [15]. Transient expression also provides the preliminary evaluation of the correct expression of the gene before starting generation of the transgenic plants.

Plant viral vectors are also being used for the production of recombinant proteins from crop plants. The expression of the antigen epitope was demonstrated by using the tobacco mosaic virus i.e. TMV coat protein in a plant system as a candidate for the polio vaccine [16].Severe Acute Respiratory Syndrome (SARS) appeared in 2002 in China. The causative agent of the syndrome is the coronavirus or SARS-CoV was then identified [17]. The large SARS-CoV genome of 29,727 nucleotides, which encodes four important viral structural components known as Envelop (E), Spike (S), Nucleocapsid (N),Membrane (M),Proteins, and the 16 nonstructural proteins[18]. Plant transient expression systems which is Potato Virus X i.e. PVX mediated infection and agroinfiltration, are feasible to produce the two SARS-CoV antigens, N and M proteins, acts as useful tool to face the SARS-CoV infection [19].

Plants acts as an ideal biofactory for the synthesis of antigen which play an important role in development of diagnostic test, as well as it is important to produce vaccines that are directed against diseases with epidemic potential, like influenza. Four companies in the US involved in production of 100 million dosages of influenza vaccine in a month[20]. This make a start to use this technology for the other diseases for example, Ebola, avian flu etc.

Strategies for Pathogens

In the twenty-first century, the important development of effective preventive and therapeutic strategies applied to new emerging pathogens emerged in humans by two new coronaviruses such as SARS-CoV (Severe Acute Respiratory Syndrome Coronavirus) and MERS-CoV (Middle East Respiratory Syndrome Coronavirus). A respiratory distress syndrome is caused by these two viruses and also associated with mortality rates. There are no approved antiviral drugs or vaccines available for these infections [21].

Severe acute respiratory syndrome coronavirus was outbreak as first infectious disease and still cause a large scale epidemic. The controlling and preventing key of SARS develop rapid diagnostic method for the assession of suspected patients. Moreover, damage of pandemic also minimized by the safe and effective treatments. Many efforts have been made. The four diagnostic methods for SARS are available such as viral detection by the RT PCR, enzyme linked immunosorbent assay of the nucleocapsid proteins, virus induced antibodies by the immunofluorescence assay and inoculation of specimen of patients in cell culture [22].

The expression of N protein of SARS-CoV occur at early stages and trigger the host antibody response[23]. Nano wire transistors and biosensors based on Plasmon resonance were also developed for detection of the N protein of SARS-CoV[24,25]. A study of N protein phosphorylation with its antibody recognition on specificity and antigenicity[6]. Then N protein was expressed as agroinfiltration in plants [26].

Recombinant Proteins

Recombinant protein production is increased by plant viral vector and HFB1s combination that also improve to bioprocessing. Different factors, including optimization of codon, specific expression of organ and organelle and the proteases are noticeable in case of the expression of the recombinant protein. In the case of influenza plants are useful for the vaccination that is quick and reliable.

Recombinant N protein has been achieved in a variety of heterologous expression system. In *E. coli* a synthetic gene with optimize codon has been expressed at high yield but it was demonstrated that bacterially expressed N protein produces false seropositivity owing to interference of bacterially derived antigen or cross react with antisera of human coronaviruses (HCoV-OC43 and HCoV-229E) infected patients [27,28]. These data underline the importance of producing recombinant protein in eukaryotic platform such as insect cells, yeast and plants to set up more efficient and specific diagnostic test [29].

Plant Derived Biopharmaceutical

The expression of vaccine linked protein in transgenic form of tobacco plants enhanced the usage of plants as biofactories[4]. After this important development, the expression of HBsAg in plants of tobacco also appeared as a big outcome[30]. This was followed by the epitope presentation of malarial parasites [31]. LTB is a heat labile form of enterotoxin B that was produced in the potato plants[32]. The functional expression in *E. coli* was appeared and plant derivatives of LTB undergo phase I and II human clinical trials [33]. Recombinant proteins expression at low level hindered its development until the antigen of anthrax succeeded to express in chloroplast based systems for the immunization of mice. At that time, the expression of heterologous proteins was enhanced by the introduction of magnifection technology[34]. This technology increased the expression of recombinant proteins with various simultaneous modifications. Asingle dose of plant derived type of vaccine in intranasal form produced in the tobacco activated the CD4+ T cells and mice antibodies against the toxin tetanus [35].

This field gained progress with the reports of plant based epitope presentation of CRPV-L1 (cottontail rabbit papilloma virus) and Rabbit immunization with CRPV-L1 confirm the effectiveness of plant derived HPV vaccine [36]. Then plant derived vaccine also approved for the immunization of chicken against the Newcastle viral disease [37].This paved a way to edible vaccines for their production and design. Then clinical trials of phase I and phase II of plant based therapeutics for carrot cell suspension culture were also undertaken against Gaucher's disease [33].The first clinical trial of human phase I for anti- idiotype vaccine was also performed against non-Hodgkin's lymphoma[38]. Then particles of virus undergo preclinical and clinical trials against H5N1 influenza and a clinical trial phase II also undertaken on caroRX (a monoclonal antibody directed against Streptococcus mutants)against the dental decay.Virus-like particles, VLPs also approved in human phase II clinical trials against H5N1 by FDA[39].

Stability of Plant Vaccines

Plant based vaccines can be stored at room temperature for long time in dried form. Many reports have been made to determine the stable expression of proteins in plants at room temperature even at elevated temperature for a long period of the time. An algal form of chloroplast derived vaccine remained stable for the 20 months in lyophilized form at room temperature and was immunogenic in comparison of antigens that were stored at 4 ℃ [40].

Efficiency of plant based vaccines has also shown great importance in animal models. Many reports have shown the high

immunogenicity of vaccine candidates in different models of animals.

Antigen Expression on *NicotianaTabacum*

Before 14 years, initial expressions of the antigens in the vaccine bearers against the human disease were seen in the *Nicotianatabacum*[41].

Later Research about the various properties and the expression proved to get improved protocols as well as the high expression level in the chloroplast of plant. Mostly the tobacco plants are used to check the expressions that are based on the chloroplast as compare to other plants species.

Tobacco plants have more information to produce the vaccines that is based on the chloroplast. Moreover, though a lot of research has been done despite this even a single vaccine is not available in the market against the human health risks. It is all about due to the lack of interest in the industrial work. There are a number of pharma companies established to produce the pharmaceutical compounds.

There is an establishment of the tobacco prototype that is already present it might be helpful in industrial advancement. Basically chloroplast transformation of tobacco have benefits it does not take as meal due to which food is not spoil and second one is the absence of transgene transfer because of the maternal inheritance pattern of the chloroplast that helpful to determine the biosafety in those countries and also higher the production at large scale in desired fields. There are two aspects of the tobacco plants that play vital role in the production of the vaccines at large scale one of is the high expression of plant and the second one is the more biomass production. Lettuce is more useful and reliable for large scale production.

Plant Vaccines in Animal Models

Plant-based vaccines are tested on the animal model to find out the reaction capacity. Vaccines are checked and reported the efficacy of the vaccine nominates and the immune effect in the various animal models [20].

There is a main problem retain in the glycosylation pathway it has great resistance to make difference that's why the process of post transcriptional modification of the animals as well as the humans is low.

This problem in plants can be resolved by introducing a special glycosylation pathway. The biomass production is increased due to the photorespiration suppression pathway that is introduced in the *Arabidopsis thaliana*. In the areas where disease rate is much higher, provide them to very cheap drugs to handle health concern problems by using plant made vaccines as well as the therapeutic agents commercially.

Conclusion

Many vaccines and therapeutic compounds can be obtained from plants by many ways in green house, in the field and in cell or root cultures. Heterologous protein production hindered the plant pharmaceutical commercialization for a long time but recent development increased the expression by novel techniques such as magnifection and optimization. Government should allocate more funds for plant based vaccines research and for further commercialization.

Future Aspects

It is necessary for pharma companies to develop plants based system to expense money. Because there is need of money and time for clinical trials and for approvals. Ultimately today's small industries get more output than the expenditure due to the establishment of the plant based system. The cooperation of the research groups and the industries make it possible to establish a platform which would be awarded with fund for the treatment of disease in all over the world. There is a need of cost effective vaccines in the establish states, at the start the platforms could be established in the areas where vaccine is needed.

In the local areas comparatively to the develop countries plants are grown, harvest and processed through the facultative ways that is not laborious and land cost economically. There is a need to get attention of the pharmacy companies by revenue that obtained through the vaccines product and provided to the far most areas and those areas where the vaccines could not be afforded. So that by providing the cheapest or alternative vaccines will give a favorable increase in the output of companies. There should be establishment of local pharma companies that provide the vaccines in less amount than to export from the industries of the far most areas. Therefore we save the time and money that used in the maintenance and transportation process. Because of the local companies the under developed countries economically get rise. After all different technologies are used in the lab as well in the industries for the maintenance of the genomics in seeds by getting more funding for research in those countries that are developed.

All the local organization could be established a platform at an industrial scale for the production of the vaccines. All the world health organizations are involved in the usefulness of the previous setups of vaccines that are stored and transported or administered. Sometimes there is need of the modification of the protocols that already present.

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