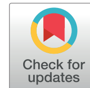

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Review
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Production of Recombinant Anti-Cancer Vaccines in Plants

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Abstract

Plant expression systems have been developed to produce anti-cancer vaccines. Plants have several advantages as bioreactors for the production of subunit vaccines: they are considered safe, and may be used to produce recombinant proteins at low production cost. However, several technical issues hinder large-scale production of anti-cancer vaccines in plants. The present review covers design strategies to enhance the immunogenicity and therapeutic potency of anti-cancer vaccines, methods to increase vaccine-expressing plant biomass, and challenges facing the production of anti-cancer vaccines in plants. Specifically, the issues such as low expression levels and plant-specific glycosylation are described, along with their potential solutions.

Key Words: Cancer, Vaccine, Glycosylation, Plant expression system, Subunit vaccine, Recombinant protein

INTRODUCTION

Advances in molecular biology and immunology have led to the successful development of recombinant subunit vaccines, which effectively use antigenic epitopes and proteins to prevent infectious diseases, unlike conventional vaccines made of killed or attenuated pathogens (Rappuoli, 2007). Vaccines that can express and process recombinant proteins similar to those of the naïve pathogenic organisms have been produced in bacterial, insect, mammalian, and yeast cells (Monsurrò *et al*., 2002; Radford *et al*., 2002; Fazlalipour *et al*., 2015; Qiao *et al*., 2015). However, the practical value of these expression systems is limited, as they require a large volume of media for biomass production and require purification steps, which can increase the overall production cost. Plant expression systems for vaccine production, in which plants are used as bioreactors, may be a promising alternative that can produce vaccines at low cost. Production in plants also prevents the risk of pathogen contamination associated with the use of conventional or recombinant subunit vaccines produced from mammalian systems (Ma *et al*., 2005; Park *et al*., 2015; Kim *et al*., 2016).

The first plant-derived vaccine was produced in tobacco plants transformed with the *Streptococcus mutans* surface protein antigen A in 1990 (Curtiss and Cardineau, 1997). Since then, antigenic proteins of viruses, bacteria, and enteric and non-enteric pathogens, as well as tumor-associated antigens, have been produced in a wide range of plant species using stable or transient expression systems (Kurokawa *et al*., 2013; Lee *et al*., 2013; Lim *et al*., 2015). Immunization with plant-derived antigenic proteins such as vaccines may someday prevent millions of people from getting infectious diseases. Furthermore, the idea of vaccination against cancer has led to a potentially novel strategy to produce tumor-associated antigens (TAAs) in plant systems (Lu *et al*., 2012). Many anticancer vaccines expressed in plants have been studied and even approved for clinical trials (Tacket, 2009).

However, for the large-scale production of anti-cancer vaccines in plants to be viable, it is necessary to understand the advantages and disadvantages of these systems. The present review covers issues associated with the production of anticancer vaccines in plants.

IN VITRO AND *IN VIVO* PLANT PRODUCTION SYSTEMS

Vaccines can be produced using *in vivo* and *in vitro* plant systems. Whole plant expression platforms, including stable transgenic and transient plant systems, are *in vivo* systems. In these systems, environmental cultivation conditions such as temperature, light, water, and nutrients in the air and soil

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should be properly controlled, as they affect vaccine protein production levels and their functionality (Jamal *et al*., 2009). Although transgenic or transiently expressing plants are generally cultivated in containment greenhouses or chamber rooms, plants can be grown under variable microenvironment conditions that may enable pathogen infections, with consequences for plant biomass, transgene expression, and quality control of plant-derived vaccines (Jamal *et al*., 2009). Thus, several institutes, including the Center for Molecular Biotechnology (Fraunhofer USA, Newark, DE, USA) and the Biodesign Institute at Arizona State University (Tempe, AZ, USA), as well as companies, such as Kentucky BioProcessing (Owensboro, KY, USA) and Medicago (Quebec, Canada), use greenhouse complexes in which internal conditions are controlled and external pollutants and insects are excluded, consistent with Current Good Manufacturing Practice standards, as well as employing recombinant protein purification.

In *in vitro* systems, microenvironment conditions can be precisely controlled without the risk of pathogenic contamination. These systems culture plant cells and organs in aseptic conditions. In plant cell culture systems, dedifferentiated cells can be cultivated in callus form, using callus culture or initiating the single cells obtained from fragmentation of a callus into cell suspension culture. In general, cells divide more rapidly in cell suspension cultures in liquid media than in callus cultures on agar (Evans *et al*., 2003). Examples of the products of *in vitro* systems include many recombinant proteins that are vaccine candidates, such as the Hepatitis B surface antigen (Smith *et al*., 2002) in *Glycine max* and *Nicotiana tabacum*, human granulocyte-macrophage colony stimulating factor (hGM-CSF) in *N. tabacum* (Lee *et al*., 2004; Hong *et al*., 2006), viral protein 1 (VP1) epitope of foot-and-mouth disease virus (FMDV) in *Nicotiana benthamiana* (Zhang *et al*., 2010), recombinant glucocerebrosidase in *Daucus carota* (Shaaltiel *et al*., 2007), and human interleukin (IL)-2 and IL-4 in *N. tabacum* (Magnuson *et al*., 1998). Bright Yellow-2 (BY-2) and NT1 tobacco lines, which lack the ability to regenerate, are often used in plant cell suspension cultures (Mayo *et al*., 2006; Vasilev *et al*., 2013).

The affinity column purification process for recombinant therapeutic proteins expressed from tobacco leaves can remove alkaloids (Ko *et al*., 2004). In addition, low-alkaloid tobacco plant varieties can be used in the production of recombinant vaccine proteins (Rymerson *et al*., 2003). However, there are still safety concerns related to alkaloid contamination of tobacco cell-based oral vaccines. Edible oral vaccines based on rice seed thus offer a safe, simple, and cost-effective approach (Takaiwa, 2007; Kuo *et al*., 2013; Kurokawa *et al*., 2013). Furthermore, suspension rice cell cultures have other advantages, such as precisely controlled environments, faster increase in cell biomass, and lack of alkaloid contaminants in orally administered therapeutic proteins (Huang and McDonald, 2012; Kuo *et al*., 2013).

These suspension cultures can even secrete recombinant proteins out of the cell into the culture medium when the signal peptide is fused to the proteins, making the cell lysis step unnecessary for extraction (Liu *et al*., 2012). Carrot cells have been used for *in vitro* suspension-based production of diverse recombinant therapeutic proteins (Shaaltiel *et al*., 2007), building on the successful establishment of a carrot cell suspension culture in 1970 (Nishi and Sugano, 1970). The recombinant human enzyme glucocerebrosidase has been successfully produced in a carrot cell suspension culture (Shaaltiel *et al*., 2007). The carrot cell-derived glucocerebrosidase was developed by the Israeli biotechnology company, approved as a drug for humans, and licensed in the United States. The plant cell-derived is considered an alternative therapeutic option to production in the Chinese hamster ovary cell system to treat Gaucher's disease.

Two types of plant organs, shoots and roots, can be cultivated *in vitro* (Paz-Maldonado and González-Ramírez, 2014). Hairy root culture systems have been established in many plant species. Hairy roots can be indefinitely propagated in liquid medium with stable morphologies; however, hairy root growth is somewhat slower than growth in shoot organ culture and suspension cell culture (Rigano and Walmsley, 2005). The hairy root culture system was originally applied to produce secondary phytometabolites, which are considered natural biopharmaceuticals and include antimicrobial flavonoids, Ginkgolide A, L-3,4-Dihydroxyphenylalanine (L-Dopa), and saponin in plants (Li *et al*., 1998; Hwang *et al*., 1999; Hussain *et al*., 2012). *Agrobacterium rhizogenes*-mediated transformation can generate transgenic hairy root expression systems, which can be applied to produce recombinant therapeutic proteins (Tepfer and Casse-Delbart, 1987; Sharp and Doran, 2001; Gaume *et al*., 2003; Medina-Bolivar *et al*., 2003). Therefore, the hairy root culture-based production system is more genetically stable than cell suspension culture (Rao and Ravishankar, 2002). The hairy root organ culture system is thus a reliable technology for use with *A. rhizogenes*-mediated transformation. This expression system should be used when media and containment environmental space are relatively cheap.

Although the cell suspension culture system has important advantages, there are still several challenges to be overcome, such as improvement of protein yield and production cost effectiveness and humanization of the glycosylation patterns of proteins. For recombinant protein yield, the factors of strong expression promoter, codon choice, and subcellular localization (Jamal *et al*., 2009; Lee *et al*., 2013) have been optimized. For glycosylation humanization, studies have explored the removal of glycosylation reactions for xylosyltransferase and fucosyltransferase by knock-out/knock-down approaches using RNAi technology (Loos and Steinkellner, 2014) and the Clustered regularly interspaced short palindromic repeats associated protein-9 nuclease (CRISPR/Cas9) system for plant genome editing, as well as introduction of human glycosylation enzymes such as sialic acid transferases and galactosyltransferase using transient and stable transformations (Strasser *et al*., 2007; Dicker *et al*., 2016).

PLANT EXPRESSION SYSTEMS FOR VACCINE PRODUCTION

Subunit vaccines can be produced by plant transformation methods using either stable transformation or transient expression. In the stable transformation system, vaccine genes are inserted into plant genomes for stable transgene expression, whereas in the transient expression system, genes are expressed in the cytoplasm without stable genomic insertion (i.e., the plant is not genetically modified). The expression of recombinant vaccine proteins has been achieved primarily using stable gene transformation. *Agrobacterium*-mediated

transformation is the main tool to transfer the transfer DNA (T-DNA) region, which contains the gene expression cassette consisting of promoter, recombinant vaccine protein gene of interest, and terminator, into the plant genomic DNA. This technique has allowed the stable transformation of many plant species to express recombinant anti-cancer vaccine proteins (Brodzik *et al*., 2008; Lu *et al*., 2012; Lim *et al*., 2015; Kang *et al*., 2016; Kim *et al*., 2016).

Transgenic plants with stable gene expression enable rapid biomass increases and consequent production of recombinant vaccine proteins with low cost inputs (e.g., soil, water, sunlight, fertilizers). Stable gene insertion in plant genomes can transfer recombinant protein genetic traits to the next generation via the seed. Seed storage is a huge advantage of stable transgenic plant systems. Seeds with high protein content can stably accumulate seed storage proteins containing recombinant vaccine proteins in an intact form when the proteins are localized to the endoplasmic reticulum subcellular organelles in soybean (Maruyama *et al*., 2014). In addition, corn has been considered a promising transgenic plant species for production of recombinant therapeutic proteins because it produces large volumes of seeds that may be stored at low cost. Genetically stable plant lines can be used to generate seed banks for large-scale commercial production. Another advantage of stable transgenic plant expression systems is that changing plant biomass production levels is easily achieved by controlling the size of the seedling cultivation field. Furthermore, cross-fertilization between transgenic plants expressing two different recombinant proteins can generate a sibling plant to produce multiple genes, such as monoclonal antibodies containing heavy and light chains and multimeric complex proteins for enhancing immune responses (Jamal *et al*., 2012).

Chloroplast transformation is an attractive alternative in which the gene of interest is inserted not into the nuclear genomic DNA but into the chloroplast DNA. This DNA is maternally inherited and is not transmitted through pollen. Hence, in transplastomic plants obtained from chloroplast transformation, gene flow does not occur (Daniell *et al*., 1998; Ruf *et al*., 2001). Furthermore, transplastomic plants have no gene silencing or positional effects due to site-specific transgene integration. However, post-translational modification in chloroplasts differs from that in eukaryotes, in which glycosylation occurs; instead, it is more analogous to that in prokaryotes, which do not perform glycosylation. Therefore, chloroplast transformation is not an appropriate approach for the production of therapeutic proteins that require glycosylation for biological activities. In addition, although some plant species, such as tobacco, lettuce (*Lactuca sativa*), and cotton (*Gossypium hirsutum*), can be transformed through chloroplast transformation, few plant species are suitable for plastid transformation compared with the number that are suitable for genomic transformation (Meyers *et al*., 2010).

Stable transgenic plants have several limitations. First, it is time-consuming to establish transgenicity; in addition, these plants have low expression capability. They also have low production efficiency of recombinant proteins compared with transient expression plant systems and plastid transgenic plants.

There are several approaches to improve recombinant vaccine production efficiency in stable transgenic plants. Ideal plant species for stable transformation contain high levels of total soluble proteins (Song *et al*., 2015), grow easily and rapidly, and produce large amounts of seeds in a short period of time. Tobacco possesses these qualities and has been regarded as an ideal plant system for recombinant protein production (Song *et al*., 2015). If the roots are left intact, the tobacco plant can regrow from the stem after the upper portion of the plant has been harvested, making it unnecessary to plant new seedlings (Kim *et al*., 2016) and saving cultivation time. The entire biomass of this plant evenly expresses a recombinant colorectal cancer vaccine candidate, including the leaves and stem (Lim *et al*., 2015), and it grows rapidly to 1.2-1.8 m in height with 20-35 leaves (30-75 cm length and 25-45 cm width). In addition, tobacco plants express the systematically recombinant colorectal cancer vaccine protein gastrointestinal carcinoma antigen fused to the immunoglobulin fragment crystallizable region (GA733-Fc) in the whole plant, including the leaves and the stem, throughout the growth period until floral expression (Lim *et al*., 2015). To improve production efficiency, biomass harvesting time and harvesting of specific plant tissues should be optimized (Lim *et al*., 2015).

Transient expression can be achieved by agroinfiltration of *Agrobacterium tumefaciens*, carrying plant expression binary vectors or plant viral vectors into intercellular spaces instead of integrating them into the plant genome. This technique results in the transient production of recombinant vaccine proteins and is comparable to microbial or mammalian *in vitro* expression systems. Transient expression using *Agrobacterium* infiltration technology has mainly been applied to the production of vaccines and antibodies that are ready for commercialization. The best plant species for transient expression is *N. benthamiana*, which is susceptible to diverse plant viruses (Sheludko *et al*., 2007; Goodin *et al*., 2008; Conley *et al*., 2011) and is amenable to systemic infiltration by *Agrobacterium*.

Agroinfiltration is induced with both syringe and vacuum methods. The syringe infiltration method is simple and cost effective and does not require any special equipment. However, syringe infiltration must be conducted on individual leaves unless *Agrobacterium* or viral expression vectors are systematically spread to the whole plant. It is time consuming and inefficient when an industrial amount of plant biomass needs to be infiltrated. However, this method can be often utilized as a quick test tool to confirm transgene expression before stable transformation is carried out. In contrast, vacuum infiltration is more reliable for scaled-up production of recombinant proteins in plants.

In general, transient gene expression offers the advantages of higher protein accumulation levels and faster production processes over stable transgenic expression. Several transient expression approaches have therefore been developed for use in large-scale production. Two deconstructed viral vectors, one based on a tobacco mosaic virus RNA replicon system and the other derived from the bean yellow dwarf virus DNA replicon system (geminiviral vectors), have been established (Matzeit *et al*., 1991; Liu *et al*., 1998; Zhang and Mason, 2006; Goodin *et al*., 2008; Lico *et al*., 2008). In the vector system, the so-called proviral expression vectors carrying genes of interest do not harbor essential viral functions for replication of wild-type virus (Marillonnet *et al*., 2004). This deconstructed viral vector platform can express multimeric vaccine and antibody proteins within 2 weeks at 5 g of protein per kg of fresh plant biomass. It is a versatile technology that is suitable for the production of seasonal and pandemic flu vaccines, as well as of recombinant proteins for patient-specific immunotherapy, which must be generated quickly for ap-

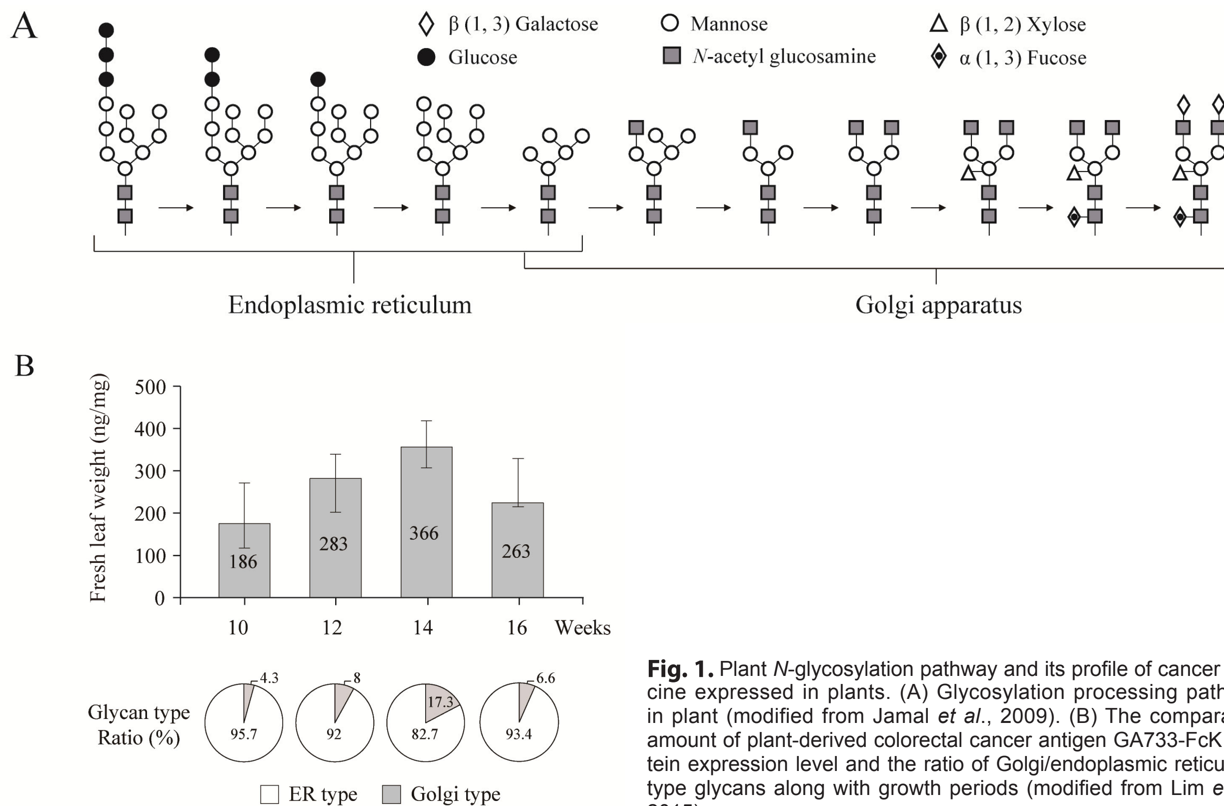

Fig. 1. Plant *N*-glycosylation pathway and its profile of cancer vaccine expressed in plants. (A) Glycosylation processing pathway in plant (modified from Jamal *et al*., 2009). (B) The comparative amount of plant-derived colorectal cancer antigen GA733-FcK protein expression level and the ratio of Golgi/endoplasmic reticulum-type glycans along with growth periods (modified from Lim *et al*., 2015).

plication (Klimyuk *et al*., 2014). Current Good Manufacturing Process-compliant facilities for large-scale manufacturing of recombinant antigens and antibodies in *N. benthamiana* using a tobacco mosaic virus RNA replicon system technology are being constructed and run worldwide (Kentucky BioProcessing; iBio Inc., Newark, DE, USA; Bio-Manguinhos/Fiocruz, RJ, Brazil; Fraunhofer USA). Viral vector expression systems can avoid issues associated with genetically modified plants, as viral vector-derived recombinants can be obtained immediately after simple virus transfection. This system does not require entire transformation processes, such as transformation, *in vitro* regeneration, rooting induction, or plant rehabilitation as in *in vivo* culture. It requires only the cloning of the plant viral vector carrying the gene of interest and a plant host. However, compatibility between the virus and plant host is required, which limits applicable plant hosts.

EFFECT OF DEVELOPMENTAL AND ENVIRONMENTAL FACTORS ON VACCINE PROTEIN EXPRESSION

Plant growth conditions and harvest times, as well as tissue positions, affect protein expression levels and glycosylation structures of cancer vaccine proteins in plant (Gomord *et al*., 2005; Lim *et al*., 2015). When recombinant cancer vaccines are produced in plants, these diverse factors should be optimized for quantity and quality control.

To improve recombinant vaccine production efficiency in stable transgenic plants, it is important to choose ideal plant species for stable transformation and to control environmental factors that affect plant health conditions. Biomass harvesting time and specific plant tissue are other important factors that should be optimized to improve the efficiency of recombinant vaccine protein production. Plant biomass should be harvested before flowering to avoid transgene flow (Jensen *et al*., 2004; Wang and Ge, 2006; Spangenberg *et al*., 2012). Colorectal cancer vaccine protein levels in leaves and stems harvested after flower fertilization were lower than those in plant material harvested before the blossoming period. The highest level of expression of a colorectal cancer vaccine protein was observed in the 12 weeks after transplanting from the *in vitro* plant seedlings (Lim *et al*., 2015).

Glycosylation, which is essential for proper biological activities of the recombinant vaccine proteins, can be affected by the photosynthetic ability of plant cells, as the glucose obtained from photosynthesis is an essential element in the *N*-glycosylation pathway (Jamal *et al*., 2009). Blue light (440 nm) and far red light (660 nm) are selectively applied as an energy source for photosynthesis to enable plant growth (Singh *et al*., 2015). Environmental factors such as temperature, soil nutrition, salinity, and drought stress all affect glycosylation, as well as protein qualities and quantities (Jamal *et al*., 2009; Kang *et al*., 2016). Glycosylation patterns in the anti-cancer vaccine GA733-FcK in tobacco vary at the developmental stages (Lim *et al*., 2015). The GA733-FcK protein harbors the endoplasmic reticulum retention motif KDEL (Lys-Asp-Glu-Leu); theoretically, proteins with KDEL are oligomannose-glycosylated in plants. However, these glycosylation patterns are plant specific, in contrast with oligomannose-type glycosylation patterns (Lim *et al*., 2015). In particular, when the plants are in

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full bloom, the glycosylation pattern is slightly shifted from the oligomannose-type glycoforms (glycoforms of endoplasmic reticulum-retained protein) to plant-specific glycoforms (glycoforms of secreted protein) (Fig. 1). The glycosylation rate of the colorectal cancer vaccine protein GA733-Fc fused with the KDEL (GA733-FcK) is much lower in yellow leaves, which contain low numbers of chloroplasts for photosynthesis, than in green leaves, which contain high numbers of chloroplasts, indicating the importance of photosynthesis for the glycosylation of recombinant cancer vaccines in plants (Lim *et al*., 2015). Furthermore, glycosylation patterns are plant tissue-specific (Lim *et al*., 2015). The plant leaves growing from the upper stem produced more recombinant glycoproteins of the oligomannose type than did leaves attached to the lower portion of the stem (Donaldson *et al*., 1999).

CANCER VACCINES IN PLANTS

The production of cancer vaccines in plant expression systems must consider several specific factors. First, in order to successfully prevent and cure diseases, proper vaccine candidates should be chosen and designed to induce potent immune responses against these diseases. Second, it is essential to choose proper antigenic proteins that the immune system can target.

Immune responses to cancer vaccines include mucosal and systemic challenges to a vaccine after its direct application to mucosal surfaces or parenteral injection, respectively (Rigano and Walmsley, 2005). Direct application of a vaccine to mucosal sites that can induce a quick mucosal immune response, including protective humoral, cell-mediated, and later systemic immune responses, may be the best choice in some enteric and respiratory diseases (Rigano and Walmsley, 2005). Rapid advancements in the fields of molecular immunology and cancer biology have improved our understanding of the role of the immune system in cancer and the development of therapeutic and preventive vaccines. Therapeutic cancer vaccines, which are administered to current cancer patients, stimulate the protective ability of the immune system to specifically recognize, attack, and kill tumor cells (Cripps *et al*., 2001). Cancer preventive vaccines are administered to healthy people to prevent cancer from developing (Bachmann and Jennings, 2010).

During the body's immune response to cancer, antigen-presenting cells display antigens that are complexed with major histocompatibility complexes (MHC II and I) on their surfaces, which are recognized by T-lymphocytes, and which eventually stimulate these T-lymphocytes (CD4+ or CD8+) to become mature helper and cytotoxic T cells, respectively. The mature helper T cells recruit and prime cytotoxic T lymphocytes to kill tumor cells (Frazer *et al*., 2007). Anti-cancer vaccines produced in plants must be oriented to proper antigenic proteins that the immune system can target. Tumor antigenic proteins may be classified into two types: tumor-specific antigens (TSAs) and TAAs. TSAs are specifically expressed on the tumor cells and trigger better immune responses than do TAAs. However, it is difficult to identify TSAs as vaccine candidates, as they are very uncommon. TAAs are expressed on both tumor and normal cells and stimulate a weaker immune response than do TAAs; however, they are commonly identified on tumor cells.

Table 1. Cancer vaccines expressed in plants

Target/Antigen/Strategy	Host plant	Transformation platform	Immunogenicity/efficacy (development status)	References
Tumor-associated colorectal cancer antigen: Fused to Fc high mannose type glycan	*N. tabacum*	Stable	Induced anti-cancer IgGs (pre-clinical)	Brodzik *et al*., 2006; Lu *et al*., 2012; Lim *et al*., 2015
Prostatic acid phosphatase (PAP) antigen: Fused to IgM Fc	*N. tabacum*	Stable	Induced anti-PAP IgGs (pre-clinical)	Kang *et al*., 2016
Tumor-associated colorectal cancer antigen	*N. benthamiana*	Transient	Serum in vaccinated mice inhibited colorectal tumor in nude mice (pre-clinical)	Verch *et al*., 2004
Human papilloma virus 16 L1/L2 chimaeras	*N. benthamiana*	Transient	Not tested	Pineo *et al*., 2013
Non-Hodgkin's lymphoma (NHL)	*N. benthamiana*	Transient	Induced immune responses (Phase II)	McCormick *et al*., 2011
E7 oncoprotein from human papilloma virus 16	*N. benthamiana*	Transient	Induced E7-specific IgG and prevented tumor development	Plchova *et al*., 2011
Her2 protein	*N. benthamiana*	Transient	Induced anti-Her2 antibody including trastuzumab-like activities	Chotprakaikiat *et al*., 2016
Plant virus particle-based cancer immunotherapy	*N. benthamiana*	Transient	Activated TLR7 and Induced high levels of protective antibody	Massa *et al*., 2007; Jobsri *et al*., 2015; Peruzzi and Chiocca, 2016
MUC1-based plant vaccine for breast cancer	*N. benthamiana*	Transient	Induced anti-MUC1 serum antibodies	Pinkhasov *et al*., 2011

Table 2. Advantages of various systems for expressing recombinant proteins (modified from Raskin *et al*., 2002)

Host	Advantages of recombinant protein expression in various systems							
	Speed	Operating cost	Capital cost	Glycosylation	Multimeric assembly	Folding	Safety	Scalability
Bacteria	+ + +	+ +	+ +	+	+	+	+ +	+
Yeast	+ +	+ +	+	+	+	+	+ +	+ +
Insect cell culture	+ + +	+ +	+	+	+ +	+	+	+ +
Plant	+ +	+ + +	+ + +	+ +	+ +	+ +	+ + +	+ + +
Mammalian cell culture	+	+	+	+ +	+ +	+ + +	+	+
Transgenic animal	+	+	+ +	+ + +	+ + +	+ +	+	+ +

Many anti-cancer vaccines have been expressed in plants (Table 1). Non-Hodgkin's lymphoma (Zhang *et al*., 2009), colorectal cancer (Bendandi *et al*., 2010; Lim *et al*., 2015), and cervical cancer (Smith *et al*., 2002; Verch *et al*., 2004) have been targeted. For non-Hodgkin's lymphoma, individualized (patient-specific) recombinant idiotype vaccines against follicular B cell lymphoma were produced in *N. benthamiana* leaves using the transient plant expression system (Zhang *et al*., 2009; Pineo *et al*., 2013). This approach allowed rapid production and recovery of idiotypic single-chain antibodies (scFv) derived from each patient's tumor and immunization with their own individual therapeutic antigen. Variation in glycan structures on the antigen do not impair immunogenicity or affect vaccine safety.

Another transient expression system was applied to produce the colorectal cancer antigen GA733 using tobacco mosaic virus plant viral vector (McCormick *et al*., 2008; Bendandi *et al*., 2010). Plant-derived GA773 stimulated humoral immune responses in mice; however, sera from mice injected with the plant-derived version did not show sufficient Antibody-dependent cell-mediated cytotoxicity (ADCC) and Complement Dependent Cytotoxicity (CDC) activity compared with the response to mammalian-derived GA733 (Shivprasad *et al*., 1999). In contrast, sera from mice injected with GA733 that was transiently expressed in Swiss chard (*Beta vulgaris* var. cicla) and low alkaloid tobacco (*N. tabacum* var. LAMD609) showed inhibition of growth of SW948 human colorectal cancer cells that were xenografted on to nude mice (Brodzik *et al*., 2008). The GA733 expression level was 5 mg per kg of fresh plant leaf tissue, which is not sufficient for commercialization; therefore, GA733 was fused to the immunoglobulin Fc fragment to enhance protein stability and create a better yield from the plant (Staib *et al*., 2001). Indeed, the expression level of the GA733 fused to Fc was 10-fold higher than that without the Fc (Brodzik *et al*., 2008). In addition, the Fc fused to vaccines can facilitate easier purification by protein-A or G affinity chromatography (Lu *et al*., 2012; Lim *et al*., 2015; Park *et al*., 2015).

The constant region Fc fragment can enhance the immune response as a result of an increased Fc receptor-mediated uptake by antigen-presenting cells, such as dendritic cells, on which Fc receptors exist (Andrianov *et al*., 2010; Lu *et al*., 2012). The antigen-antibody complexes can assemble to become multimerized peptide antigens in plants, eventually enhancing immune responses (Pleass, 2009). The antigens were fused to the C-terminus of the heavy chain of the antibody and expressed with light chains in transgenic plants (Bhoo *et al*., 2011). The antigen-antibody fusion proteins are assembled to form virus-like multimeric structures, which are more immunogenic than the antigen itself (Chargelegue *et al*., 2005).

CONCLUSIONS

Production of anti-cancer vaccines in plant systems has several advantages over animal-based systems, including lower upstream process costs, reduced risk of mammalian pathogen contamination, and ease of scalability (Table 2). Nevertheless, to fully commercialize plant systems for anti-cancer vaccine production, two major concerns should be eliminated: low expression of recombinant vaccine proteins and unwanted plant-specific glycosylation. Advance in plant biotechnology, glycoengineering, and molecular immunology can overcome such drawbacks in plant systems for recombinant vaccine production. Thus, plants can be considered to be a potential alternative to current mammalian based vaccine production system.

CONFLICT OF INTEREST

The authors confirm that they have no conflicts of interest.

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