Improving the Yields of Protein Production in Plants

Co-expression of the human molecular chaperones in plants to improve heterologous glycoproteins yields.

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Background

As the global demand for quality pharmaceutical products continues to grow, conventional production platforms are facing severe limitations in terms of scalability, long production times and high costs.

In recent years, the production of heterologous proteins using plant expression systems have come to light as a cost-effective and scalable alternative, leading to a paradigm shift for the pharmaceutical industry.

Plant-based expression systems are particularly appealing to resource-limited countries, as their raw material and infrastructure cost requirements are much lower than those of conventional pharmaceutical production.

The commercial viability of any plant-made pharmaceutical is largely governed by the expression yield which generally needs to exceed 1% of the total soluble protein.

However, this threshold is seldom realised and expression levels remain a challenge for many heterologous proteins, particularly viral glycoproteins.

Instead, a threshold of 50 mg/kg has inadvertently become the gold standard after the development of a plant-produced influenza haemagglutinin candidate for clinical trials by top industry players.

Technology Overview

A recent trend to improve the yield and quality of plant-made pharmaceuticals (PMPs) has resulted in extensive efforts to manipulate the plant host cell environment beyond just the expression of the heterologous protein of choice.

Researchers at the University of Cape Town (UCT) have developed a method for increasing the expression of a heterologous polypeptide of interest in plant cells.

The invention encompasses the co-expression of the human molecular chaperones (calreticulin, calnexin, GRP78/BiP, protein disulphide isomerase and/or ERp57) to improve the expression of heterologous glycoproteins in plants.

The inventors have demonstrated the utility of this approach using an engineered soluble HIV Envelope glycoprotein as a model antigen and subsequently demonstrated the broader applicability of this approach.

To enable human calnexin to improve the expression yields of HIV Envelope glycoprotein, a near full-length HIV antigen was designed based on the CAP256 SU Envelope. The sequence was modified, and the native signal peptide was replaced with a monoclonal antibody for expression in plants. The sequence was also truncated to improve expression levels.

According to the invention, human calnexin improves the expression yields of a membrane-bound HIV Envelope glycoprotein co-expressed with HIV Gag for efficient production of virus-like particles.

The researchers have shown that gp150 may be expressed alone, or in the presence of HIV Gag to produce a virus-like particle presenting HIV Envelope. The Gag antigen may be a naturally occurring protein isolated from a virus or a synthetic antigen designed in silico. A Gag subtype C mosaic antigen designed in silico to maximize the coverage of potential T cell epitopes was used. The Envelope gp150 sequence was modified to replace the native cleavage sequence with a glycine-rich linker peptide to circumvent the need for furin-mediated cleavage which does not occur in plants.

The co-expression of chaperones is not limited to viral glycoproteins and may likely work for other heterologous glycoproteins. Non-glycosylated proteins undergo chaperone-mediated folding. The co-expression of cytosolic chaperones may promote the assembly of virus-like particles.

Benefits

The inventors have proven the positive impact of co-expressing heterologous mammalian chaperones *in plants* to improve the production of a recombinant protein in terms of both yield and protein folding, which is an important factor in the correct expression of proteins.

They have also highlighted the remarkable plasticity of the plant proteome, which can be manipulated for the production of high levels of pharmaceutically relevant proteins.

The technology is also applicable for the production of virus-like particles.

Applications

- Enabling the production of low yielding vaccine antigens, or other reagents as well as diagnostic proteins, at commercially viable levels.
- This approach is broadly applicable to other heterologous proteins, especially those usually produced in mammalian expression systems such as mammal-infecting viruses.
- The co-expression of a heterologous glycoprotein with its cognate chaperone establishes a new paradigm for the production of viral glycoproteins in plants.

Opportunity

UCT is looking for industrial partners especially those in the plant expression space to commercialise the technology

Patents

- Priority Founding Patent Application GB 1708866.7
- International Patent Application PCT/IB2018/053944
- Canada - national phase patent application number 3065738 (filed)
- United States - national phase patent application number 16,618,704 (filed)
- South African national phase patent application number 2019/08177 (granted)

IP Status

- Patent application submitted

Seeking

- Licensing