PRODUCTION OF THERAPEUTIC GLYCOPROTEINS IN MUSHROOMS

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ABSTRACT

The market for N-glycosylated therapeutic proteins represents multi-billion dollars in sales and is growing more than 3% each year. We investigated the potential of mushroom-forming basidiomycetes for the production of these drugs.

Keywords: N-glycosylation; Therapeutic proteins

INTRODUCTION

Proteins can be used as drugs. Examples include antibodies, insulin, erythropoietin (EPO). They are used for the treatment of many diseases, including cancer, cardiovascular and (auto) immune disorders. Protein therapeutics is currently the fastest growing class of human drugs. It is growing about threefold above the average growth rate for pharmaceuticals in general[1]. Over 160 protein products are marketed representing USD 50-60 billion [1,2] and over 500 proteins are still in the developmental pipeline. Most of these products are N-glycoproteins (60%).

N-glycoproteins are proteins decorated with sugar groups (glycans). The glycans determine behavior of the protein in the human body. When proteins are produced in another host cell than human or mammalian, the glycans that are added to the protein are different, e.g. plants add plant-specific glycans and yeasts add yeast-specific glycans. This changes the behavior of the protein and makes it difficult to control the specific activity of the drug. In some cases production in other hosts even makes the protein immunogenic; the human body will recognize the protein as foreign or non-human. It is desirable to have protein production systems with (1) no non-human glycosylation, and (2) homogeneous glycosylation.

Currently, most therapeutic glycoproteins are produced in mammalian cells. However, costs of mammalian cell fermentation technology are high, and also the strict testing needed as result of the risk for human viral contaminations of the product increase costs. In addition, stable and homogeneous glycosylation is difficult to achieve, especially upon changes in culture conditions, upscaling etc.

Transgenic animals and plants are alternative production platforms. The human therapeutic protein glucocerebrosidase produced in carrot cells (Protallix, Israel) and also, AtrynT derived from the milk of transgenic goats (GTC biotherapeutics, Massachusetts, USA) are approved human drugs.

Yeast and filamentous fungi are under investigation for production of glycoproteins, as these organisms have been extensively used for industrial protein production. One product has been approved so far that was produced in the yeast *Pichia pastoris* (Ecallantide; Dyax, Cambridge, USA).

TRENDS FOR PRODUCTION OF THERAPEUTIC GLYCOPROTEINS IN MUSHROOMS

Mushroom-forming basidiomycetes as cell factories. Mushroom-forming basidiomycetes may represent an interesting platform for protein production. They have a high natural protein-secretion capacity. The mycelium can be grown in liquid fermentations and the fruiting bodies can be formed on solid state fermentation. This latter yields interesting opportunities, e.g. fast and flexible scaling, and conservation of the principle production unit during up- or downscaling (i.e. the individual fruiting body). Mushrooms are grown at large scale for food purposes already. Technology for growing and also for stable storage of production strains is at hand. Spore-less varieties of *Agaricus* and *Pleurotus* could be used for production of drugs to prevent spread of GMO's, and production should be done on sterile synthetic media. Genetic modification of mushroom-forming fungi is relatively easy. Many suitable expression signals are also available.

Time to market of novel transgenic strains of mushroom-forming fungi is expectably relatively short. The gene of interest is transformed. After 16 h colonies can be selected using available selection markers. Within four days, colonies can be screened for protein production and selected for further propagation.

Therapeutic glycoprotein production in basidiomycetes. For the specific purpose of producing ***glyco***proteins mushrooms were shown to have a highly suitable starting situation[3]. This is caused by the following. As indicated, different species produce different glycans, which may change the behavior of a protein to be used in therapy. We have made the surprising observation that mushroom-forming basidiomycetes have an N-glycosylation profile much more similar to humans than yeasts and ascomycetes; in contrast to these species, mushrooms produce no non-human glycans. In addition, the glycans that they do produce can very easily be changed into all possibly desired glycans needed on therapeutic glycoproteins [3].

Humanization of N-glycosylation in mushrooms. By the deletion and introduction of genes involved in formation of glycans we were able to change the N-glycosylation of the model organism *Schizophyllum commune*. We have developed different strains that homogeneously produce specific glycans. For instance, we recently produced a strain that shows >80% of a glycan moiety required for some commercial products on produced proteins. We could also show that this glycan was indeed attached to a human therapeutic protein that we produced in this strain.

CONCLUSION

Mushrooms may be further developed to provide a novel alternative production platform for therapeutic glycoproteins. Intrinsic features of mushroom-forming fungi, longstanding mushroom culturing and processing, the suitable natural N-glycosylation and the possibility to adapt the glycan profile towards highly homogeneous production of human-like glycans strongly support further investigation into this field.

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