1. Vitiligo

Vitiligo (leukoderma) is a skin disorder causing patches of the skin to turn white, resulting from the destruction of melanocytes, responsible for the pigment molecule melanin.

Three major theories have been proposed: (1) a neural theory suggesting an accumulation of neurochemical substances from nerve endings, (2) a biochemical theory suggesting an accumulation of toxic molecules such as reactive oxygen species, and (3) an autoimmune theory suggesting an accumulation of immunoglobulin G (IgG) anti-self antibodies targeting the melanocytes.

There is no cure for vitiligo and current treatments are inefficient, carrying many side effects.

2. Therapeutic approach

We propose a novel treatment for vitiligo, based on bacterial therapy. Bacteria secreting proteins beneficial for vitiligo re-pigmentation are applied onto affected skin. The proteins then relocate through the epidermal skin layers to the melanocytes, where they act towards skin re-pigmentation.

3. Proteins

For the autoimmune theory, proteins masking the autoimmune response by either binding or cleaving the Fc region of IgG antibodies were selected [3, 4]. A protein chimera of the Protein A Z-domain (PZA-Z) and a bacterial IgG protease (IdeS) was proposed.

For the biochemical theory, a few key proteins important for the well-being of melanocytes were chosen. Superoxide dismutase (SOD1) is an important antioxidant converting superoxide into oxygen and hydrogen peroxide [5]. It requires a copper chaperone (yCCS) to gain full activity [6]. Basic fibroblast growth factor (bFGF) promotes melanin synthesis and mitosis [7]. MITF is an important transcription factor for melanocyte development [8]. Tyrosinase catalyzes the production of melanin [9].

4. Cell-penetrating peptides

Skin is an effective barrier for all macromolecules. A needle-free approach for protein delivery into the skin can be achieved by coupling the proteins to cell-penetrating peptides (CPPs), small molecules shown to penetrate into cells and in some cases cross the skin barrier [10]. The CPPs Trans-Activating Transcriptional Activator (TAT), Low Molecular Weight Proteamine (LMWP), and Transportan 10 (TP10) were chosen.

5. Lab work

Cloning

Escherichia coli was selected as our cloning chassis. Assembly Standard 25 was used, as it enables protein fusions [11]. Each CPP was fused to both the N- and C-termini of all proteins due to the uncertainty of their efficiency at each terminus.

Our cloning chassis secreted from bacteria, eliminating the need for protein purification. Also, to obtain full SOD1 activity, operons containing SOD1 and yCCS were assembled.

Protein expression

Protein overexpressions were performed in the expression vector pEX to verify protein production [12]. Resulting expression levels were insufficient for all tested constructs, inconsistent with levels from a similar commercially available vector using the same promoter (Pbad) [13].

Purification

To enable activity and penetration assays, efforts were put into purifying our His tagged proteins using nickel-affinity columns. These attempts were unsuccessful, most likely due to low expression levels.

Activity assays

Activity of SOD1 was investigated using a SOD determination kit (Sigma-Aldrich) [14]. The kit measures the abundance of O2^- which is reversibly proportional to SOD1’s levels. No elevated SOD1 activity was recorded.

IgG protease activity was tested for IdeS by digesting peroxidase-conjugated IgG antibodies and recording the resulting peroxidase activity. Although activity was recorded, His was attributed to unspecific E. coli proteases.

Cell- and skin penetration

Immunohistochemistry-based experiments were designed for testing cell and skin penetration of CPPs. Cultured melanocytes and keratinocytes had been prepared, as well as human artificial skin. Also, skin would be harvested from dead mice.

6. Modeling

To enable regulation of bacterial protein production on skin, a model for a safe induction of protein expression from our Pex vector was developed from work by Ahmadzadeh et al. [15]. The proposed toy model is based on the well-studied E. coli lac operon with lactose as inducer.

7. Transfer to Lactobacillus

In our final model, CPP-bound proteins are naturally secreted from bacteria, eliminating the need for protein purification. Our cloning chassis E. coli does not normally secrete proteins extracellularly. Although a number of treatments have been developed to promote extracellular secretion, secretion tags are not sufficiently efficient and convenient in E. coli [16]. However, they have been used with more success in gram-positive bacteria such as Lactobacillus [16].

In our model we propose application of a bacterial sentiment directly onto the skin. This requires that the choice of bacteria be carefully taken into consideration. Lactobacillus have already been successfully used in bacteriotherapy [17]. This further suggests that Lactobacillus is a good choice as our future cellular chassis.

References

[6] Coexpression of yeast copper chaperone (yCCS) and CuZn-superoxide dismutases in.
[16] Expression vector.
[17] We want to thank.

We want to thank:

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