Journal of Dermatological Science (2008) **50**, 87—98

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Journal of Dermatological SCIENCE

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INVITED REVIEW ARTICLE

Cutaneous gene delivery

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Received 6 June 2007; received in revised form 10 July 2007; accepted 20 July 2007

KEYWORDS
Gene therapy;
Barrier function;
Genodermatosis

Summary Over the past decade, many approaches to transferring genes into the skin have been investigated. However, most such approaches have been specifically aimed against genodermatosis, and have not produced sufficient results. The goal of such research is to develop a method in which genes are transferred easily, efficiently and stably into keratinocytes, especially into keratinocyte stem cells, and in which the transgene expression persists without a reaction from the host immune response. Although accidental development of cancer has occurred in trials of gene therapy for X-linked severe combined immunodeficiency (X-SCID), resulting in slowing of the progress of this research, the lessons of these setbacks have been applied to further research. Moreover, combined with the techniques acquired from tissue engineering, recent developments in our knowledge about stem cells will lead to new treatments for genodermatoses.

The present review summarizes the methods by which therapeutic genes can be transferred into keratinocytes, with discussion of how gene transfer efficiency can be improved, with particular emphasis on disruption of the skin barrier function. It concludes with discussion of the challenges and prospects of keratinocyte gene therapy, in terms of achieving efficient and long-lasting therapeutic effects.

Contents

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0923-1811/$30.00
doi:10.1016/j.jdermsci.2007.07.006

1. Introduction

Efficient cutaneous gene delivery *in vivo* requires precise knowledge of the mechanisms of the barrier function of the skin. The skin is the most superficial part of the body, and is an attractive target for therapeutic gene transfer due to its easy accessibility, ease of observation and easy removal of genetically modified areas if necessary. However, the skin functions as a barrier against invasion by chemicals and pathogens, and it can be difficult for gene transfer methods to overcome this barrier. Many approaches have been tried to improve the efficiency of skin gene transfer, and some of them have been applied to the treatment of diseases by using the skin as a bioreactor or vaccination site. However, such approaches are not effective for clinical treatment of inherited skin diseases. In the present review, we summarize the current state of methods for delivering genes into the skin.

2. Structural barrier against gene delivery into the skin

Human skin is composed of four different layers: basal, squamous, granular and horny layers. Keratinocytes are derived from epithelial stem cells that have the ability to proliferate and differentiate into all of these cell types. Keratinocyte stem cells are thought to be located in the basal layer of the epidermis and in the hair follicle bulge. When a defect appears in the skin, keratinocyte stem cells give rise to daughter keratinocytes that migrate to the skin defect [1].

To efficiently transfer genes into keratinocytes, we must overcome the tissue barrier and the cellular barrier. The tissue barrier physically prevents invasion by foreign bodies from the environment, and the cellular barrier impedes intracellular trafficking. Due to these barriers, only molecules smaller than 500 Da can pass through the skin [2].

The tissue barrier function of the skin is mainly due to three structures of the skin. The first is the horny layer, which is the outermost layer of the skin. It contains dead cells that have differentiated from granular keratinocytes, interspersed within a lipid-rich matrix, and functions as a cutaneous barrier preventing water loss and invasion by chemicals and pathogens. The horny layer is selectively permeable, and allows only relatively lipophilic compounds to diffuse into the lower layers.

The second structure is tight junctions. To reach the underlying epidermis or dermis, molecules passing through the paracellular space must pass specialized junctions. Tight junctions create a primary barrier to diffusion of solutes via the paracellular route. Recently, claudin was identified as a component of tight junctions [3]. In one study, claudin-1-deficient mice died within 1 day of birth, and exhibited severe defects in the epidermal permeability barrier [4].

The third structure is the desmosome, which mediates cell—cell adhesion and these are located in the intercellular space between adjacent keratinocytes. In desmosomes, intermediate filaments are connected to the transmembrane proteins desmoglein and desmocollin, which belong to the cadherin family. The importance of desmosome junctions is demonstrated in the skin disease pemphigus, in which severe bullous lesions caused by production of an anti-desmoglein autoantibodies lead to leakage of body fluids. Desmosomal cadherins not only mediate cell—cell adhesion but also affect epidermal barrier function. For example, transgenic mice that expressed desmoglein 3 throughout the entire epidermis died within the first 10 days of life; presumably, the structure of their horny layer was altered, leading to changes in the permeability barrier [5].

3. Approaches to gene transfer into the skin

The aim of cutaneous gene therapy is to improve skin phenotype via the effects of proteins derived from genes delivered into skin cells. There are two general methods of gene transfer, transient and stable gene transfer, which are selected depending on the basis of the target disease. Transient transgene expression is used for tissue repair, vaccination and anticancer treatment. Stable transgene expression, which involves integration of the transgene into the cell genome, is used for life-long correction of inherited or acquired conditions. In diseases involving recessive loss-of-function mutations such as those involved in many genodermatoses, simple re-introduction of the wild-type gene via viral or non-viral insertion may be sufficient for correction of the phenotype. The two basic approaches to delivery of therapeutic genes into the skin are *ex vivo* and *in vivo* gene delivery.

3.1. Ex vivo transfer of genes into the skin

In *ex vivo* gene transfer, skin obtained from the patient is expanded *in vitro*. A gene is introduced into the expanded skin cells, which are then re-grafted onto the patient. An advantage of this approach is the ease with which keratinocytes can