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We hope that making available the relevant information on Pachyonychia Congenita will be a means of furthering research to find effective therapies and a cure for PC.
Direct injection of naked DNA and cytokine transgene expression: implications for keratinocyte gene therapy

D. Sawamura, M. Akiyama and H. Shimizu
Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Summary
Intradermally injected DNA diffuses into the epidermis and can then enter keratinocytes and become expressed by these cells. Using this method, plasmids containing cytokine genes that have been introduced into keratinocytes can induce a level of cytokine expression sufficient to provide biological effects in the treated skin. Furthermore, transgenic cytokines released from the transduced keratinocytes can also enter the circulation and have downstream effects on other target organs. Thus far, naked DNA injection appears to be a safe, simple, and relatively efficient method that enables genes to be expressed in transplanted human skin on immunosuppressed animals. In humans, keratinocyte gene therapy using the cytokine gene DNA injection method has the potential to become a powerful therapeutic tool for dermatologists in the management of certain inflammatory and other dermatoses.

Introduction
Keratinocyte gene therapy appears to be a promising treatment goal for improving management of intractable genetic, neoplastic and inflammatory skin diseases.¹-³ Several advantages have been proposed in using keratinocytes as targets for gene therapy. First, their ease of accessibility. Epidermal keratinocytes are readily accessible and transgene expression can be easily monitored. If problems arise, the treated area can be excised. Secondly, keratinocytes have several functions including mechanical, structural, and immunological tasks. Such pluripotent cell populations can synthesize mature proteins from a wider variety of transgenes. Thirdly, keratinocytes have a high proliferative potential. This aspect is advantageous for gene therapy, as strategies to target gene transfer to the proliferative compartment of keratinocytes can be used. For these reasons, keratinocytes appear most suitable target cells for gene therapy.

Cytokines are soluble proteins that in pico- or nanomolar amounts induce changes in a variety of cells. It is known that many kinds of cytokines work together to form a complex network regulating both immunological and nonimmunological cell responses, which are probably associated with many disease processes.

As recombinant cytokines are now used clinically to treat patients with cancers and viral diseases, gene therapy using cytokine genes is an extremely worthwhile approach. In the dermatology field, for example, basic fibroblast growth factor is extremely effective for skin ulcers⁴ and several cytokines are expected to be efficient in the treatment of malignant melanoma.⁵ As keratinocytes are known to synthesize many cytokines that modulate keratinocyte functions, keratinocyte gene therapy using cytokine genes has a great potential for the treatment of certain intractable skin diseases. Furthermore, keratinocytes could be used also as bioreactor targets for gene therapy in the treatment of systemic diseases caused by insufficient amounts of circulating proteins: the appropriate genes would be introduced into keratinocytes, the gene products synthesized and secreted into the circulation.¹ ² ⁶

Injection of naked DNA into the skin
Successful keratinocyte gene therapy requires the development of highly efficient gene transfer methods into epidermal keratinocytes. Several viral and nonviral
methods have been reported for in vivo gene transfer into keratinocytes. Of these, injection of naked DNA is the simplest, yet it is still relatively efficient. Plasmid DNA encoding the gene is injected intradermally, it then diffuses through the basement membrane zone into the epidermis, and subsequently is taken into the keratinocyte cytoplasm and nucleus, where the gene is transcribed into mRNA and the protein is synthesized (Fig. 1).

We have injected intradermally a LacZ plasmid complexed with the nuclear protein, high mobility group 1. The transgene expression with the protein was two to three times higher than that of the control plasmid without the protein, suggesting that high mobility group 1 protein increased transfer of the DNA from the cytoplasm to the nucleus. We also added a 69% bovine papilloma virus 1 gene (BPV) DNA into a LacZ expression vector and transferred it into keratinocytes in vivo using the naked DNA method. This fragment contains seven distinct genes (E1–E7) and sustains episomic amplification of the DNA plasmids in which it is cloned. The results demonstrated that the transgene expression of vectors with the BPV DNA was higher than that without the BPV gene, suggesting that the BPV fragment may increase stability of the transferred DNA. These modifications were indicated to enhance the efficacy of the naked DNA method although we may address side-effects associated with the use of these substances in vivo.

In the following sections, the naked DNA method and its applications to the field of keratinocyte gene therapy will be discussed.

Suitable promoter/enhancer cassettes

Transcriptional cassettes that express the therapeutic gene properly in keratinocytes in vivo are critical for effective keratinocyte gene therapy. Several expression cassettes may have to be used, depending on the situation requiring keratinocyte gene therapy in clinical practice. We usually use cassettes that result in high transgene expression levels and ideally choose inducible cassettes that express transgenes for an appropriate period and at suitable levels. In order to establish a battery of useful, robust cassettes that are effective in keratinocytes in vivo, we experimented with plasmids that were constructed by introducing various promoter/enhancer cassette combinations and used these for the direct injection of naked DNA. These experiments identified the best cassette, which contains the cytomegalovirus immediate early enhancer, the modified β-actin promoter and the 3′-flanking sequence of the β-globin gene. Recently, we have transferred inducible promoters, including metallothionein and 1,24-vitamin D₃(OH)₂ dehydroxylase promoters, and have demonstrated that topical application of the inducing agents increases the promoter activity, highlighting a method of controlling of transgene expression by exogenous agents.

Expression of cytokine genes in keratinocytes

When considering keratinocyte gene therapy using cytokine genes in clinical practice, we should evaluate those effects using an in vivo system. Hengev et al. first succeeded in expressing the interleukin (IL)-8 gene and detected its biological effects in the skin. We also cloned cDNAs of several cytokines and have introduced these genes into rat keratinocytes and evaluated the epidermal changes. IL-6 is a cytokine that has pleiotropic effects on a wide range of target cells including growth and differentiation of B lymphocytes, differentiation and activation of T lymphocytes, enhancement of multi-potential haematopoietic colony formation and the induction of acute-phase proteins in the liver. After injection of an IL-6-plasmid, we observed erythema in the treated area of rat skin. The biopsy specimens showed thickening of the epidermis and a lymphocytic infiltration in the upper dermis. These histological changes exhibited a close resemblance to those seen in psoriasis and lichen planus, in which keratinocyte IL-6
production is known to be enhanced. Our results provide the first direct evidence that IL-6 is responsible for the development of inflammatory skin diseases. We have performed similar experiments using tumour necrosis factor (TNF-α) and found apoptotic keratinocytes after the introduction of the TNF-α gene. These data indicate that the injection of plasmids containing cytokine genes can induce sufficient levels of clinically detectable cytokine expression in the treated skin. Recently we succeeded in transferring the 9 kb type VII collagen cDNA, suggest that the size of DNA was not critical using the naked DNA method compared to the other viral methods.

Release of transgenic cytokines into the circulation

Keratinocytes can be used as bioreactors to release transgenic protein products directly into the circulation. Such gene products can have endocrine and systemic effects that may be helpful for disease treatment (Fig. 2). We performed experiments using the IL-10 gene to show these effects. IL-10 is known to play a major role in suppressing immune and inflammatory responses by inhibiting the production of pro-inflammatory cytokines. Recent studies have shown that a local application or intraperitoneal injection of recombinant IL-10 suppresses the effector phase of contact hypersensitivity (CHS). We measured the amount of human IL-10 in rat serum after the injection of a human IL-10 plasmid into rat epidermis. ELISA detected considerable amounts of IL-10 in the serum of the treated rats, but did not do so in the serum of normal rats. To determine whether the transgenic IL-10 could have a suppressive effect on the CHS response in a different area of the skin, we sensitized hairless rats using dinitrochlorobenzene (DNCB) and injected the IL-10 plasmid into the dorsal skin before DNCB challenge on the ear and measured ear swelling after the challenge. Gene transfer of the plasmid significantly inhibited the effector phase of the CHS response. Keratinocyte gene therapy could theoretically be applied to systemic diseases in the future.

Towards this end, we determined whether all transgenic cytokine released from the transduced keratinocytes could enter into the circulation. In total, we analysed a further seven cytokines including IL-4, IL-6, transforming growth factor (TGF)-β1, monocyte chemotactic and activating factor (MCAF), granulocyte-macrophage colony stimulating factor (GM-CSF), TNF-α, and interferon (IFN)-γ. Following introduction of cytokine plasmids, we examined mRNA and protein expression in the treated epidermis and levels of these cytokines in the systemic circulation. These results showed that considerable amounts of both mRNA and protein of all of these cytokines were detected within the local epidermis. However, only the serum concentrations of IL-4, IL-6, IL-10 and TGF-β1 reached high enough levels to exert biological effects, whereas those of MCAF, GM-CSF, TNF-α and INF-γ were very low or undetectable. The properties of individual cytokine genes or transgenic cytokines may cause differences in their systemic expression levels, although the detailed mechanisms have not yet been fully elucidated.

Gene introduction into human skin

To achieve successful keratinocyte gene therapy in the future, fundamental experiments have to be performed using human skin. Hengge et al. transplanted human skin onto a SCID mouse and succeeded in transferring the bacterial β-galactosidase gene into the human keratinocytes in vivo using naked DNA injection. We transplanted human skin onto a nude rat and injected a β-galactosidase expression vector into the human skin, and then detected the activity in the transplanted skin. However, these experiments using the bacterial gene did not demonstrate that the transgenic protein produced from the transgene was biologically active in vivo. We therefore injected an IL-6 expression plasmid into the human skin grafts on nude rats and observed certain changes including epidermal-thickening. Immunohistochemical studies using an anti-proliferating cell nuclear antigen (PCNA) antibody showed strong focal PCNA expression in the area of epidermal thickening where the injection of the IL-6 gene was performed.
Keratinocyte gene therapy using cytokine gene in clinical practice

We have demonstrated that it is possible to treat patients with some intractable skin and systemic diseases by introducing cytokine transgenes into keratinocytes. As mentioned above, various methods for in vivo gene transfer into epidermal cells have been proposed for clinical trials. Recently, two groups have demonstrated that direct injection of lentivirus vector can induce local expression of transgene in the treated site. This method has a great potential to achieve persistent expression of transgene. Of all possible methods, the injection of naked DNA is the simplest and yet it is still relatively efficient. Furthermore, it is an effective method of introducing genes into human skin cells. On the other hand, there are several disadvantages of this method: it gives a short period of transgene expression, transfers a gene to a small number of cells at the treated site and cannot introduce a gene to the cells in the whole body at once.

In terms of clinical trials, are there any critical side-effects with this method? The method does not induce genetic or chromosomal changes in host keratinocytes, as the integration of the transgene into the host chromosome does not take place. Furthermore, it does not induce the production of antibodies to DNA itself because of a lack of any adjuvant. We have already injected naked DNA into about 400 hairless rats and observed no side-effects so far in these animals. Clinically, as physicians, we have routinely injected inactivated bacteria and viruses as vaccination strategies into the skin, all of which contain DNA. This suggests that injection method is safe for human beings, although we should repeat such fundamental experiments to further confirm the level of safety of this procedure.

The method of naked DNA injection may also be performed with muscle. Indeed, injection of a vascular endothelial growth factor (VEGF) plasmid into muscle has been applied to patients with critical limb ischaemia and provides excellent treatment efficacy. We as dermatologists should take further advantage of this relatively easy method of keratinocyte gene transfer. Furthermore, we now know that cytokines possess a variety of biological activities within the skin. We believe that keratinocyte gene therapy with the cytokine gene DNA injection method will be powerful technique in dermatological practice in the near future.

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References


18 Sawamura D, Yasukawa K, Kodama K et al. The majority of keratinocytes incorporate intradermally injected plasmid DNA regardless of size but only a small proportion of cells can express the gene product. J Invest Dermatol 2002; 118: 967–71.


