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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.
Keratinocyte Gene Therapy

Jonathan C. Vogel, MD

Background: Gene therapy is currently being used in clinical trials to treat a variety of diseases. In keratinocyte gene therapy, the gene that will correct the disease by expressing the normal protein or enzyme is inserted and expressed in keratinocytes. Keratinocytes have significant potential, as a target cell of gene therapy, in the treatment of both systemic diseases as well as skin diseases caused by a genetic defect in keratinocytes.

Observations: Although keratinocyte gene therapy is not yet being tested in clinical trials, animal models do exist where keratinocytes are being used to secrete factors such as human growth hormone and factor IX (for hemophilia) into the systemic circulation. Genetic diseases of the skin such as recessive epidermolysis bullosa dystrophica or xeroderma pigmentosum have not yet been treated with keratinocyte gene therapy in animal models.

Conclusions: Keratinocytes have many advantages as a target cell in gene therapy, and progress has been made using animal models. However, the sustained and efficient delivery of factors to the bloodstream by keratinocytes expressing a transgene has not yet been accomplished. Future goals are to obtain adequate levels of the desired factors, hormones, or enzymes for sustained periods of time, either in keratinocytes or in the vascular system.

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The prospect of using gene therapy to treat diseases has become an exciting possibility as the underlying genetic basis of many different diseases, including skin disease, is being elucidated.4 Gene therapy is currently being used to correct diseases in both animal models and human clinical trials.5,6 The most straightforward definition of gene therapy is the insertion of a new gene (transgene) into the target cells to treat a disease. After insertion, the new transgene would express a needed protein product in the target cell and alleviate the disease.

Keratinocytes have not yet been used in gene therapy clinical trials, but they have tremendous potential as a target cell for gene therapy treatment of systemic diseases as well as skin diseases caused by a genetic defect in keratinocytes.5,6 In systemic diseases caused by insufficient amounts of a factor or enzyme in the circulation, such as insufficient factor VIII or factor IX in hemophilia, the gene for the missing enzyme or factor would be inserted into keratinocytes that would then secrete the factor into the systemic circulation. Genetic skin diseases are caused by genetic defects that result in abnormal proteins or enzymes in keratinocytes. One example of a genetic skin disease that could be treated with keratinocyte gene therapy is the disease xeroderma pigmentosum where skin lesions are due to a genetic defect that results in abnormal or missing DNA repair enzymes. Xeroderma pigmentosum could be treated by inserting the normal gene for the DNA repair enzyme in affected keratinocytes.

Animal models where keratinocytes are being used to secrete factors such as factor IX and human growth hormone (hGH) into the systemic circulation do exist. However, there are no examples of animal models where keratinocyte gene therapy has been used to treat genetic diseases of keratinocytes. Nevertheless, transgenic animal models of some keratinocyte genetic diseases (such as epidermolysis bullosa sim...
METHODS OF INTRODUCING GENES

The main method of introducing genes into keratinocytes is referred to as the ex vivo approach. Keratinocytes are harvested from a skin biopsy specimen, and the desired transgene is inserted into the keratinocytes while they are being propagated in tissue culture. These genetically modified keratinocytes are grown into confluent sheets and grafted back onto the donor. The desired end result is a skin graft expressing the new gene at high levels for long periods of time (Figure 1).

Retroviral vectors are retroviruses that have been designed to carry the desired normal gene (transgene), and they are the most common method of introducing genes into keratinocytes in tissue culture. Retroviruses readily infect many different cell types and are able to stably integrate themselves into the cellular chromosomes when the cell divides in a process called transduction. It is this transduction process that allows retroviral vectors to stably introduce transgenes into dividing keratinocytes. In the future, other viral vectors such as adenovirus, which have been modified to carry normal transgenes, increasingly may be used to introduce genes into keratinocytes.

By manipulating keratinocytes in tissue culture, the ex vivo approach can ensure high frequencies of transduction, which means that a high percentage of the genes have been integrated into their chromosomes and are expressing it in a stable fashion. In contrast, keratinocytes containing the desired transgene can be selected for (enriched) in tissue culture prior to reimplantation back onto the donor. It is desirable to have an inserted transgene in a high percentage of the keratinocytes because this will help ensure high levels of expression and persistence of the gene in the graft. Additional clinical examples using the ex vivo approach include treatment of immunodeficiency associated with adenosine deaminase deficiency by inserting the normal adenosine deaminase gene into lymphocytes and the treatment of hypercholesterolemia by targeting a normal low-density lipoprotein (LDL) receptor gene to hepatocytes. In general, the main difficulty of ex vivo approaches is the technical problem of successfully transplanting the transduced cells back into the original donor.

In contrast to the ex vivo approach, the in vivo approach directly introduces the transgene into a target cell of an individual and does not require removal of cells for in vitro manipulation. It is the preferred method for protocols such as delivering the cystic fibrosis transmembrane conductance regulator gene to pulmonary epithelium for treatment of cystic fibrosis. However, the percentage of cells containing the inserted transgene are usually not as high as achieved with the ex vivo approaches and the expression of the gene is usually transient.

Figure 1. In ex vivo therapy, the cells or tissues destined to be genetically modified are removed from the donor, propagated in tissue culture, and transduced with a retrovirus containing the desired transgene. The transduced cells are then reimplanted back into the donor.

ADVANTAGES OF KERATINOCYTE GENE THERAPY

Compared with other target cells of gene therapy, keratinocytes have many compelling and attractive features. Keratinocytes can be easily obtained and readily expanded from small skin biopsy specimens using defined culture conditions and techniques, and these proliferating keratinocytes are efficiently transduced with retroviral vectors containing the transgene. Techniques for grafting epidermis back onto patients are available and constantly improving, circumventing the problem of transplanting the transduced cells back into the donor in ex vivo therapy. Because skin is readily visible, it is easy to assess the status of the graft over time and a biopsy specimen can be easily obtained from the graft to determine if the inserted gene is still present and being expressed. The accessibility of the graft makes it feasible to modulate the expression of the inserted gene and, depending on the regulatory region (promoter) of the inserted gene, expression could be increased or decreased. For example, a steroid-responsive promoter or regulatory region that increases gene expression in the presence of steroids could be used to regulate the expression of the transgene. After insertion into keratinocytes, topical steroids could be applied to increase expression of the steroid-regulated transgene. Easy accessibility of the skin also means that the graft of transduced keratinocytes could be removed if there is an undesired response to the gene therapy treatment. Finally, keratinocytes may be quite proficient at expressing and secreting factors for systemic delivery because they are potent producers of an ever increasing number of cytokines and growth factors.

CHALLENGES OF KERATINOCYTE GENE THERAPY

A number of technical issues need to be addressed when considering gene therapy using keratinocytes. The first of these relates to the rapid proliferation, differentiation, and ultimately, turnover of epithelial tissues, including kera-
tinocytes. Persistence and continued expression of the gene product is important in gene therapy, and, in epidermal gene therapy, the transgene will have to be introduced into early keratinocyte precursor or stem cells to prevent its rapid loss from the skin during normal desquamation. Stem cells maintain an unlimited capacity for self-renewal and give rise to the proliferating keratinocytes that eventually differentiate. If the transgene is present in stem cells, it will also be in the differentiating keratinocytes derived from the stem cell and will not be eliminated by desquamation.

Isolating or enriching for stem cells in tissue culture has been an overriding goal in gene therapy and would enable investigators to insert genes into pure populations of stem cells. Unfortunately, there are no specific molecular or histocchemical markers for identifying and purifying keratinocyte stem cells in vivo or in tissue culture. Identification of stem cells (or stem-cell markers) is also hindered by the lack of good bioassays for stem-cell activity; in vitro assays of stem cells that measure the proliferative capacity of cells in tissue culture have to be assessed with caution. One good measure of keratinocyte stem-cell activity is to follow the graft of transduced keratinocytes over time for the persistence of the transgene. Long-term persistence of the transgene in vivo will indicate that a stem-cell population was targeted. Some progress can be reported in enriching and identifying epidermal stem cells in tissue culture. Investigators found that keratinocytes that expressed higher levels of β3 integrins and adhered rapidly to substrates such as the keratinocyte extracellular matrix and type IV collagen had the highest proliferative capacity and highest colony-forming efficiency in tissue culture. These characteristics of keratinocytes that exhibit stem-cell-like behavior could be used to enrich or isolate stem cells prior to retroviral transduction.

A second consideration for effective keratinocyte-mediated gene therapy is the choice of promoter or regulatory region that regulates or drives the expression of the transgene. It is important to understand that different promoters or regulatory regions can be connected to the transgene. The promoter one chooses will determine what cell type will express the transgene. Some promoters may only allow transgene expression in a specific cell type (keratin promoters in keratinocytes or insulin promoters in pancreatic islet cells), while promoters derived from viruses usually promote expression of the transgene in many different cell types at high levels. Since it is critical to get high levels of expression of the transgene over a long period of time, viral promoters have frequently been used in gene therapy because they are active in many different cell types. Even though viral promoters promote expression at very high levels in tissue culture, when used in vivo, they may be inactivated or down regulated over time leading to decreased expression of the transgene. This phenomenon of in vivo down regulation may be more problematic in some cell types than others, but it has led to the argument that endogenous or cellular (tissue-specific) promoters should be used instead of viral promoters to ensure long-term expression of the transgene in keratinocyte grafts. In keratinocytes, one might want to use promoters of structural genes normally expressed in large amounts such as the keratins or involucrin. Considerable knowledge about different keratin promoters has been accumulated, and it has been demonstrated that keratin promoters will selectively express genes in the epidermis in vivo (in transgenic mice). These endogenous cellular promoters presumably would be active in keratinocytes over a long period of time.

An additional characteristic of keratinocytes that must be addressed is the fact that since the epidermis is not vascularized, proteins produced by transduced keratinocytes and intended for the systemic vascular system must traverse the dermoepidermal basement membrane. We will have to become more knowledgeable about the characteristics that permit diffusion of proteins through the dermoepidermal junction. The relative importance of characteristics such as size, charge, or relative hydrophobicity-hydrophilicity has not yet been determined. Once a better understanding of these parameters exists, it may be possible to predict how efficiently a particular protein will cross the dermoepidermal junction.

A final consideration is whether any adverse effects on the transduced keratinocytes result when a transgene is expressed at high levels. Expressing large amounts of a novel protein in differentiating keratinocytes may interfere with the normal differentiation process and may be deleterious for those keratinocytes with the transgene and select against them. This of course will depend on what protein the transgene actually is producing and is a general problem of all gene therapy approaches regardless of the cell being targeted for transgene expression. To some degree, this concern may be studied in tissue culture but ultimately, in vivo experience with animal models will be required.

ANIMAL MODELS USING KERATINOCYTES TO DELIVER PROTEINS SYSTEMICALLY

Several groups have begun work on animal models using keratinocyte gene therapy to express proteins (growth hormone and factor IX) for the systemic vascular system. The main goals of these studies are to develop the methods necessary to achieve high levels of transgene expression in vivo and to obtain adequate serum levels of the proteins. Another important goal is to obtain expression over a prolonged period of time.

In one study, cultured human keratinocytes were transduced with retrovirus containing an hGH gene that was regulated by a viral promoter (the long terminal repeat
were producing was the normal endogenous apoE. Also, the reappearance of normal basement membrane following the grafting procedure was documented. Passage of apoE through a normal basement membrane is a significant finding since it conclusively proves that keratinocyte-derived proteins can reach the systemic circulation. Since apoE is relatively large (299 amino acids), it is possible that molecular size is not the major limiting constraint of the dermoepidermal barrier.

ALTHOUGH PROGRESS has been made, the sustained and efficient delivery of factors to the bloodstream by keratinocytes expressing a transgene has not yet been accomplished. Future issues to be addressed include identification of the promoters that are optimal for expressing transgenes in keratinocytes; stem-cell isolation and transduction to obtain long-term transgene expression; and understanding how the diffusion of proteins through the dermoepidermal junction is regulated.

NONKERATINOYTE GENE THERAPY INVOLVING THE SKIN

An alternative approach in gene therapy involving the skin is to use skin dermal fibroblasts instead of keratinocytes as a target for gene insertion, followed by subcutaneous engraftment of special collagen matrices that contain the transduced fibroblasts. Skin fibroblasts are an attractive target because they are easily obtained and grown in tissue culture and can produce and secrete large amounts of biologically active factor IX and adenosine deaminase for adenosine deaminase deficiency, following transduction with retroviruses containing these genes. When these transduced fibroblasts are embedded in a collagen matrix and transplanted subcutaneously back into the donor animal, expression of the transgene persists for longer periods of time when endogenous cellular promoters such as dihydrofolate reductase are used instead of viral promoters that tend to be inactivated. Recently, a lysosomal storage disorder in mice (type VII mucopolysaccharidosis, Sly syndrome) was corrected by retrovirally inserting the gene for β-glucuronidase into mutant mouse fibroblasts and engrafting these fibroblasts back into the mutant mice. Therefore, cell types other than keratinocytes have also been used successfully in the skin, and more experiments using skin fibroblasts will undoubtedly be performed in the future.

FUTURE DIRECTIONS: GENETIC SKIN DISEASES

One goal of keratinocyte gene therapy mentioned above is to correct known genetic defects of the keratinocytes themselves. It may be possible to correct these diseases...
because the genetic basis of many epidermal diseases are now being defined. While it will probably never be practical to perform gene therapy on the entire epidermis, areas of skin where the disease manifestations are most severe could be targeted. 49 If the genetic defect involving the keratinocyte is recessive (i.e., disease results when both copies of the gene or allele are abnormal), then the disease should be treatable by inserting and expressing a normal copy of the gene in keratinocytes. Examples of recessive genetic diseases amenable to this form of keratinocyte gene therapy would include xeroderma pigmentosum, which was described above, 61 and the recessive form of dystrophic epidermolysis bullosa caused by a defect in type VII collagen. 43 In recessive dystrophic epidermolysis bullosa, inserting a normal type VII collagen gene in the keratinocytes should prevent the epidermal disease.

Genetic defects that result in dominant negative mutations would be much more difficult to treat with standard keratinocyte gene therapy. In dominant negative mutations, one abnormal gene (allele) and one normal gene (allele) is present in the cell. However, the protein product made by the abnormal gene or allele is dominant and capable of causing disease even though a normal protein produced by the remaining normal gene is also present in the keratinocyte. One example of a disease with a dominant negative mutation is epidermolysis bullosa simplex, where a genetic defect in one of the keratin genes or alleles (either K5 or K14) produces an abnormal keratin that interferes with normal keratin intermediate filament formation and causes skin lesions. 44-48 This abnormal keratin protein has a dominant effect because it can disrupt or prevent keratin intermediate filament formation even though a normal keratin protein is also present. In other words, even though only one of the two keratin alleles is abnormal or mutated, a phenotype of skin disease still results. This presents a problem for the standard gene therapy approach because the insertion of a normal keratin gene inside the cell would not be sufficient in preventing disease because the mutant protein would still be present and would continue to interfere with normal keratin formation (Figure 2). Therefore, the abnormal keratin gene would have to be removed, leaving one normal keratin allele that should produce enough normal keratin protein for correct keratin filament formation. 50-54 (Figure 2). The techniques involved in selectively removing an abnormal gene from a keratinocyte are much more difficult than inserting a normal keratin gene, but such therapies may be feasible in the future. Other epidermal diseases with dominant negative mutations include epidermolytic hyperkeratosis 55-59 and dominant epidermolysis bullosa dystrophica. 60 This list of keratinocyte genetic diseases will almost certainly grow in the near future.

CONCLUSIONS

Although many technical hurdles remain, progress is definitely being made toward keratinocyte gene therapy. Keratinocytes are being used to deliver products systematically in animal models. At present, no animal models exist for gene therapy of specific keratinocyte genetic diseases but these can also be anticipated in the future. Before human trials can be considered, however, adequate systemic levels of the desired factors, hormones, or enzymes will have to be obtained for sustained period of time in these animal models. This goal is not yet a reality, but hopefully, soon will be.

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Reprint requests to Dermatology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 (Dr Vogel).

REFERENCES


Rudolf JM, Green H. Serial cultivation of strains of human epidermal 


Humes ES, Gordon DA, Pershing LK, Williams DI, Taichman LB. Systemic delivery of 


Ollendorf L, Sheng S, Dong G, Sun L, Lavker RM. Existence of a single progenitor 
cell that can be preferentially stimulated to proliferate. J Cell Biol. 1986;207:201-209.


Jones PH, Watt FM. Separation of human epidermal stem cells from transit 


Termin TO, Rosenberg GJ, Osborne WR, Miller AD. Genetically modified skin 

Rajaram K, Lai S, Palmer T, Stock R, Osborne WR. High-level expression of 
human keratin expression in dog skin fibroblasts is not sustained following 


Lasko A, Rosenberg M, Vassar R, Fuchs E. Regulation of a human epidermal 
keratin gene: sequences and nuclear factors involved in keratin-specific 


Duncan JR, Barrandon Y, Green N, Mulligan RC. Expression of an exogenous 

Hurnt J, Lindahl A, Green H. Human growth hormone in the blood of 


Hornick JL, Rand MD, Brinkman KM, Jernig J. Phenotypic correction of factor 

Scharfman RE, Hornick JL, Jernig J. Long-term in vivo expression of retrovirus-mediated 

Palmer TD, Hock RA, Osborne WR, Miller AD. Efficient retrovirus-mediated 
transfer and expression of a human adenosine deaminase gene in diploid skin 

Palmer TD, Thompson AR, Miller AD. Production of human factor IX in animals 

Mouiller P, Bohl D, Heard BN, Dahan C. Correction of hagemost storage in 


ERCC-3 is involved in the repair of DNA crosslinks in cells from 

Langer R, Peterson C. Expression of a human DNA repair gene 

Hoeimann A, Duquesnoy P, Blanchet-Bardon C, et al. Genetic linkage of 

Bonifas JM, Rothman LA, Epstein EHJ. Epidermolysis bullosa simplex: evidence 


Lane EB, Rupp EL, Nuvessa H, et al. A mutation in the conserved helix 

Coulombe PA, Hatton ME, Lott A, et al. Point mutations in human keratin 14 

Lott A, Coulombe PA, McCormick MB, et al. Disease severity correlates with 


Graff EJ, Collinge J, Markus N, et al. A leucine-proline mutation in the H1 

Rymann MD, Nygren J, Selberg S, et al. Genetic linkage of type VII 
collagen (COL7A1) to dominant dystrophic epidermolysis bullosa in families with 