Epidermis as a Secretory Tissue

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Compelling evidence has accumulated during recent years to support a theory that epidermal keratinocytes synthesize and deliver to the extracellular space a wide variety of gene products. Each of these cellular products has unique physical-chemical properties resulting from its biochemical structure that define its a) relative solubility and ability to diffuse under physiologic conditions; b) probability of spontaneous assembly into higher order structures; c) specific affinity for ligands or cell surface receptors of target cells; and/or d) specific catalytic activity. Depending on the concentration of the gene product relative to its receptor or substrate, it may regulate stimulation or inhibition of cellular function. Gene products of keratinocytes known to be delivered to the extracellular space include cytokines, enzymes, and adhesion molecules, which together constitute the majority of the regulatory environment of epidermal tissue. Uninjured skin maintains a steady-state equilibrium of relatively slow metabolism of gene products. By comparison, injury or disease initiates expression by keratinocytes of several potent inflammatory mediators including interleukins (ILs)-1, -3, -6, -7, -8, -10, granulocyte colony stimulating factor (G-CSF), granulocyte-monocyte stimulating factor (GM-CSF), and arachidonic acid metabolites that are associated with acute phase dermatitis and systemic response [1,2]. After whole body irradiation with ultraviolet (UV) light, significantly elevated circulating levels of ILs-1 and -6 correlate with induction of fever in humans [3,4]. Keratinocytes in culture also produce and release parathyroid hormone-related protein (PTHrP), which is believed to participate in regulation of epidermal differentiation [5]. Collagenases and tissue plasminogen activator (TPA) are synthesized and secreted by cultured keratinocytes [6,7], and transforming growth factor beta 1 (TGF-β1) has been shown to stimulate collagenase expression in keratinocytes [8]. Increased expression of intercellular adhesion molecule-1 (ICAM-1) by keratinocytes after injury has been suggested to facilitate T-lymphocyte infiltration in contact dermatitis, perhaps by establishment of a gradient toward the site of injury [9]. Several basement membrane components are secreted by keratinocytes, including the hemidesmosome protein, kalinin [10]. Taichman and colleagues have demonstrated circulating levels of human apolipoprotein E (Apo E) derived from epidermal keratinocytes *in vitro* [11,12], and in serum after grafting of cultured epidermal keratinocytes to athymic mice [13]. From these kinds of examples, it follows intuitively that epidermis may secrete other gene products that may regulate extracellular or systemic responses.

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Abbreviations: Apo E, apolipoprotein E; G-CSF, granulocyte colony stimulating factor; MCAF, monocyte chemotaxis and activating factor; PTHrP, parathyroid hormone-related protein; TPA, tissue plasminogen activator.

WHAT IS SECRETION?

To estimate the extent to which epidermis may function as a secretory tissue, it is important to define secretion. Cytokines or adhesion molecules that are externalized but bind immediately to receptors and act on the cell that produce them are *autocrine,* or *juxtacrine* if acting on cells with which they are in contact. In the case of transforming growth factor alpha (TGF-α), affinities between externalized ligand and receptors for epidermal growth factor (EGF) are sufficiently high that the probability for escape of free TGF-α into the environment is diminishingly small [14]. *Paracrine* action in the skin is exemplified by the complementary exchange of factors in the epithelial-mesenchymal axis [15] that neither remain in their respective tissue compartment nor arrive in the lymphatic or vascular circulations. Heparin-binding growth factors [16] are examples of cytokines that are released but are bound locally to tissue matrix. Gene products of keratinocytes that enter the circulation and induce specific biologic responses in target tissues act by *endocrine* mechanisms. Glandular epithelia (sebaceous and sweat) of the skin are specialized for *exocrine* functions of tissue homeostasis. Most reports of expression of gene products by keratinocytes describe autocrine, juxtacrine, or paracrine activities. But, if secretion is defined as molecular release and translocation, then paracrine, endocrine, and exocrine activities are most consistent with this definition. However, with regard to systemic responses or prospective therapies, endocrine delivery of gene products by keratinocytes is the most pertinent mechanism of secretion.

EPIDERMIS AS AN ENDOCRINE TISSUE

Because uninjured epidermis functions primarily as a self-regenerating protective barrier of the body with the external environment, its participation in regulation of systemic physiology is restricted to control of fluid loss and microbial infection. These homeostatic functions do not require induction of local or systemic responses, and at present, uninjured epidermis is not known to deliver physiologically relevant concentrations of any peptide, lipid, or carbohydrate mediators into the circulation. In sharp contrast, after injury, epidermal cytokines are known to stimulate local and systemic responses, for recruitment of immune effector cells from the circulation [2], and for proliferation and differentiated function of parenchymal cells (e.g., fibroblasts, vascular endothelium, nerve). Local responses of keratinocytes to injury stimulate acute or chronic inflammation, which leads to either resolution or persistence of the local tissue symptoms. However, if the disease involves extensive proportions of the body surface (e.g., major burns, auto-immune conditions, congenital anomalies, anaphylaxis), it has been postulated that the absolute levels of keratinocyte gene products (i.e., IL-1 and other inflammatory mediators) released into the circulation may contribute directly to acute phase response and hypermetabolism [17]. It must also be appreciated that high circulating levels of keratinocyte gene products in disease that is extensive and acute

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may result as much or more from keratinocyte lysis as from actual secretion by intact epithelium.

Notwithstanding the native biology and pathology of epidermis, emerging technologies have opened new possibilities for consideration of epidermis as an endocrine tissue. Advances in culture and transplantation of epidermal keratinocytes [18,19] have demonstrated regeneration of epidermis that restores homeostatic barrier to fluid loss and microbial infection. Although the kinetics of wound healing with cultured epithelium are slower than with native skin grafts, comparable processes of inflammation, hypertrophy of parenchymal cells, and tissue remodelling occur in skin regeneration with cultured skin substitutes. The persistence of autologous cultured keratinocytes after healing of wounds has been substantiated indirectly by indefinite closure of large, full-thickness burns with cultured epithelium. This hypothesis is also supported by the persistence of grafts of cultured human keratinocytes on athymic mice for their entire lifespan [20]. Grafting studies have now been extended to transplantation of cultured skin substitutes containing allogeneic cultured keratinocytes [21]. In contrast to autologous keratinocytes, DNA restriction endonuclease analysis [22] after treatment of skin ulcers or partial-thickness burns suggests strongly that allogeneic keratinocytes engraft, form epidermis, and then are replaced by ingrowth of autologous keratinocytes from the host. However, replacement of allogeneic keratinocytes does not stimulate typical T-cell–mediated graft rejection. This may be accounted for, in part, because allogeneic Langerhans cells, rich in HLA-DR antigens, do not survive in keratinocyte cultures [23]. Rather, it is postulated that allogeneic keratinocytes facilitate wound healing by delivery of cytokines, which promote the growth of fibrovascular tissue, and by the regeneration of basement membrane on which autologous keratinocytes can migrate [24]. If these mechanisms are found to be consistent for treatment of acute and chronic wounds, then cultured allogeneic keratinocytes may serve as vehicles for temporary delivery of therapeutic gene products.

Recognition of the indefinite or temporary persistence of cultured autologous or allogeneic keratinocytes, respectively, has sparked the attention of molecular biologists. An exciting demonstration for feasibility of gene therapy has been made by the grafting to athymic mice of human keratinocytes after transfection in culture with the gene for human growth hormone [25]. Products of the transfected gene have been detected in the transplanted epithelium, but not in the blood of the host. Similar studies have been performed recently by transfection of cultured human keratinocytes with the human β-chorionic gonadotrophin (β-hCG) gene [26]. Accumulation of the transfected gene product in culture medium increased 15 times over a 21-d period. These important studies indicate that gene therapy by transplantation of transfected keratinocytes is feasible. Epidermis regenerated from transfected keratinocytes could function as endocrine tissue for prospective treatment of certain heritable disorders including, but not limited to, hemophilia, diabetes, hypothyroidism, dwarfism, cystic fibrosis, and infertility.

GENETICALLY ENGINEERED EPIDERMIS TO TREAT DISEASE

Somatic gene therapy is an ultimate goal of modern medicine because it promises to correct the biologic basis of disease, rather than to treat its symptoms. However, some clinicians may consider that many congenital diseases are treated safely and effectively by pharmaceutical therapy with recombinant peptides. Hemophilia can be treated with factor VIII, diabetes with insulin, and dwarfism with growth hormone. Although most of these disorders may be managed effectively by periodic medication, the need for regulation of physiologic blood levels of the respective compounds has resulted in the development of complex infusion devices, which impose chronic inconvenience and expense to the patient. Biologic regulation of drug delivery is an essential advantage, and perhaps the greatest challenge of gene therapy.

If native structure, function, delivery, and regulation of endocrine gene products are assumed to be acceptable models for the delivery of keratinocyte gene products from transfected epidermis, then a multiplicity of requirements must be satisfied [27,28]. First, the biologic stability of the transfected genes must be established within the transfected cells, and the stability of transfected cells within transplanted tissue. Second, quantitative and kinetic expression of the gene product must be sufficient to meet the metabolic requirements of the host for resolution of the disease symptoms. Depending on the type of gene vector and its regulation, the proliferative or differentiated state of the transfected cells may influence dramatically expression of the transfected gene. Third, structural homology of the transfected gene product with its native counterpart must be sufficient to provide comparable biologic activity. Fourth, delivery of the peptide to the blood should not result in excessive local accumulations between the secretory epithelium and vascular plexus. And very importantly, negative feedback mechanisms should exist to downregulate delivery of the drug. Native antagonists, or neuro-endocrine mechanisms for downregulation, may not function in transfected epidermis.

Katz and Taichman [12] report in this issue systematic studies to address some of these fundamental questions. Recognizing that dermis acts as a filter between epidermis and vascular circulation, an *in vitro* model was constructed for direct detection and measurement of secretion by cultured human epidermal keratinocytes. Total protein secreted by keratinocytes was shown to be 10–20 $\mu g/10^6$ cells within 24 h. Accumulation of ^{35}S-labeled protein was time dependent, and about 70 molecular species were resolved by two-dimensional electrophoresis. Protein accumulation in culture medium was blocked by exposure to Brefeldin A, an inhibitor of secretion. Using the same model, Barra and colleagues [11] demonstrated convincingly that keratinocyte size correlates inversely with secretion of ^{35}S-Apo E, but was not related to labeling index with bromo-deoxyuridine (BrdU). These findings indicate that expression of certain keratinocyte gene products is independent of cell replication, but localized to the smaller, basal-like cells. This implies that the reduced metabolic rate of uninjured epidermis may not reduce constitutive secretion by keratinocytes. From a modeling perspective, this approach is convincing, but does not allow estimation of the fraction of secreted ApoE that may reach the vascular circulation *in vivo*. And, although Apo E from normal human keratinocytes has been detected in blood of athymic mice grafted with cultured human epithelium [13], detection of gene products of transfected keratinocytes in the blood has not been shown. Assuming that delivery to the blood of gene products from transfected keratinocytes can be accomplished, plasma levels of drug would be directly proportional to the area of grafted epithelium required for therapeutic efficacy. For clinical practicality, the expression of transfected genes would need to be sufficiently high to minimize the area grafted with genetically modified epithelium.

Potentials for permanent correction of congenital disorders have promoted more extensive study of gene therapy in other medical disciplines than in dermatology. Prospective applications of somatic gene therapy include transfection of bone marrow or liver to maximize systemic output [29,30]. Congenital disorders of specific organs (e.g., brain, lung, heart, kidney, muscle) may best be served by targeting transfected genes to respective organs [31]. Although most models of somatic gene therapy use retroviral vectors, they could be eliminated by homologous recombination of a functional gene into a defective gene [28]. Many impressive examples of phenotypic modification have resulted from studies with transgenic mice, but most of these genetic modifications are not heritable, confer sterility, or are lethal [32]. If genetic modifications were stable and heritable, then far-reaching ethical questions would be raised regarding population eugenics. Among these considerations, epidermis is highly probable to be utilized as a delivery device for somatic gene therapy. Because the medical prospects for gene therapy are broad, convergent knowledge from parallel efforts in unrelated disciplines of medicine may be expected to contribute to advances toward the understanding, regulation, and engineering of epidermis as a secretory tissue.

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