Review paper

THE ACTIVATED KERATINOCYTES

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ABSTRACT

The passive protective role of epidermal keratinocytes has been appreciated for a long time, but recently another, active role is also gaining prominence. Activated keratinocytes are migratory and produce as well as respond to a cornucopia of cytokines and growth factors. Interleukin IL-1 and members of the epidermal growth factor family initiate and maintain keratinocytes in activated state, respectively. As a marker of activated keratinocytes we use the expression of keratins K#6 and K#16, proteins not found in healthy skin, but found in wound healing, psoriasis, carcinomas etc. The extracellular signals that activate keratinocytes cause intracellular signal transduction cascades to activate transcription factors necessary for the expression of keratins K#6 and K#16.

KEY WORDS

keratinocyte, activated, epidermal growth factor (EGF), EGF receptor, interleukin 2, keratin 6 and 16

Although it has been appreciated for a long time that epidermal keratinocytes defend us from mechanical injury and desiccation, only recently has their role in immunological defense became apparent, when it was realized that keratinocytes can produce a cornucopia of cytokines, chemoattractants and growth factors (1). Keratinocytes express receptors for many polypeptide factors, respond to signals produced by the immune system and respond to autocrine stimulation (2), because of which keratinocytes are thought to be one part of the immune system. The signaling between keratinocytes and lymphocytes is apparent in those disorders that involve both cell types, such as psoriasis, delayed-type hypersensitivity, atopic dermatitis and cutaneous T-cell lymphoma (3-5). Keratinocytes stimulated by lymphocytes start to produce cytokines and growth factors, which then become autocrine stimulating factors.

In response to epidermal injury, e.g. during wound healing, or in allergic and inflammatory reactions, keratinocytes become "activated": they respond to and produce growth factors and cytokines, they produce components of the basement membrane and they become migratory (l, 6-9). A specific pair of keratin proteins, K#6 and K#16, is expressed in activated keratinocytes, different from the keratin proteins found in the healthy epidermis (10).

a) Markers of Activated Keratinocytes

The initial signal for activation of keratinocytes may be the release of activated IL-1. This cytokine is produced by keratinocytes themselves, stored in the cytoplasm and released when they are injured by mechanical, chemical or immune factors (6).

Once activated, keratinocytes synthesize additional signaling growth factors and cytokines. These include TGFα, IL-3, IL-6, IL-8, G-CSF, GM-CSF, and M-CSF (11). The effects of these signaling molecules produced by keratinocytes are not only paracrine and chemotactic for white blood cells, but also autocrine for keratinocytes themselves. They may lead to secondary effects of keratinocyte activation. Several extracellular markers are specifically expressed by the activated keratinocytes. These include cell surface proteins CD13, CD14, CD68, ICAM-1 and HLA-DR, components of the extracellular matrix, integrins, as well as receptors for both the autocrine factors and those produced by infiltrating immune cells (12).

In a feed-back loop, the increase in the expression of cell surface receptors may augment the initial activation signal. The various signaling molecules may be synergistic or antagonistic with each other. This allows the activated phenotype to be specifically modified, which can lead to different activated phenotypes. In other words, keratinocytes activated in psoriasis are different from keratinocytes activated during wound healing.

b) Signaling via the EGF Receptor

One of the most extensively studied cellular receptor signaling pathways are those involving epidermal growth factor, EGF, and its receptor EGFR (13, 14). Binding of the appropriate ligands to EGFR can activate keratinocytes. Upon ligand binding, the EGFR dimerizes, activating its intracellular protein tyrosine kinase (15, 16). EGFR activation signals are conveyed to the nucleus by a system of protein phosphorylation and dephosphorylation signals, which are eventually conveyed to nuclear proteins that regulate both gene expression and cell division (13). Among the regulated genes are those encoding additional regulators that, when activated, cause major morphological, developmental and differentiation changes. In many cell types, activation of EGFR results in major pleiotropic changes including increased motility, degradation of extracellular matrix and proliferation (17, 18).

In response to the activation of the EGFR, keratinocytes proliferate, degrade components of the extracellular matrix and become migratory (17, 18). In adult epidermis EGFR is primarily expressed in the basal and, to a lesser degree, the deepest suprabasal layers (19).

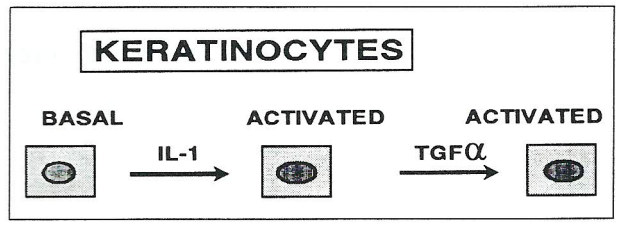

Fig. 1. Keratinocyte Activation
Cytokine IL-1 initiates keratinocytes activation, TGFα maintains keratinocytes in activated state

Several polypeptides can signal to keratinocytes via the EGFR, including transforming growth factor α (TGFα), amphiregulin and heparin binding EGF. TGFα is a polypeptide that interacts with the EGFR with effects similar to those of EGF (14, 20). Epidermal keratinocytes both produce and respond to TGFα (21). The production of TGFα is often elevated in carcinomas (22, 23) and in psoriasis (24). Its synthesis can be induced by tumor promoters (25). Amphiregulin and heparin binding EGF are autocrine activators of keratinocytes found in wound healing and other processes (26, 27). Their function may be modulated by the extracellular matrix proteins.

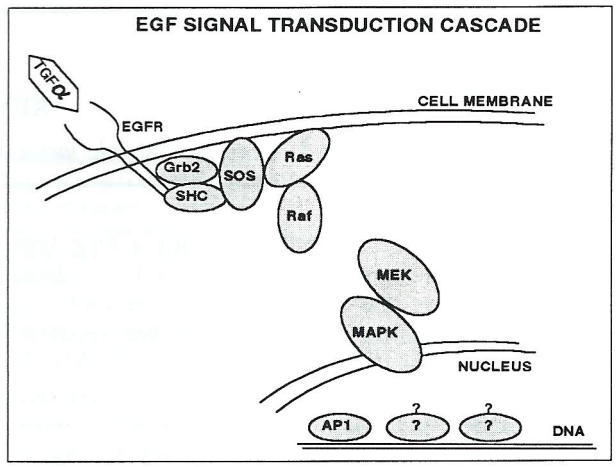

Fig. 2. EGF signal Transduction Cascade
Binding the ligand, such as TGF, activates the receptor, EGFR which then complexes with several proteins such as Grb2, SHC and SOS. These activate Ras, which via Raf, MEK and MAPK, phosphorylates AP1 and other, unknown transcription factors.

Keratin expression has been used as marker of keratinocyte phenotype. Basal keratinocytes express keratins K#5 and K#14, differentiating ones K#1 and K#10, while in psoriasis, wound healing etc. keratins K#6 and K#16 are expressed (28). Thus K#6 and K#16 keratins can be used as markers of activated keratinocytes. Results from our laboratory indicate that the activation of EGFR has no effect on the promoters of simple epithelial, the basal layer specific or differentiation specific keratins. Importantly, however, the expression of K#6 and K#16 keratin genes is induced strongly and specifically. A short DNA segment in each promoter conveys the EGF regulation, and therefore constitutes an EGF responsive element. A nuclear transcription factor specifically binds to these elements. Thus, one of the endpoints of the EGFR signal transduction cascade results in activation of nuclear proteins that bind to EGF responsive elements in the promoters of the K#6 and K#16 keratin genes, markers of activated keratinocytes (29).

c) Initiators of Keratinocyte Activation

Although the growth factors produced specifically by the activated keratinocytes, such as amphiregulin, heparin binding EGF and TGFα, can maintain the cells in an activated state, the initiation of activation must be caused by an endogenous effector, i.e. a signal already present in the quiescent keratinocytes. The cytokine interleukin 1 is the most likely candidate for such a signal. IL-1 is produced and stored in keratinocyte cytoplasm (30-32). Damage to keratinocytes releases IL-1 into the intercellular space, where it can interact with the receptor on neighboring cells. The original damage can be mechanical, chemical, or physical, e.g. by UV light (12). The release of IL-1 causes the activation of neighboring cells, which then produce factors that activate EGFR and thus maintain the activated state. IL-1 induces synthesis of both soluble factors, such as IL-8, and cell surface markers characteristic of activated keratinocytes, such as ICAM-1 (33).

At present it is not clear how activated keratinocytes return to their normal, differentiation state. One possibility is that there is a negative feedback loop within the keratinocyte-lymphocyte interaction. Injury to keratinocytes also causes production of immunosuppressive cytokines such as IL-10 and IL-1ra, which are known to suppress lymphocyte activity and inhibit the IL-1 effect, respectively. They are thought to limit the extent of inflammation and also cause recovery from inflamed state. It is also known that many of the growth factor receptors, such as the EGF receptor, are internalized and degraded after binding to their ligand and conveying the signal to the cytoplasm. This internalization of the receptors may be a more general phenomenon that allows keratinocytes to return to normal differentiation.

ABBREVIATIONS

CD-13	Cell differentiation antigen 13
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte and macrophage colony stimulating factor
HLA-DR	Human Leukocyte antigen DR
ICAM-1	Intercellular cell adhesion molecule 1
IL-1	Interleukin 1
IL-1ra	Interleukin 1 receptor antagonist
M-CSF	Macrophage colony stimulating factor
TGFα	Transforming growth factor α
UV	Ultra-violet

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