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SIGNAL TRANSDUCTION IN KERATINOCYTE PROLIFERATION AND DIFFERENTIATION

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SUMMARY

• *Keratinocytes, a key cellular component for both homeostasis and pathology of the skin, secrete a number of growth factors and cytokines, and their proliferation and differentiation are stimulated by a variety of biological factors. The major mechanism by which keratinocytes respond to extracellular signals is change in protein phospho-rylation. In this review, we focus on factors known to influence keratinocyte proliferation and differentiation, such as epidermal frowth factor family, nerve growth factor, transforming growth factor-fil, insulin-like growth factor-I, keratinocyte growth factor, hepatocyte growth factor, cytokines. A hypothesis for a dual role of epidermal frowth factor in keratinocyte proliferation and differentiation is proposed. (Biomed Rev 1997; 8: 73-85)*

INTRODUCTION

• Cell proliferation plays a fundamental role in the development and maintenance of organisms. Keratinocytes, a key component for both homeostasis and pathology of the skin, secrete and are a target of various biological factors that influence their proliferation (Table 1). Endogenous for the keratinocyte factors inducing proliferation of these cells are epidermal growth factor (EOF), transforming growth factor-a (TGF-a), nerve growth factor (NGF), basic fibroblast growth factor (bFGF), proopiomelanocortin (1), heparin-binding EGF-like growth factor (HB-EGF) (2), amphiregulin (AR) (3), while transforming growth factor-(3 (TGF-(3) is a keratinocyte proliferation inhibitor. Endocrine regulation on keratinocyte proliferation is also performed, in both positive (androgens, corticotropin, bombesin, estrogens, glucocorticoids, insulin, neurokinins, placental extract, progestins) and negative (calcitriol, glucocorticoids, epinephrine, parathyroid hormone-related protein, vitamin A hormone) manner. Keratinocyte differentiation inducers are androgens, corticotropin, calcitriol, glucocorticoids, epinephrine, parathyroid hormone-related protein, vitamin A hormone, thyroxine (reviewed in 4). The basis for differential cell responsiveness to growth factors is complex, and results from the selective receptor expression on the cell surface, the tonic influence of other growth regulatory factors in the biological milieu, and the presence of intrinsic signalling or processing mechanisms. Disruption of these regulatory processes leads to absent (aplastic), inappropriate (metaplastic), or persistent (neoplastic) cell proliferation.

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EPIDERMAL GROWTH FACTOR FAMILY

• EGF is a member of the family of EGF-like molecules, which also includes TGF-a, poxvirus growth factors, AR, epi-regulin, HB-EGF, and betacellulin, all encoded by separate genes. Normal epidermal proliferation and differentiation appear to be integrated and regulated structurally and functionally by a local production of TGF-a and a selective expression of its congate receptor, the EGF receptor, within the germinative layers of epidermis. By contrast, psoriasis is

Selected abbreviations

CDK - cyclin-dependent kinase
EOF - epidermal growth factor
FGF - fibroblast growth factor
IL - interleukin
INF - interferon
MAPK - mitogen-activated protein kinase
NFKB - nuclear factor KB
NGF - nerve growth factor
PKC - protein kinase C
TGF - transforming growth factor
VEGF - vascular endothelial growth factor
1,25(OH),D_3 - 1,25 Dihydroxyvitamin D_3

associated with unbalanced proliferation and differentiation, evidenced by both enhanced expression of TGF-a and persistent expression of EGF receptor in the upper stratified layer of psoriasis epidermis (5).

- **Epidermal growth factor**

EGF was the second growth factor to be discovered (Stanley Cohen, 1962) and purified, and is considered a prototype growth factor. It is one of the most thoroughly investigated peptide growth factors, and in many ways has been a model for revealing the biological andbiochemical mechanisms of growth control. EGF binds to its receptor, inducing receptor dimerization, activation of receptor tyrosine kinase activity, and autophosphorylation of multiple tyrosine residues in the cytoplasmic domain of the receptor (6). These residues, and surrounding amino acids serve as binding sites for the SH2 domains of several proteins such as She (a prolin-rich adaptor protein), the p85 subunit of phosphatidylinositol 3' kinase, phospholipase C (PLC), and the protein tyrosine kinase Src. , The tyrosine phosphorylation site on She serves for binding of a praline-rich binding motif of the SOS exchange protein, leading to activation of Ras, which in turn binds to the Raf protein. Raf phosphorylates and activates the serine/threo-nine kinase MEK. Activated MEK phosphorylates and activates the mitogen-activated protein kinase (MAPK), which regulates multiple intracellular pathways involving transcrip-tional activation, cell proliferation, and cytoskeletal rearrangements (7).

• Epidermal growth factor as a differentiation factor in keratinocytes

EGF is a well known keratinocyte proliferation (8) and migration (9) factor. Our recent studies suggested that, in addition to these effects, EGF seems to play a role in epidermal differentiation. We demonstrated that in contrast to human dermal fibroblasts (10), EGF activated MAPK activity in human keratinocytes by a protein kinase C (PKC)-dependent pathway (11). This result was somewhat surprising, in view of the ability of PKC to control negatively human keratinocyte growth in response to EGF (12). In addition, evidence strongly supports the necessity of PKC activation in the initial signal ling events of keratinocyte differentiation (12-15). EGF can stimulate the transcription of the immediate-early response genes *c-fos* and *c-jun* (6). It has been shown that *c-fos* and *c-jun,* whose expression is controlled by PKC (13), are involved in the differentiation process of keratinocytes, rather than their proliferation (16). In addition, *c-fos* and *c-jun* expression (17) and PKC activity (18) are decreased in psoriasis. Thus, EGF, PKC, MAPK cascade, *c-fos,* and *c-jun* may play a role in human keratinocyte differentiation. Our results also suggest that MAPK (in our experimental conditions) is probably not in volved in triggering EGF-stimulated proliferation of keratino cytes. In support of this idea is the finding that pituitary extract (a commonly used mitogen for the serum-free culture of human keratinocytes) stimulated the proliferation of human keratino cyte cultures, while inhibited the basal MAPK activity. EGF was not able to stimulate proliferation, but stimulated MAPK activity. EGF also abrogated the stimulatory effect of the pituitary extract, restoring the MAPK stimulation (unpub lished results).

The effects on keratinocyte growth or differentiation may be dependent on mitogen concentrations. In human keratinocytes, EGF at 25ng/mL, a concentration commonly used in cell cultures, did not stimulate but actually decreased the incorporation of ^{3}H-thymidine into DNA, while EGF greatly enhanced the antiproliferative activity of 1,25 dihydroxyvitamine D_3 ($1,25(OH)_2D_3$) (19). In HN6 and HN30 keratinocytes, EGF can stimulate or inhibit cell growth depending on its doses. p21 (WAF1/CIP1/SDI1) is a likely mediator of EGF-induced growth-inhibition, probably through mechanisms involving sequestration of the PCNA protein, and inhibition of cyclin-dependent kinase (CDK) activity (20). A biphasic DNA synthesis response to EGF and TGF-a has been observed. PKC down-regulation was also dependent on the growth factor concentrations (21). Bovine pituitary extract may have stimulatory or inhibitory effects on keratinocyte growth, depending upon its concentration as well as the presence of other components and their respective concentrations (22).

The effects of the mitogens could be also dependent on the cell culture state. Thus, the direction of the proliferative response to $1,25(OH)_2D_3$ in cultured human keratinocytes depends on the extracellular Ca^{2+} concentration and the degree of cell differentiation (23). In subconfluent human keratinocytes expressing basal cell phenotype, an autocrine growth factor of the EGF family suppressed the terminal differentiation (24). It is possible that in confluent andpostconfluent cultures, corresponding to a differentiation phenotype, EGF acts on

Table 1. *Stimulators and inhibitors of keratinocyte proliferation*

Factors	References
Stimulators of keratinocyte proliferation	
Epidermal growth factor, Heparin-binding EGF-like growth factor	2, 8, 9, 12
Transforming growth factor-α, amphiregulin	1, 3, 9, 49
Insulin	72
Insulin-like growth factor-I	44, 72
Fibroblast growth factor, acidic	27
Fibroblast growth factor, basic	1, 27
Keratinocyte growth factor	12, 47
Interleukin-1, -3, -4, -6, -8	1, 49, 53-55
Nerve growth factor	1, 33, 36, 38
Granulocyte-macrophage colony-stimulating factor	1
Bombesin	71
Neu differentiation factor (heregulin)-β	45
Bradykinin	64-66
Hepatocyte growth factor	48, 49
Prostaglandin E_2	76
1,25-Dihydroxyvitamin D_3	73, 75
Prolactin, growth hormone	4, 67, 68
Androgens, corticotropin, estrogens, neurokinins, placental extract, progestins	4
Substance P, substance K	68
Vasoactive intestinal polypeptide	70
Thapsigargin, lysophosphatidic acid, H_2O_2, UVB, thalidomide	2, 60, 78, 79
Inhibitors of keratinocyte proliferation	
Transforming growth factor-β1/β2	39-42, 79
Interferon-α/β	27, 56
Interferon-β	27, 56
Tumor necrosis factor-α	27, 80
Neu differentiation factor (heregulin)-α	45
1,25-Dihydroxyvitamin D_3	19, 23, 73
Epidermal growth factor	11, 20, 24, 25
Parathyroid hormone-related protein fragment 107-111	63
Interferon-γ	1, 57, 60
Gangliosides	82
Glucocorticoids, epinephrine, vitamin A hormone	4
Eicosapentaenoic acid	77
Catecholamines	83
Adenosine, adenine nucleotides	84
Platelet-activating factor	81

keratinocyte differentiation *via* a PKC-M APK dependent pathway (11), and the inhibition of this pathway leads to a stimulatory effect ofEGF on keratinocyte proliferation (12). Ourhypothesis was recently supported in papers showing that Ca^{2}* increased TGF-cdevels, which in turn stimulated tyrosine phosphorylation of the EOF receptor and PKC-8 during keratinocyte differentiation (25). Also, EOF supressed the expression of the suprabasal keratins (early markers of terminal differentiation), while enhancing involucrinandtransglutaminase(latemarkers)(26).

- **Transforming growth factor-a**

TGF-a is synthesized in many human tissues and is structurally related to EGF. They are both highly homologous peptides with substantial solution structure similarity dependent on conserved cystein residues, and have an antigenic relationship to EGF as well as binding capacity to its receptor now called the EGF/TGF-a receptor (27). EGF and TGF-a affect cultured keratinocytes mainly by increasing their rate of migration (9). They may elicit divergent effects in certain biologi-

cal processes (TGF-cc was more effective thanEGF in promoting colony dispersion, *in vitro* wound closure, and single-cell migration), but retain similar responses in another context (EGF and TGF-a evoked identical profiles of DNA synthesis) (28). TGF-a induced interleukin-6 (IL-6) inapermanenthumankeratinocyte cell line (HaCaT) possibly by transcriptional activation of nuclear factor(NF)-KB, andNF-IL-6 (29). Low basal levels of TGF-a but not of EGF are produced by keratinocytes and may contribute to the regulation of skin growth. TGF-ods likely to be an autocrine growth factor for normal human keratinocytes as well as for trans-formed cells. There is general agreementmat TGF-a isoverexpressed inthepsoriaticskin.

Keratinocytes regulate production of TGF-a through a den sity-dependent mechanism (30). In human foreskin keratino cytes, a biphasic DNA synthesis response to EGF and TGF-a has been observed: low concentrations stimulated DNA syn thesis, while high levels (>3ng/mL) decreased DNA synthe sis. However, when keratinocytes were pretreated with PKC inhibitors, DNA synthesis remained elevated even at high growth factor concentrations (21). Vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) plays a major role in diseases associated with increased vascular permeabil ity and angiogenesis. It is a 40-45 kDa glycosylated cytokine that potently and selectively enhances microvascular endo thelial cell proliferation and migration. Overexpression of VEGF is evident in many pathologic skin disorders, in particu lar psoriasis. TGF-a has been shown to induce VEGF expres sion in normal human epidermal keratinocytes *in vitro* (31). In normal human epidermal keratinocytes TGF-a activated the nucleartransription factors API, C/EBPp, andNFicB, while EGF activated API and NFKB, and bo vine calf serum -SP1 and API (32).

a Heparin-binding EGF-like growth factor

HB-EGF belongs to the EGF family, and is an autocrine mito-gen for keratinocytes that is overexpressed during wound healing and inflammatory processes. Low doses ultraviolet B (UVB), and H_2O_2 induce HB-EGF expression and keratino-cyte proliferation (2).

- **Amphiregulin**

AR (initially called keratinocyte autocrine factor) is a hepa-rin-binding, heparin-inhibited member of the EGF family, the predominant autocrine growth factor for cultured human kera tinocytes. AR is specifically expressed during epidermal de velopment *in utero,* and has also been shown to be increased in psoriatic epidermis and other epidermal proliferative disor ders. In the skin of transgenic mouse whose epidermis is targetedto overexpressAR,AR exerts distinctactivities (leukocyte infiltration) fromtransgenicTGF-aorothercytokines(3).

NERVE GROWTH FACTOR

- NGF isknowntoinducegrowthofnervefibers,andplays acrucialrole in the development and maintenance of sensory and sympathetic neurons. NGF is synthesized in the skin and basal keratinocytes express the low-affinity nerve growth factor receptor (NGFR).Culturedhumanepidermalkeratinocytes synthesize and release NGF (33). NGF could act as a cytokine in human skin and take part in disorders ofkeratinocyte proliferation. It is known to be mitogenic for keratinocytes, anditseffectonkeratinocyteprolifera-tion is mediated through the phosphorylation of its high-affinity tyrosinekinase receptor (trk). UVB can inhibit bothNGF synthesis and function in human keratinocytes (34). NGF has recently been shown to be overproduced by keratinocytes inpsoriasis, andmay be involved in the epidermal hyperplasia seen in this disorder, playing a pivotal role in the development and maintenance of psoriatic lesions (35). Normal human keratinocytes in culture express both the low- and high-affinity NGFR at mRNA level. NGF significantly stimulated the proliferation of normal human keratinocytes in culture in a dose-dependent manner, its effect mediatedby the high-affinity NGFR. Moreover, NGF mRNA was expressedinnormal human keratinocytes, and increasing amounts of NGF were secretedby keratinocytes during growth (3 6). NGF may play animportantrole in epidermal-dermal interactions, rein-nervation, and reepithelisation occuring in wound healing and psoriasis, aT-cell mediated disease with intrinsic wound healing phenotype(77).

Despite the fact that several cell types residing permanently or transiently in the skin, are sources and/or targets of NGF, little is known about the role of NGF in skin development, physiology and disease. 10-200 ng/mL of NGF stimulated epidermal keratinocyte proliferation in organ-cultured C57 BL-6 mouse skin in the telogen phase of the hair cycle. Follicle keratinocyte proliferation was stimulated by 100 ng/mL NGF in telogen skin organ culture, but this concentration inhibited both epidermal and follicle keratinocyte proliferation in organ culture of anagen skin (38).

TRANSFORMING GROWTH FACTOR-ftl

• TGF-P superfamily comprises a broad variety of poly-peptides with multiple biologic activities: TGF- (31-5, bone mor-phogenic proteins, decapentaplegic, Vg 1, mullerian-inhibiting substance as well as activins and inhibins. TGF-p increases proliferation of dermal fibroblasts and stimulates deposition of extracellular matrix molecules and integrins by these cells. By contrast, it inhibitits proliferation of keratinocytes, and induces their differentiation. TGF-J3 is secretedby most cultured cells in an inactive (latent) form, converted to the active polypeptide by proteases. The binding of the latent polypeptide to a mannose-6-phosphate receptor facilitates the activation of TGF-P by proteases, including plasmin and cathepsin. Once activated, TGF-P binds to a transmembrane

serine/threonine kinase receptor (type I or type II), responsible for signal transduction. TGF-p 1 (a homodimeric 25 kDaprotein) is known to inhibit epithelial cell growth by inducing a G1 cell cycle arrest, and suppression of *c-myc* transcription has been implicated in the mechanism ofTGF-pl inhibition ofkeratinocytegrowth(39). Treatment of early passage human keratinocytes and HaCaT cells with TGF-P1 resulted in formation of a DNA binding complex between theretinoblastomasusceptibility(Rb)-relatedproteinpl30 andE2F. This correlated with inhibition of cell cycle progression at G1, and suppression of the E2F-regulated *cdc2* gene, p 130 is a downstream target of TGF-p 1 and apossible mediator of the G1 -airest(40). Thetranscriptional suppression of *c-myc* is important in the TGF-p 1 growth inhibition pathway. The inhibition of B-myb and cyclin A (growth factor-inducible products that are critical regulators of G1 /S transition) may contribute to the late G1 -arrest causedbyTGF-pl. Theseeventsmaybelinkedthroughtheactions of Rb or a Rb family member (41).

Normal progression through Gl is promoted by the activity of CDK4 and CDK6, which are inhibited by the protein p 16INK4. p!5INK4B is a new member of the p!6INK4 family, and its expression is induced approximately 30-fold in human keratinocytes after treatment with TGF-P 1, suggesting that pi5 may act as an effector of TGF-P 1-mediated cell cycle arrest. The gene encoding pi5 is located on chromosome 9 adjacent to the pi 6 gene, at a frequent site of chromosomal abnormality in human tumors (9p21) (42). The inhibition of human keratinocyte growth by $1,25(OH)_2D_3$) is associated with a time- and dose-dependent increase in the concentrations of TGF-P2 but not TGF-pl (43).

INSULIN-LIKE GROWTH FACTOR-1

• Insulin-like growth factor-I (IGF-I) is a single chain polypeptide of 70 amino acids that belongs to insulin-related proteins, which also include IGF-II, insulin, and relaxin. It is a primary mediator of growth hormone action, produced by many tissues as an autocrine/paracrine regulator of local tissue events. In the skin, IGF-Iis amajorkeratinocytemitogen, which also augments the mitogenic effects of EOF. It is produced by melanocytes and fibroblasts but not by keratinocytes, suggesting that it may be a paracrine regulator of keratinocyte proliferation. In the epidermis, the IGF-I receptor is expressed predominantly by basal keratinocytes, while in psoriasis expression is increased and also found in suprabasal keratinocytes. However, IGF-I alone is not sufficient for keratinocyte growth, requiring also EGF and bovine pituitary extract added (44).

NEU DIFFERENTIATION FACTORS

• The family ofNeu differentiation factors (NDF or heregulins) includes several glycoproteins, whose receptor binding domain displays two variants, a and p, which bind two receptor tyrosine kinases, ErbB-3 and ErbB-4. Different NDF isoforms induce distinct growth regulatory effects on cultured keratinocytes *via* direct activation of ErbB-3. Certain isoforms were reported to induce growth arrest and differentiation of mammary tumor cells, whileother breast cancer cell lines respondedmitogenically. Normal EGF-dependent epithelial cells, Balb/MKkeratinocytes, can undergo eitherproliferation or differentiation inresponse to various NDF isoforms. p Isoforms ofNDF had mitogenic effect, which was significantly weakerthan the maximal response to EGF. By contrast, ocNDF isoforms exerted almost no mitogenic activity, but were sufficient to maintain keratinocytes in culture (45).

KERATINOCYTE GROWTH FACTOR

• Keratinocyte growth factor (KGF) is a paracrine factor secreted by fibroblasts that stimulates keratinocyte growth. It belongs to the FGF family. The paracrine effect of KGF on epithelial cells is due to an alternative splicing variant of the FGF receptor-2, which can bind KGF or acidic FGF (aFGF) (12). In monolayer cultures, KGF expression by quiescent fibroblasts was stimulated by serum, EGF, and bFGF. However, in dermal equivalents, the collagen matrix negatively modulated KGF mRNA expression, and then only the serum slightly stimulated KGF expression. The induction of KGF gene was mediated by at least 2 different signalling pathways, involving PKC and cyclic adenosine monophosphate (cAMP) (46). In subconfluent keratinocytes, KGF did not show any significant increase of proliferation compared to EGF. However, at confluency, KGF stimulated keratinocyte proliferation stronglier than EGF. At higher concentrations, EGF showed greater potency than KGF (47).

HEPATOCYTE GROWTH FACTOR

• Hepatocyte growth factor/scatter factor (HGF/SF) has also been reported to promote keratinocyte migration and proliferation. It is a heterodimer composed of a 69 kDa oc-chain and a 34 kDa p-chain and was originally identified as a potent mitogen for hepatocytes, but now is thought to be a pleiotropic factor acting as mitogen, motogen, and morphogen for various epithelial cells. HGF is produced by dermal fibroblasts, and its c-Met receptor (a transmembrane protein tyrosine kinase) is expressed by basal keratinocytes in wounded skin (48). HGF can stimulate keratinocyte collagenase-1 and stromelysin-1 production in a dose- and matrix-dependent manner. The regulation of collagenase-1 expression is trans-criptionally mediated, and requires tyrosine kinase and PKC activities (49). In mouse keratinocytes (PAM-212), HGF and EGF, both potent mitogens for these cells, stimulated the DNA synthesis and tyrosine phosphorylation of MAPK (48).

CYTOKINES

• Cytokinesare 10-50kDaproteinmoleculeswhichexert regulatory effects on a variety of cell types, including immune cells. Inflammatory cytokines produced by keratinocytes are IL-1 a, IL-1 (3, IL-3, IL-6, IL-8, granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF),granulocyte-macrophage colony-stimulatingfactor (GM-CSF), hepatocyte stimu lating factor III, tumornecrosis factor-a(TNF-a). IL-1 stimulates keratinocyte proliferation and chemotaxis, while IL-3, IL-6, and GM-CSF are autocrine growth factors for keratinocytes. The epidermis is also affectedby products of activated T-cells, com prising a large deal of the infiltrates seen in dermatoses; one such product, interferon (IFN)-y, is an importantnegative growthregu-lator of keratinocyte proliferation. IL-1, IL-3, and GM-CSF are alsoactivatedT-cellproducts(l).

Upon stimulation, keratinocytes produce a range of cytokines including IL-6, IL-8, TGF-a, and GM-CSF. IL-6, IL-8, and TGF-a stimulate keratinocyte proliferation. The psoriatic keratinocytes were as responsive as normal keratinocytes to the stimulatory effects of TGF-a and IL-8, but were less susceptible to stimulation by IL-6, and both normal and psoriatic keratinocytes were generally unresponsive to GM-CSF (49). The proliferative effects of IL-6 were mediated indirectly *via* the EGF/TGF-a receptor, and autocrine overexpression of IL-6 may be limited in psoriatic keratinocytes (50). These findings suggest that IL-6 is not a major autocrine mitogen for psoriatic keratinocytes (51).

IL-4 is known as a cytokine playing a central role in the regulation of immune response. Two cytoplasmic proteins, STAT6 and IL-4-induced phosphotyrosine substrate/insulin receptor substrate! (4PS/IRS2), are activated in IL-4 signal transduc-tion. STAT6 has also been demonstrated to be important for effects of IL-13 related to IL-4 (52). IL-4 stimulated the keratinocyte proliferation *in vitro* by inducing *c-myc* expression (53).

Increased expression of IL-6 and IL-8 may indicate transbasal cell carcinoma cell lineormation of normal keratinocytes in locally aggressive basal cell carcinoma (54). IL-8 is a potent proinflammatory molecule present in high amounts in psoriatic skin. There, it may play an important role in the keratinocyte hyperproliferation, and the neutrophil and T-cell infiltration associated with the disease. IL-8 synthesis may be regulated by PKC either positively (by phorbol 12-myristate 13-ac-etate) or negatively (by IL-1J3 or TNF-a) (55).

IFN represent a family of polypeptides with antiviral activity initially subdivided on the basis of their cellular source, IFN-oc secreted mainly by leukocytes, IFN-J3 - by fibroblasts, and IFN-y- by T-cells. IFN-oc/P are also synthesized and secreted by human keratinocytes, and may be involved in host defence mechanisms. UVB irradiation, IL-1 a, TNF-a, and lipopolysac-charide increased IFN-oc/P mRNA expression in human keratinocytes (56). IFN-7 is a potent inducer of squamous differentiation in normal human epidermal keratinocytes, its effect characterized by a 95% or more decrease in the expression of two growth regulatory genes, cdc2 and E2F-1, and a 7-15-fold increase in the expression of two squamous cell-specific genes, TGase-1 and cornifm. It was hypothesised that in normal human epidermal keratinocytes, an irreversible growth arrest precedes the expression of the squamous-differentiated phenotype. The action of IFN-y on the expression of squamous cell-specific genes was antagonized by retinoic acid and TGF-P1. Both factors are potent suppressors of TGase-1/cornifm induction; however, they did not prevent the commitment to irreversible growth arrest (57). Activated T-cells produced INF-y (58), which activated sphingomyelin hydrolysis in keratinocytes, thus generating ceramide, which may function as a cytokine second messenger in keratinocytes
(59). The induction of inducible nitric oxide synthase (iNOS) by INF-7 in human keratinocytes was differentiation-dependent. This effect was antagonized by INF-oc, the latter also inducing growth arrest but not differentiation in these cells
(60). IFN-y markedly increased the expression of mRNA of Fas (a cell membrane protein known to mediate apoptosis) in SV40-transformed human keratinocytes, thus inducing a Fas-dependent programmed cell death in these cells, augmented by 12-0-tetradecanoyl-phorbol-13-acetate (TPA) *via* activation of PKC (61).

MACROPHAGE-STIMULATING PROTEIN

• Macrophage-stimulatingprotein(MSP)wasoriginallyiden-tified as an inducer of murine peritoneal macrophage responsive-ness to chemoattractants. It is structurally related to HGF and plasminogen. The product of RON, a protein tyrosine kinase cloned from a human keratinocyte library, is the MSP receptor, expressedby keratinocytes (62).

PARATHYROID HORMONE-RELATED PROTEIN FRAGMENT

• Low concentrations of the C-terminal parathyroid hormone-related protein (PTHrP) fragments, PTHrP 107-111 and PTHrP 107-139, stimulated membrane-associated PKC, but not adenylate cyclase or an internal Ca^{2}^ surge, in early passage human and BALB/MK-2 murine keratinocytes. The maximally PKC-stimulating concentrations ofPTHrP 107-111 stopped or stimulated BALB/MK-2 keratinocyte proliferation depending on whether the cells were, respectively, cycling or quiescent at the time of exposure(63).

BRADYKININ

• Kinins are potent vasoactive oligopeptides thatmay act as mediators in a variety of inflammatory skin diseases. The mammalian bradykinin is produced as a cleavage product by the action of kallikrein-like enzymes at sites of inflammation and injury, and causes pain, vasodilation, and smooth muscle contraction. Like the neuropeptides bombesin, vasopressin, and endothelin, ithas been reported to act as a growth factor, and implicated in various physiological andpathological conditions. Bradykinin is one of the key mediators of inflammation, and a weak mitogen. In a human keratinocyte cell line (HaCaT),bradykinin promoted expression of the proto-oncogenes *c-fos, c-jun,* and *c-myc,* but this did not correlate with cell proliferation (64). Bradykinin induced the generation of inositol 1,4,5-trisphosphate, which caused Ca^{2+} mobilization negatively modulated by PKC in primary cultured human keratinocytes (65). Stimulation of its receptor led to activation of a tyrosine kinase activity through a PKC-dependent pathway (66).

WEUROPEPTIDES

• Brain hormones may directly regulate skin functions. It was recently postulated that prolactin from anterior pituitary acts as a neuroendocrine modulator of skin epithelial cell proliferation carrying signals between the brain and the skin (67). Physiological concentrations of prolactin markedly stimulated the proliferation of newborn foreskin keratinocytes in serum-free medium (68). Dermal fibroblasts could be a potential local source of prolactin in the skin (69). Growth hormone receptors have been demonstrated in the epidermis, suggesting that keratinocyte proliferation could be also under control of this hormone (4). Unmyelinated cutaneus C-fibers secrete neurokinins of the tachykinin family, such as substance P and substance K, which were also found to promote keratinocyte growth (4).

GUT HORMONES

• Neuropeptides of gastrointestinal tract, termed *gut hormones,* have also been found in human skin. Vasoactive intestinal polypeptide stimulated cell proliferation and adenylate cyclase activity (70), while bombesin increased thymidme incorporation by cultured keratinocytes (71). Insulin, the only endocrine hormone absolutely required for support of keratinocyte proliferation, transmodulated EGF receptor expression in a dose-dependent manner without altering EGF-binding activity (72).

1.25-DIHYDROXYVITAMIN D

• $1,25(OH)_2D_3$ has been proposed as aphysiologic regulator ofkeratinocyte growth and differentiation. Ithas abiphasic effecton human keratinocyte growth. In medium free of serum, sterol, and pituitary extract, $1,25(OH)_2D_3$ inhibited keratinocyte growth at concentrations greater than 10^{-8} M whereas it stimulated growth of these cells at concentrations lower than 10^{-9}. In serum-containing medium, the hormone inhibitedkeratinocyte growth at all concentrations, effect associated with a decrease in C.-myc mRNA. Increased PKC activity and translocation of this enzyme to the plasmalemmahavebeenreported. The growth-inhibitory effectof $1,25(OH)_2D_3$ involved, at least in part, an increase in TGF-l32 release (43).

Physiological concentrations of $1,25(OH)_2D_3$ are required for optimal mouse keratinocyte growth. In proliferative serum-free culture system, physiological (picomolar) concentrations of this hormone stimulated proliferation of primary mouse epidermal keratinocytes, while at higher (nanomolar to micromolar) doses, growth was inhibited. The response to $1,25(OH)_2D_3$ was mediated by a slow transduction pathway, such as that activated by its intracellular receptor (73). The effects of $1,25(OH)_2D_3$ in cultured human keratinocytes depended also on the extracellular Ca^{2T} concentration, and the stage of differentiation (23). $1,25(OH)_2D_3$ (10^{-12} M to 10^{-8} M) caused a dose-dependent increase of PKC activity in the so-lubilized membrane fractions of cultured human keratinocytes, and in the cytosolic fractions of cultured human fibroblasts (74). $1,25(OH)_2D_3$ stimulated DNA synthesis *via* sequential activation of Raf and MAPK (75).

PROSTAGLANDIN E

• Prostaglandins (PG) are lipid-soluble hormone-like com pounds that bind to cell-surface receptors. They are synthesized from arachidonic acid, and at least 16 different PG in nine different chemical classes, designated PGA-PGI, are recognized. Four different genes encoding heterotrimeric G-protein linked PGE receptors have recently been cloned. The receptors are linked to stimulatory and inhibitory cAMP andphosphatidylinositol second messengerpathways. Ligands forEP2, EP4, and EP3c receptors were detected in non-confluent keratinocytes. EP3c-mediated signalling decreased cAMP, while EP2 and EP4 increased it. Growth of non-confluent keratinocytes was inhibited in 50% by treatment with indomethacin, and restored after addition of PGE_2 (but not other PG), which propably acted through the EP2 receptor (76).

EICOSAPENTAENOIC ACID

• Eicosapentaenoic acid (EPA) is a 20-carbonpolyunsatu-rated fatty acid that is structurally very similar to arachidonic acid. Itisfoundalmost exclusively inmarinefish oil. EPAhasaninhibitory effect on the human keratinocyte growth *in vitro.* Psoriasis is rare among Greenland Eskimos who eat EP A-rich diets, indicating that EPA may exert a protective effect against this disease (77).

THAPS1GARGIN

• Thapsigargin, a sesquiterpene lactone, is the constituent of the plant *Thapsiagarganica* responsible for its potent skin- irritating effect. Thapsigarginhas demonstrated tumor-promoting activity in a two-stage model of skin carcinogenesis, andbecause it did not bind or activate PKC, the compound has been classified as anon-TPAtype turn or promoter. Thapsigargin activates cells *via* arapid and very marked increase of intracellular free Ca^{2}~ concentration ([Ca^{2}~].) by intracellular stores, without hydrolysis of phospho-inositides. This is due to a specific inhibition of the endoplasmatic reticulum ATPase, which tips the equilibrium in favour of Ca^{2+} release from this intracellular store. Acute thapsigargin-induced [Ca^{2}~], elevation inhibitedkeratinocyteproliferation, possibly due to induction *ofc-fos* and inhibition of protein synthesis. In contrast, sustained elevation of [Ca^{2+}]. in response to thapsigargin was associated with increased keratinocyte proliferation *in vitro* and may, at least in part, mediate thapsigargin-induced epidermal hyperplasia and tumor promotion *in vivo* (78).

LYSOPHOSPHATIDIC ACID

• Lysophosphatidic acid(LPA) is abiologically active phospholipid known to have growth factor-like activity on fibroblasts. The effects of LPA are comparable with all-trans-retinoic acid (RA), a structurally unrelated lipid that has been previously shown to induce both TGF-oc and TGF-J3, andhas pronounced effects on keratinocyte proliferation and differentiation. Treatment of cultured human keratinocytes with LPA or RA elevated TGF-oc production 4-8-fold. A number of structurally related phospholipids did not mimic the TGF-oc-inducing activity of LPA. LPA is mitogenic for keratinocytes, and its stimulatory effect could be blocked with an antibody to the EGFATGF-areceptor, suggesting that LPA-stimulated keratinocyte proliferation is mediated by TGF-oc. LPA and RA also induced both the active and latent forms of TGF-p in cultured keratinocytes. TGF-P induction may mediate the LPA effects on keratinocyte differentiation, namely inhibi tion of proliferation (confluent cultures) and increased involucrin synthesis. Also, after LPA treatment, dramatic morpho logical changes were observed. Mechanistic studies suggested that LPA activates both pertussis toxin-sensitive and insensi tive signalling pathways involving PKC activation and protein tyrosine phosphorylation. The effects of LPA on TGF-a and TGF-P production by keratinocytes likely have *in vivo* rele vance as concluded from rodent studies with topical LPA treatment (79).

TUMOR NECROSIS FACTOR-PC

• Tumor necrosis factor-a (TNF-a) was found to induce certain keratinocyte differentiation products, including plas-minogen activator-inhibitor type 2 (PAI-2), which plays a role in keratinocyte terminal differentiation. PAI-2 is synthesized in high amounts in the superficial layers of normal human epidermis, and is concentrated along the periphery of keratinocytes in the granular layer. In cultured human keratinocytes, PAI-2 was induced by elevated $Ca^{2"}$ concentration, promoting a more differentiated epidermal phenotype. TNF-a increased PAI-2 mRNA and protein in keratinocytes incubated in both low Ca^{2+} medium (in which most cells have a basal-like phenotype) and high Ca^{2T} medium (in which cells stratify and express various differentiation makers) (80).

PLATELET-ACTIVATING FACTOR

• Platelet-activating factor (PAF) is apotent lipid mediator involved in inflammation which interacts with a specific receptor. Cultured keratinocytes and fibroblasts were reported to produce PAF, and the PAF receptor is constitutively expressed in the epidermis. When PAF (0-1 OOnM) was added to human keratinocyte cultures, cell proliferation was inhibited dose-dependently, effect recovered by a PAF antagonist, WEB2086 (81).

GAMGLIOSIDES

• The alteredpatterns of ganglioside expression during density-dependent growth inhibition, oncogenic transformation, and embryogenesis suggest that gangliosides, sialylatedmembrane glycolipids, may influence cell proliferation and differentiation. Gangliosides of the "b" synthetic pathway, including GM3, GD3, and GD1 b, inhibit the proliferation of cultured keratinocytes without affecting differentiation. In contrast, significant induction of keratinocyte differentiation by GTlb (a more highly sialylated ganglioside of the "b" synthetic pathway that is also present in cultured keratinocytes) has been noted, as evidenced by early desmosome formation, increased cornified envelope formation, and expression of involucrin and the differentiation-specific keratin Kl. The addition of GT1 b did not cause a shift in [$Ca^{2"}$]. or affect PKC activity. Thus, alterations in the membrane concentration of GT Ib, a minor ganglioside component of the keratinocyte membrane, may modulate keratinocyte differentiation (82).

CATECHOLAMINES

• Human epidermis has capacity of total catecholamine biosynthesis. Catecholamine biosynthesis depends on the substrate supply (i.e. L-tyrosine), and an essential cofactor for tyrosine hydroxylase and phenylalanine hydroxylase (6R 5,6,7,8 tetrahydrobiopterin). In the epidermis, epinephrine leads to *in vivo* expression of ahigh density of p2-adrenoreceptorsinkeratinocytes. Upon adrenergic stimulation with epinephrine, a significant increase of both [Ca^{2}*]. and cAMP occurred. Further, a connection has been demonstrated between catecholamine biosynthesis, p2-adrenoreceptor expression, Ca^{2+} flux, and the differentiation of keratinocytes inhuman epidermis (83).

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ADENOSINE AND ADENINE HUCLEOTIDES

• Adeninenucleosidesandnucleotidescanregulateavariety ofphysiological processes, including inhibition of cellproliferation, and immune cell function. They may be important regulators of keratinocyte growth *in vivo.* Adenosine and adenine nucleotides abrogate exogenous EGF-dependent or independent keratinocyte proliferation at submillimolar concentrations. Such compounds may find an application in the treatment of epidermal proliferative disorders, in which the EGF receptor signalling pathway is overactivated(84). Adenosine, adenosinemonophosphate, adenosine diphosphate, and adenosine triphosphate may be released into the extracellular space at sites of tissue destruction by the disrupted cells, or at sites of inflammation by eosinophils and neutrophils. Transport of adenosine (or adenosine enzymatically-derived from adenine nucleotides) into keratinocytes severely limits their ability to produce cytokines (IL-la, IL-8) in response to phorbol ester or TNF-oc stimulation (85).

H_20_2 UVB, THALIDOMIDE

• UVB and H_2O_2 induce HB-EGF expression, and thus keratinocyte proliferation (2), EGF receptor phosphorylation (86), and TGF-p-inducible early gene expression (87). Thalidomide is a powerful therapeutic agent for pyoderma gangrenosum and other skin wounds. It modulated human keratinocyte proliferation *via* a chemokine-dependent pathway, stimulating secretion of IL-8 known to increase human keratinocyte proliferation and migration (

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