The EGF/TGFα Receptor in Skin

Lloyd E. King, Jr., M.D., Ph.D., Ronald E. Gates, Ph.D., Christa M. Stoscheck, Ph.D., and Lillian B. Nanney, Ph.D.

Departments of Medicine (Dermatology) (LEK, REG, CMS), and Plastic Surgery and Cell Biology (LBN), Vanderbilt University and Department of Veterans Affairs Medical Centers, Nashville, Tennessee, U.S.A.

In responsive cells, all known effects of epidermal growth factor (EGF), transforming growth factor α (TGFα), and related proteins are mediated through binding to a specific membrane receptor. The EGF/TGFα receptor is a single-chain glycoprotein (1186 amino acids) containing three functional domains: 1) an extracellular, glycosylated portion that binds EGF; 2) a small transmembrane portion; and 3) a cytoplasmic portion that has the intrinsic tyrosine kinase activity and multiple sites that can be phosphorylated. When EGF binds to the receptor its intrinsic tyrosine kinase is activated, resulting in increased phosphorylation of intracellular tyrosine residues both on the receptor (autophosphorylation sites) and on exogenous proteins involved in regulating cellular functions. Site-specific mutagenesis has established that the tyrosine-kinase activity of the receptor is essential for nearly all of the effects of EGF including its ability to elevate cellular calcium levels and to induce DNA synthesis. The binding of EGF and the kinase activity of the receptor are both regulated by the phosphorylation of the receptor on specific threonine/serine sites catalyzed by other protein ki-nases. Specific lipids such as sphingosine also can regulate kinase activity. Tyrosine-specific phosphoprotein phosphatases and perhaps proteases must be important in terminating the cellular response to EGF.

In human skin, the response to EGF/TGFα is determined by the location and number of receptors and is modulated by processes affecting the binding affinity, internalization, and tyrosine-kinase activity of the receptor. Specific patterns of EGF binding and of immunoreactive receptors characterize normal growth and differentiation and these are altered during the abnormal growth and differentiation associated with diseases such as psoriasis, viral infections, neoplasms, and paraneoplastic syndromes. It is not clear if the altered patterns reflect the consequence of the disease or are the cause of the disease. As a cause, the EGF receptor may have undetected point mutations that result in internalization and degradation defects, aberrant phosphorylation, and dephosphorylation or abnormal glycosylation. *J Invest Dermatol 94:164S–170S, 1990*

The interest in receptors for growth factors as well as those for related cytokines, interleukins, interferons, and cellular adhesion molecules has exploded in the past few years. As specific information becomes available about one type of receptor, there is a marked increase in activity aimed at determining if this same mechanism is operative for related receptors. Nowhere else has this been more true than in studying the tyrosine-kinase–containing growth factor receptors such as those for EGF, insulin, and platelet-derived growth factor (PDGF). There are a number of excellent reviews on specific aspects of the structure of the EGF receptor [1–4].

There is a growing tendency to group growth factors into the family of epidermal growth factors [3] (Fig 1). Given the complexity of growth factors, interleukins, interferons, and related molecules, it may be useful to think of proteins that act through specific membrane receptors as receptor-activated multifunctional peptide systems (RAMPS). EGF was initially thought to be the only ligand that bound to the EGF receptor, but this proved not to be true. TGFα and certain viral growth-factor–like proteins from *Vaccinia* and *Molluscum contagiosum* also bind to the EGF receptor and may mediate internalization of and infection by these viruses. These findings imply that other such receptors have alternate ligands that may be very important in the pathogenesis of some human diseases. Similarly, growth factors and related cytokines often have opposite effects on differing cell types and induce different cellular processes or functions. As an indication of the multi-ligand and multi-functional aspects (RAMPS), the specific receptor involved in EGF effects will be designated as the EGF/TGFα receptor. This review will focus on the biochemical processes that affect EGF/TGFα receptors in human skin as a model for how these and related RAMPS may be regulated in health and disease.

STRUCTURE OF THE EGF RECEPTOR

The pioneering experiments of Stanley Cohen and his colleagues at Vanderbilt University showed that the EGF-binding activity could be solubilized while still retaining the EGF-stimulated phosphorylation activity [5]. Subsequently, the receptor was affinity purified to homogeneity, antibodies to it were developed, it was cloned, and its amino acid sequence was deduced [1–4]. From this sequence it was apparent that the EGF/TGFα receptor is a single-chain protein of 1186 amino acids that contains three functional domains: the amino terminal extracellular portion of 621 residues; a small transmembrane portion of 23 amino acids; and a carboxyl terminal cytoplasmic domain of 542 residues [2]. The extracellular part of the receptor binds EGF, and has two regions rich in cysteine and 12 potential asparagine (N-linked) glycosylation sites, most of which are glyco-

```
          1         5          10         15
mEGF          N S Y P G C P S S Y D G Y C L N G G V
hEGF          N S D S E C P L S H D G Y C L H D G V
rTGF    V V S H F N K C P D S H T Q Y C F H - G T
hTGF    V V S H F N D C P D S H T Q F C F H - G T
hA      . . . K K K N P C N A E F Q N F C I H - G E
VVGF    . . . P A I R L C G P E G D G Y C L H - G D
SVGF    . . . K H V K V C N H D Y E N Y C L N N G T
MVGF    . . . K R I K L C N D D Y K N Y C L N N G T
                      a                 b

         20          25         30         35
mEGF    C M H I E S L D S Y T C N C V I G Y S G
hEGF    C M Y I E A L D K Y A C N C V V G Y Y I G
rTGF    C R F L V Q E E K P A C V C H S G Y Y V G
hTGF    C R F L V Q E D K P A C V C H S G Y Y V G
hA      C K Y I E H L E A V T C K C Q Q E Y Y F G
VVGF    C I H A R D I D G M Y C R C S H G Y Y T G
SVGF    C F T I - A L D . . . P F C V C R I N Y E G
MVGF    C F T V - A L N . . . P F C A C H I N Y G G
        a                      b   c

         40          45         50         55
mEGF    D R C Q T R D L R W W E L R
hEGF    E R C Q Y R D L K W W E L R
rTGF    V R C E H A D L L A
hTGF    A R C E H A D L L A
hA      E R C G E K
VVGF    I R C Q H V V L V D Y Q R S E N P N T
SVGF    S R C Q F I N L V T Y
MVGF    S R C Q F I N L I T I K
            c
```

Figure 1. Sequence of epidermal growth factor and related peptides. The sequences of mouse EGF (mEGF), human EGF (hEGF), rat TGF (rTGF), human TGF (hTGF), human amphiregulin (hA), vaccinia virus growth factor (VVGF), Shope fibroma virus growth factor (SVGF), and Myxoma virus growth factor (MVGF) were aligned to allow maximal homology. *Dashes* represent spaces inserted for alignment and *dots* represent omitted amino acids. Invarient residues between the peptides are boxed. The VVGF, SVGF, and MVGF sequences have been truncated at the amino end (near residue 1). The letters *a, b,* and *c* show which cysteines should be matched.

sylated [6,7]. The intracellular portion of the receptor has the intrinsic tyrosine-kinase activity as well as several tyrosines that are phosphorylated by this kinase activity (autophosphorylation sites). Additionally, the intracellular portion of the receptor has regulatory sites that can be phosphorylated by other protein kinases to modify binding and kinase activity for the EGF receptor [1–4]. The domains of the receptor function in concert to initiate the response to EGF. When EGF/TGFα or related molecules bind to the extracellular domain, a signal is induced across the plasma membrane that activates the intrinsic kinase of the intracellular domain resulting in phosphorylation of itself and of other proteins on tyrosine residues.

FUNCTIONS OF THE EGF/TGFα RECEPTOR

The Extracellular Domain   This portion of the receptor clearly functions as the recognition or binding site for EGF and related ligands. Clues to the specific roles of the extracellular portion are provided by analyses of cells with specific alterations is this portion of the receptor. Oncogene products, for example, can be altered versions of normal cellular tyrosine kinases including growth-factor receptors. One such oncogene product found in a virally induced leukemia in chickens is an aberrant or abnormal form of the EGF/TGFα receptor, v-*erb*-B. Because the extracellular portion of the normal EGF/TGFα receptor is not retained in the v-*erb*-B oncogene product, its tyrosine kinase activity cannot be regulated by this portion [8]. Similar to v-*erb*-B, in vitro deletion mutants of the EGF receptor in which the extracellular domain [9,10] is removed also transform cells. How the absence of the extracellular domain leads to cell transformation is not clear. Its absence may remove an inhibition on the kinase that is normally relieved when EGF binds or may prevent internalization and degradation of the truncated receptor.

When EGF binds to the extracellular portion of the receptor, there is rapid clustering of EGF-receptor complexes into coated pits that are internalized. Ultimately, the majority of the receptors are degraded in lysosomes even though many other cell-surface receptors such as transferrin and the LDL receptor are not degraded but are instead recycled back to the plasma membrane [11]. Although the structural feature of the EGF receptor that prevents its recycling by sending it to the lysosome for degradation is not known, an interesting possibility is the mannose phosphate detected on the EGF/TGFα receptor [12]. Because mannose phosphate on extracellular proteins or on newly synthesized enzymes targets them to the lysosome through interaction with the mannose-phosphate receptor [13], a similar mechanism involving the mannose-phosphate receptor may route the EGF receptor to the lysosome after internalization. The detection of mannose phosphate on the EGF/TGFα receptor is the first example of this modification occurring on a transmembrane protein, so potentially this proposed lysosomal targeting mechanism may occur in other RAMPS.

The Transmembrane Domain   Very little is known specifically about the role of the transmembrane portion of the receptor. A recent study [14] of a deletion mutant that produces only the intracellular portion of the receptor found that the kinase retained its activity without the membrane-spanning region. Perhaps, like the glycophorin receptor in human erythrocytes, this transmembrane portion may be affected by the lipid environment and modulate the activity of the intrinsic tyrosine kinase or events important in receptor clustering and internalization.

The Intracellular Domain   Much more is known about the intracellular portion of the receptor, which contains the major autophosphorylation sites and the intrinsic tyrosine kinase. Site-specific mutations that eliminate the kinase activity of the receptor prevent virtually all of the effects of adding EGF to cells including raising of cellular levels of calcium and inducing DNA synthesis [15,16]. In contrast, site-specific mutations of the three principal autophosphorylation sites that are on tyrosines near the carboxyl teminus of the receptor affect only the sensitivity of the cellular response to EGF [15,17,18]. Furthermore, deleting all three of the principal autophosphorylation sites does not prevent the response to EGF [19]. Although the role of tyrosine kinases as oncogenes has been relatively well-studied, there are potentially many other abnormalities of EGF-receptor expression that could induce abnormal growth and differentiation. Although these have not been detected at present, naturally occurring point mutations of the receptor could result in internalization and degradation defects or in aberrant regulation of kinase activity, thereby causing human diseases.

SUBSTRATES OF THE EGF/TGFα RECEPTOR PROTEIN KINASE

The activated EGF/TGFα receptor also phosphorylates other cellular proteins on tyrosine residues, thereby transducing the extracellular signal into one that regulates cell proliferation and differentiation. There are a number of potentially very important enzymes and structural proteins that can be phosphorylated by the EGF receptor [3,4,8]. Of the various proteins that are substrates for the EGF receptor, one of the most abundant has been purified and studied at Vanderbilt and is called p35 or lipocortin I [20]. The precise role of p35 in keratinocyte function is unclear, but its distribution can be determined with antibodies. Studies have shown that p35 is present in the normal human epidermis and its appendages [21]. The distribution of immunoreactive p35 is similar to that of EGF receptors [21]. In involved psoriatic epidermis, the p35 distribution is also abnormal and increased (unpublished observations). Whether the p35 distribution reverts to normal when the psoriasis starts clearing has not yet been studied, although it is expected that the distribution will change in concert with that of the EGF receptor.

It is well-known that protein kinase C (PKC) (Fig 2) is activated after the addition of EGF. Several other serine- and threonine-specific protein kinases that are activated after the addition of EGF to intact cells, including casein kinase II, have also been identified
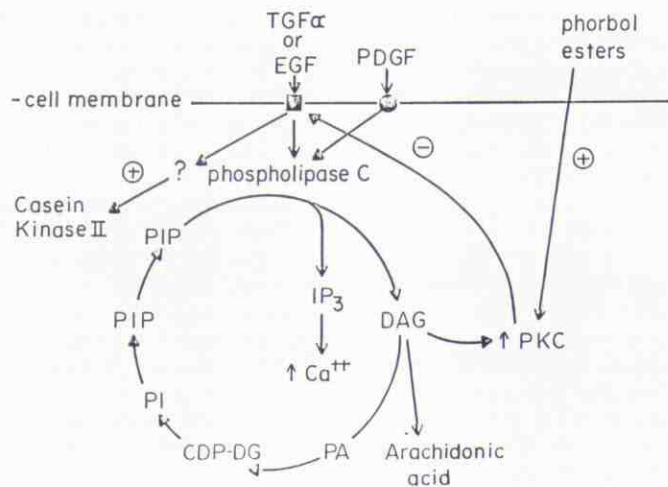
Figure 2. Interaction of the EGF receptor with inositol phospholipids. Interaction of TGFα or EGF with the EGF receptor (E) activates the receptor, which in turn activates phospholipase C. Phospholipase C produces demyo-inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) from phosphatidyl 4,5-biphosphate (PIP2). IP3 and its metabolites induce the release of intracellular stores of CA⁺⁺. DAG and CA⁺⁺ activate protein kinase C (PKC), which can phosphorylate the EGF receptor and thereby decrease receptor-kinase activity. DAG is also catabolized to arachidonic acid, which is a precursor for prostaglandins. Alternatively, DAG can be converted to phosphatidic acid (PA), which is then converted to cytosine diphosphate-diacylglycerol (CDP-DG) to phosphatidylinositol (PI), to phosphatidylinositol-4-phosphate (PIP), to phosphatidylinositol-4-5-biphosphate (PIP2). The platelet-derived growth-factor (PDGF) receptor (O) also has the capacity to activate phospholipase C. Phorbol esters directly activate PKC. The figure also shows that the EGF receptor interacts with other proteins in addition to phospholipase C. Activation of casein kinase II has been shown to be independent of protein kinase C activation [24].

[22–24]. Because there is no evidence of an EGF-dependent phosphorylation of tyrosine on any of these protein kinases, their activation must be by indirect mechanisms. The details of one such a mechanism have been recently suggested for the activation of PKC. As shown in Fig 2, a key enzyme whose action leads to increases in both calcium and diacylglycerol, each of which are activators of PKC, is phospholipase C. This enzyme hydrolyzes phosphotidylinositol 4,5-bisphosphate to produce diacylglycerol and inositol 1,4,5-trisphosphate (IP₃), the intracellular messenger that increases calcium levels inside the cell. Several lines of evidence suggest that phospholipase C is activated when the EGF receptor kinase is activated. First, the tyrosine-kinase activity of the EGF/TGFα receptor is required for nearly all of the consequences of adding EGF to cells, including the very early rise in intracellular calcium levels [15,16]. Second, alterations in phosphoinositol metabolism including increased IP₃ occurs very rapidly after adding EGF to intact cells [25–27]. Third, a specific form of phospholipase C (PKC II) is phosphorylated on tyrosine when the EGF receptor kinase [28–30] or the PDGF receptor kinase [31] is activated. This phosphorylation may activate PLC either directly or indirectly but has not been directly demonstrated. Indirect activation may result from the increased (or decreased) association of phosphorylated PLC with another activator (or inhibitor), such as has been suggested but not proved for a G protein [32]. A large number of extracellular messengers whose receptors do not have protein-kinase activity strongly activate PLC, whereas EGF is only a weak activator [33]. Thus, the indirect mechanism seems more likely. With either mechanism, the result is the increased synthesis of IP₃ and diacylglycerol [25–27] and the activation of PKC.

Because EGF and TGFα increase the motility of many cell types, including keratinocytes, and causes a pronounced reorganization of the microfilaments, cytoskeletal proteins may also be phosphoryl-

ated on tyrosine. One such cytoskeletal protein appears to be ezrin, an 80-kDa protein enriched in the microvillar core of brush borders and found in actin-containing surface structures of many cells [34]. After cells are treated with EGF, ezerin is phosphorylated on both tyrosine and serine residues in a time course consistent with its appearance in the membrane ruffles and microvillar-like structures induced by EGF [34].

LOCALIZATION OF THE EGF/TGFα RECEPTOR

Normal Human Skin The significance of EGF/TGFα receptor abnormalities in psoriasis and other hyperproliferative skin diseases can only be understood in the context of their metabolism in normal skin. To detect EGF/TGFα receptors, one must use more than one experimental method because each method identifies a different aspect of the receptor [3]. Binding of radioactive EGF may underestimate the concentration of EGF receptors because the receptors may be occupied or inaccessible to external EGF. Similarly, the presence of immunologically reactive receptors does not prove that the receptors were biochemically active (binding of EGF or kinase activity) [35,36]. Given these caveats, we have found that in normal adult human skin EGF/TGFα receptors are found in high concentrations on basal keratinocytes and decrease in number as the keratinocytes differentiate to the normal stratum corneum [35]. This ¹²⁵I-EGF binding pattern in human epidermis has been confirmed by several laboratories and in other species [36–38]. The predominant localization of EGF/TGFα receptors to basal keratinocytes suggests that these receptors are present on the rapidly dividing cell population (Table I). However, EGF/TGFα receptors are also found in normal adult human skin on cells that do not rapidly divide, such as sebocytes, arrector pilorum muscles, myoepithelial cells, and vascular smooth muscle [35]. In fact, the highest concentration of EGF/TGFα receptors are found on the slowly dividing eccrine sweat duct cells that actively transport ions [35]. The association of EGF receptors with epithelial cells actively transporting ions (choroid plexus, ependyma, parietal cells) is an important clue to its normal, perhaps multifunctional role, because EGF identified by its other name, urogastrone, inhibits gastric acid secretion [39]. The rapid effect of EGF on the Na⁺/H⁺ antiporter and cytoplasmic alkalinization in cells may provide the biochemical basis of these findings [40].

Abnormal Human Skin When skin is cut, release of growth factors from platelets including EGF, PDGF, TGFβ, and cytokines from skin cells occurs [41]. Provided that the microvasculature is relatively intact, the release of these growth factors, interleukins, interferons, and related inflammatory mediators produces a coordinated repair of dermal and epidermal wounds. Potentially the EGF/

Table I. Localization of Immunoreactive EGF/TGFα Receptors

Organ/System	Cell Type
Epidermis and appendages	Basal keratinocytes Eccrine sweat-duct cells Hair follicle outer root-sheath cells Basal sebocytes
Dermis	Apocrine myoepithelial cells Vascular smooth-muscle cells Arrector pilorum muscle cells
Liver	Hepatocytes
CNS	Ependymal cells Neurons Purkinje cells Pyramidal cells
Placenta	Amnion epithelial cells "Decidual cells"
Placenta	Syncytial trophoblasts
Smooth muscle	All organs and vessels except portions of large muscular arteries

TGFα receptor activity could be specifically altered by this physical trauma or other pathologic events such as sunburns, bacterial infections, and cancers. These changes in receptor localization and activity could be due to multiple factors including the depth of the wound. Therefore, we studied the changes in EGF/TGFα distribution in a number of abnormal skin conditions.

In skin biopsies from sites of active proliferation of normal human skin and various skin diseases, an overall pattern emerges. The EGF/TGFα receptor persists in the upper stratum of the epidermis that retains its parakeratotic features such as in psoriasis and normal adult mucosa and in rapidly growing neonatal epidermis [35,42]. There are exceptions to this broad generalization. For example, in seborrheic keratoses of long-standing duration that are a benign expansion of basaloid keratinocytes, EGF/TGFα receptors can be found primarily confined to the basal and suprabasilar keratinocytes [42]. Interestingly, in seborrheic keratoses that are clinically documented to be actively growing, EGF/TGFα receptors are found throughout the epidermis ([43], unpublished observations). In fact, the EGF/TGFα receptor distribution very accurately predicts the growth activity of these lesions, whereas routine histology does not. Moreover, when the skin lesions revert to normal due to treatment, the EGF/TGFα receptor pattern reverts to its predominantly basilar location just as in resolving psoriasis. This change in EGF/TGFα receptor distribution was also seen in resolving paraneoplastic skin lesions of a patient after excision of his melanoma [43]. Coincident with this change in receptor pattern, urinary TGFα was also decreased [43]. These findings demonstrate an active, reversible modulation of EGF/TGFα metabolism in vivo.

In psoriasis and a few other skin diseases, there appears to be a persistent growth-stimulatory signal occurring as a result of the obvious reaction to external injury (Koebner reaction) or not so obvious immune-mediated reactions. Whether a primary epidermal response such as acanthosis is seen or dermal fibrosis is the predominant response to such pathologic events must be regulated by the interplay among coagulation factors, cytokines, growth factors, and other products of inflammatory cells and the resident cells in the skin.

How these factors or combination of factors affect the synthesis, expression, internalization, persistence, and activity of the EGF/TGFα receptor and other RAMPS is a very active topic of research as it provides clues to homeostatic regulatory mechanisms. For example, persistent expression of EGF receptors and two to four times increase in receptors may indicate a failure to decrease the synthesis of new EGF/TGFα receptors or a failure to internalize and degrade already expressed receptors. Preliminary data by Elder et al [44] indicate that EGF/TGFα receptor mRNA is not increased in psoriasis, whereas there is a marked increase in TGFα but not in EGF mRNA. Thus defective internalization or degradation may play a role in the persistence of the receptors as may be suggested by the observation that chloroquine, a lysosomal inhibitor, induced worsening of psoriasis in a number of patients [45].

When treatment begins to be effective, the decrease in the persistent expression of EGF receptors appeared not to be dependent on the treatment modality, because the same results were seen when psoriasis was effectively treated with ultraviolet light, corticosteroids, or methotrexate ([46], unpublished data). At least one other cell-surface receptor, the low-density lipoprotein (LDL) receptor, is also persistently expressed throughout the involved psoriatic epidermis. It is expressed primarily on basal keratinocytes in normal skin [47]. This finding indicates that the EGF/TGFα receptor defect noted above is not unique. Because more than one extracellular receptor follows the same pattern of persistent expression in lesional psoriatic skin, these changes may simply reflect defective terminal differentiation of keratinocytes. Another explanation is that several different membrane receptors (RAMPS) persist throughout the layers of the epidermis and are involved in producing hyperproliferation of the keratinocytes directly or indirectly by their effects on calcium/phospholipid metabolism.

Regulation of Keratinocyte Proliferation   What events and molecules regulate keratinocyte growth/differentiation has always

been of interest to skin biologists. Much evidence indicates control of epidermal growth and differentiation by the vascular, nervous, and endocrine systems [48–50]. However, the epidermis can also regulate itself and function even in ischemic and denervated areas. Therefore, the production of molecules by keratinocytes that autoregulate various events in keratinocytes, so called *autocrine regulation,* has been more closely studied. Recently, the most intense interest has been on the autocrine regulation of keratinocyte proliferation by EGF and TGFα, each of which bind to the EGF receptor. Because TGFα is produced by human keratinocytes [50,51], it may play a major role in epidermal homeostasis through an autocrine mechanism. The EGF receptor plays a pivotal role in these effects of TGFα because it is the only identified receptor for TGFα. The mRNA for TGFα, which can be increased in keratinocytes by EGF/TGFα [50,52] as well as by phorbol esters was found to also be increased in psoriasis [53]. This indicates that not only is there more receptor expressed in psoriasis but there is also more ligand. Thus it is likely that the receptor is occupied and in its activated state. Another multifunctional autocrine factor that keratinocytes produce is TGFβ, which exists in at least three forms [50,54,55]. This factor is an inhibitor of keratinocyte growth or a chalone-like molecule but stimulates the growth of other cells [48,49,56]. Thus it would be of interest to determine whether there is altered or reduced expression of any form of TGFβ in psoriasis or altered ratio of the TGFβ inhibitory effects and the stimulatory effects of TGFα/EGF or cytokines.

Some experiments indicate that fibroblasts, mononuclear cells, and even neural or nevus cells might affect the progress of psoriasis [57]. These cells produce peptide factors that affect the EGF/TGFα receptor. For example, EGF binding and receptor activity may be affected by factors released from platelets and present in plasma such as EGF, TGFα, and TGFβ as well as a number of bioactive molecules such as platelet-derived growth factor (PDGF). PDGF may indirectly affect EGF/TGFα receptor phosphorylation on specific threonine residues [58] when the skin is injured in the Koebner reaction or isomorphic phenomenon. In addition, wound macrophages express messenger RNA transcripts for TGFα, TGFβ, and PDGF [59]. At a site of injury or clotting, platelets containing TGFβ could modulate the high-affinity binding of EGF and TGFα to the EGF/TGFα receptor [60]. The presence and distribution of the EGF/TGFα receptor are altered in non-infected and virally infected keratinocyte in human skin [42,61,62]. Thus, the aggravation of psoriasis by concomitant viral infections such as III HIV-1 [63] may be mediated by factors released from infected cells affecting the EGF/TGFα receptor.

Processes Regulating Kinase Activity   Although there are many different substrates for PKC, threonine 654 [64] as well as other sites [61] on the EGF/TGFα receptor itself are phosphorylated when this kinase is activated. Direct activation of PKC by agents such as phorbol esters [65] blocks the mitogenic capacity of EGF and TGFα apparently both by abolishing high-affinity binding of EGF to its receptor [66–69] and inhibiting receptor-kinase activity [69,70]. Because the binding of EGF to its receptor results in the activation of phospholipase C and then of PKC (Fig 2), a control mechanism exists whereby activating the kinase activity of the EGF receptor eventually leads to that activity being suppressed.

As predicted from Fig 2, treatment of cells with PDGF that also activates a specific form of PLC [31,71] also results in inactivation of EGF/TGFα receptors. When metabolized, some of the diacylglycerol produces arachadonic acid, a precursor of prostaglandins [33]. Prostaglandins and their metabolites can function as both intracellular and extracellular messengers, some feeding back into the inositol phospholipid pathway itself.

In vitro experiments have identified several lipids that modulate the activity of the kinase portion of the EGF/TGFα receptor. Sphingosine, which inhibits PKC, activates the receptor kinase at concentrations as low as 5 μM [14,71,72] as does the novel ganglioside, de-N-acetyl-GM$_3$, at concentrations above 100 M [73]. These effects of these lipids on EGF-receptor-kinase activity are very spe-

cific for these particular lipids and are not general characteristics of certain lipid classes [73,74].

Although the role of these biochemical interactions in the pathogenesis of psoriasis is not known, it is clear that EGF/TGFα receptors may be involved in phospholipase C activation [25–31], and are abnormal in psoriasis [45] as is PKC metabolism [75,76] and the activation of calcium-activated neutral protease [77,78]. Because calcium activates a number of enzymes such as transglutaminases, calcium-activated neutral protease (calpain), PKC, and other kinases, the potential exists for multiple effects on the EGF/TGFα receptor when calcium levels inside the cell are increased [79]. Other calcium-related enzymes such as calmodulin are also abnormally regulated in psoriasis [80], so it is unclear whether these are epi-phenomena or pathogenetic events due to an altered intraepidermal calcium gradient.

To date, no consistent effect of retinoids on EGF-receptor metabolism has been defined but this interaction is of interest because retinoids are an effective adjunctive therapy for patients with severe psoriasis. When normal human keratinocytes are cultured in the presence of EGF, low concentrations of retinoic acid that supress terminal keratinization, do not stimulate growth [81] and high retinoid concentrations inhibit growth [82]. When the same cells are quiescent because they are maintained in a basal media without growth-stimulating factors including EGF, high concentrations of retinoic acid stimulate growth [83]. Retinoic acid increases by as much as sevenfold the number of EGF/TGFα receptors expressed on some cell lines without altering their affinity for EGF [84–86]. However, this increase in EGF/TGFα receptors does not correlate with growth because some lines are growth stimulated [87] by retinoic acid whereas others are growth inhibited [85].

Phosphoprotein Phosphatases The role of phosphatases in modulating the activity and function of EGF/TGFα receptors is also important. Phosphatases can potentially remove phosphates from regulatory sites of the EGF/TGFα-receptor, thereby activating it. Phosphatases can also antagonize or reverse the effects of the EGF-activated tyrosine kinase in the receptor, thereby providing negative feedback. For example, an increase in tyrosine protein phosphatase activity was associated with the inhibition of pancreatic cancer-cell growth by somatostatin [88]. The phosphotyrosyl-protein phosphatases may have regulatory effects in mammalian skin; they have been identified in mouse epidermis, a human keratinocyte cell line [89], and normal and psoriatic skin [90]. Whether the palliative effects of somatostatin reported for psoriasis [91] are mediated through these phosphatases is an unanswered question. Tyrosyl protein kinase and protein phosphotyrosine phosphatase were both elevated in psoriatic skin [90]. Because the epidermis was not separated from the dermis, it was not clear whether this was a consequence of the increased vasculature associated with psoriasis in the dermal fraction of the samples.

REFERENCES

1. Carpenter G, Cohen S: Epidermal growth factor. Annu Rev Biochem 48:193–216, 1979

2. Stoscheck CM, King Jr LE: Functional and structural characteristics of EGF and its receptor and their relationship to transforming proteins. J Cell Biochem 31:135–152, 1986

3. King Jr LE, Stoscheck CM, Gates RE, Nanney LB: Epidermal growth factor and transforming growth factor-α. In: Goldsmith L (ed.). Biochemistry and Physiology of the Skin, 2nd ed. Oxford University Press, New York (in press)

4. Todderud G, Carpenter G: Epidermal growth factor: the receptor and its function. Biofactors 2:11–15, 1989

5. Cohen S, Carpenter G, King LE: Epidermal growth factor receptor protein kinase interactions. J Biol Chem 255(10):4834–4842, 1980

6. Cummings RD, Soderquist AM, Carpenter G: The oligosaccharide moieties of the epidermal growth factor in A-431 cells. J Biol Chem 260:11944–11952, 1985

7. Soderquist AM, Carpenter G: Glycolysation of the epidermal growth factor receptor. J Biol Chem 259:12586–12594, 1984

8. Stoscheck CM, King Jr LE: Role of epidermal growth factor in carcinogenesis. Cancer Res 46:1030–1037, 1986

9. Wells A, Bishop JM: Genetic determinants of neoplastic transformation by the retroviral oncogene v-erb-B. Proc Natl Acad Sci USA 85:7597–7601, 1988

10. Khazaie K, Dull TJ, Graf T, Schlessinger J, Ullrich A, Beug H, Vennstrom B: Truncation of the human epidermal growth factor receptor leads to differential transforming potentials in primary avian fibroblasts and erythroblasts. EMBO J 7:3061–3071, 1988

11. Goldstein JL, Brown MS, Anderson RGW, Russel DW, Schneider WJ: Receptor mediated endocytosis. Annu Rev Cell Biol 1:1–39, 1985

12. Toderrud G, Carpenter G: Presence of mannose phosphate on the EGF receptor in A-431 cells. J Biol Chem 263:17893–17896, 1988

13. von Figura K, Hasilik A: Lysosomal enzymes and their receptors. Annu Rev Biochem 55:167–193, 1986

14. Wedegaertner PB, Gill GN: Activation of the purified protein tyrosine kinase domain of the epidermal growth factor receptor. J Biol Chem 264(19):11346–11353, 1989

15. Honegger AM, Szapary D, Schmidt A, Lyall R, Van Obberghen E, Dull TJ, Ullrich A, Schlessinger J: A mutant epidermal growth factor receptor with defective protein tyrosine kinase is unable to stimulate proto-oncogene expression and DNA synthesis. Mol Cell Biol 7:4568–4571, 1987

16. Moolenaar WH, Bierman AJ, Tilly BC, Verlaan I, Defize LHK, Honegger AM, Ullrich A, Schlessinger J: A point mutation at the ATP-binding site of the EGF-receptor abolishes signal transduction. EMBO J 7:707–710, 1988

17. Honnegger A, Dull TJ, Bellot F, Obberghen EV, Szapary D, Schmidt A, Ullrich A, Schlessinger J: Biological activities of the EGF receptor mutants with individually altered autophosphorylation sites. EMBO J 7:3045–3052, 1988

18. Bertics PJ, Chen WS, Hubler L, Lazar CS, Rosenfeld MG, Gill GN: Alteration of the epidermal growth factor receptor by mutation of its primary carboxyl-terminal site of tyrosine self-phosphorylation. J Biol Chem 263:3610–3617, 1988

19. Clark S, Cheng DJ, Hsuan JJ, Haley JD, Waterfield MD: Loss of three major auto phosphorylation sites in the EGF receptor does not block the mitogenic action of EGF. J Cell Physiol 134:421–428, 1988

20. Fava RA, Cohen S: Isolation of a calcium-dependent 35 Kd substrate for the epidermal growth factor receptor kinase from A-431 cells. J Biol Chem 259:2636–2645, 1984

21. Fava RA, Nanney LB, King LE: Immunolocalization of p35 (lipocortin I) in human skin (abstr). Clin Res 36(3):643A, 1988

22. Yang SD, Chou C, Huang M, Song JS, Chen H: Epidermal growth factor induces activation of protein kinase F_A and ATP-Mg-dependent protein phosphatase in A-431 cells. J Biol Chem 264(10):5407–5411, 1989

23. Giugni TD, Chen K, Cohen S: Activation of a cytosolic serine protein kinase by epidermal growth factor. J Biol Chem 263(35):18988–18995, 1988

24. Ackerman P, Osheroff N: Regulation of casein kinase II activity by epidermal growth factor in human A-431 carcinoma cells. J Biol Chem 264:11958–11965, 1989

25. Wahl MI, Sweatt JD, Carpenter G: Epidermal growth factor (EGF) stimulates inositol triphosphate formation in cells which over express the EGF receptor. Biochem Biophys Res Commun 142:688–695, 1987

26. Wahl MI, Carpenter G: Regulation of epidermal growth factor stimulated formation of inositol phosphates in A-431 cells by calcium and protein kinase C. J Biol Chem 263:7581–7590, 1988

27. Helper JR, Nakahata N, Lovenberg TW, DiGuiseppi J, Herman B, Earp HS, Harden TK: Epidermal growth factor stimulates the rapid accumulation of inositol (1,4,5)-triphosphate and the rise in cytosolic calcium mobilized from intracellular stores in A-431 cells. J Biol Chem 262:2951–2956, 1987

28. Wahl MI, Daniel TO, Carpenter G: Anti-phosphotyrosine recovery of phospholipase C activity after EGF treatment of A-431 cells. Science 241:968–970, 1988

29. Wahl MI, Nishibe S, Suh PG, Rhee SG, Carpenter G: EGF stimulates tyrosine phosphorylation of phospholipase C-II independently of receptor internalization and extracellular calcium. Proc Natl Acad Sci USA 86:1568–1572, 1989

30. Nishibe S, Wahl MI, Rhee SG, Carpenter G: Tyrosine phosphoryla-

tion of phospholipase C-II in vitro by the epidermal growth factor receptor. J Biol Chem 264(18):10335–10338, 1989

31. Wahl MI, Olashaw NE, Nishibe S, Rhee SG, Pledger WJ, Carpenter G: Platelet derived growth factor induces rapid and sustained tyrosine phosphorylation of phospholipase C- in quiescent BALB/c 3T3 cells. Mol Cell Biol 9(7):2934–2943, 1989

32. Fain JN, Wallace MA, Wojcikiewicz: Evidence for involvement of quanine nucleotide-binding regulatory proteins in the activation of phospholipase C by hormones. FASEB J 2:2569–2574, 1988

33. Nishizuka Y: Studies and perspectives of protein kinase C. Science 233:305–312, 1986

34. Bretscher A: Rapid phosphorylation and reorganization of ezrin and spectrin accompany morphological changes induced in A-431 cells by epidermal growth factor. J Cell Biol 108:921–930, 1989

35. Nanney LB, Stoscheck CM, King Jr LE: Comparison of epidermal growth factor binding and receptor distribution in normal human epidermis and epidermal appendages. J Invest Dermatol 83:385–393, 1984

36. Green MR, Couchman JR: Differences in human skin between the epidermal growth factor receptor distribution detected by EGF binding and monoclonal antibody recognition. J Invest Dermatol 85:239–245, 1985

37. Damjanov I, Mildner B, Knowles: Immunohistochemical localization of the epidermal growth factor receptor in normal human tissues. Lab Invest 55:588–592, 1986

38. Ponec M, Kempenaar J, Weerheim A, Boonstra J: Differentiation of human keratinocytes: changes in lipid synthesis, plasma membrane lipid composition and ^{125}I-EGF binding upon administration of 25-hydroxycholesterol and mevinolin. J Cell Phys 133:358–364, 1987

39. Gregory H: Isolation and structure of urogastrone and its relationship to epidermal growth factor. Nature 257:325–327, 1975

40. Moolenar WH, Aerts RJ, Tertoolen LGJ, de Laat SW: The epidermal growth factor induced calcium signal in A-431 cells. J Biol Chem 261:279–284, 1986

41. Leof EB, Proper JA, Goustin AS, Shipley CD, DiCorletto PE, Moses HL: Induction of c-sis mRNA and activity similar to platelet-derived growth factor by transforming growth factor-beta: a proposed model for indirect mitogenesis involving autocrine activity. Proc Natl Acad Sci USA 83:2453–2557, 1986

42. Nanney LB, Ellis DL, Dale B, Stoscheck CM, Holbrook KA, King Jr LE: Epidermal growth factor receptors (EGF-R) in idiopathic and virally induced hyperproliferative skin diseases (abstr). Clin Res 36(3):678A, 1988

43. Ellis DL, Kafka SP, Chow JC, Nanney LB, Inman WH, McCadden ME, King Jr LE: Melanoma, growth factors, acanthosis nigricans, the sign of Leser-Trelat, and multiple acrochordons. N Engl J Med 317:1582–1587, 1988

44. Elder JT, Mitra RS, Voorhees JJ, Nickoloff BJ: Modulation of TGFα and EGF receptor expression by interferon gamma in normal and psoriatic human keratinocytes (abstr). J Invest Dermatol 89:548, 1989

45. Fitzpatrick T, ed.: Dermatology in General Medicine — Textbook and Atlas. McGraw Hill Book Co. Publishers, New York, 1988

46. Laskin JD: PUVA's clinical effects seen as changes in cell membrane. Dermatology News 22:2, 1988

47. Mommaas-Kiehuis AM, Grayson S, Wijsman MC, Vermeer BJ, Elias PM: Low density lipoprotein receptor expression on keratinocytes in normal and psoriatic epidermis. J Invest Dermatol 89:513–517, 1987

48. Sporn MB, Roberts AB: Peptide growth factors are multifunctional. Nature 332:217–219, 1988

49. Wilke MS, Hsu BM, Wille JJ, Pittelkow MR, Scott RE: Biologic mechanisms for the regulation of normal human keratinocyte proliferation and differentiation. Am J Pathol 131:171–181, 1988

50. Coffey RJ, Sipes NJ, Bascom CC, Graves-Deal R, Pennington CY, Weissman BE, Moses HL: Growth modulation of mouse keratinocytes by transforming growth factors. Cancer Res 48:1596–1602, 1988

51. Coffey Jr RJ, Derynck R, Wilcox JN, Bringman TS, Goustin AS, Moses HL, Pittelkow MR: Production and autoinduction of TGFα in human keratinocytes. Nature 32:817–819, 1987

52. Derynck R: Transforming growth factor α: structure and biological activities. J Cell Biochem 32:2293–2304, 1986

53. Elder JT, Fisher GJ, Lindquist PB, Bennett GL, Pittelkow MR, Coffey

RJ, Ellingsworth L, Derynck R, Voorhees JJ: Overexpression of TGF-α in psoriatic epidermis. Science 243:811–814, 1989

54. DeLarco JE, Todaro GJ: Growth factors from murine sarcoma virus transformed cells. Proc Natl Acad Sci USA 75:4001–4005, 1978

55. Dernyck R, Goeddel DV, Ullrich A, Gutterman JU, Williams RD, Bringman TS, Berger WH: Synthesis of messenger RNAs for transforming growth factors α and β and the epidermal growth factor receptor by human tumors. Cancer Res 47:707–712, 1987

56. Shipley GD, Pittelkow MR, Wille JJ, Scott RE, Moses HL: Reversible inhibition of normal human prokeratinocyte proliferation by type β transforming growth factor-growth inhibitor in serum-free medium. Cancer Res 46:2068–2071, 1986

57. Farber EM, Cox AJ, eds.: Psoriasis, Proceedings of the 3rd International Symposia, Stanford University, 1981. Gruene and Stratton, New York, 1981

58. Olashaw NE, O'Keefe EJ, Pledger WJ: Platelet-derived growth factor modulates epidermal growth factor receptors by a mechanism distinct from that of phorbol esters. Proc Natl Acad Sci 83:3834–3839, 1986

59. Rappolee DA, Mark D, Banda MJ, Werb Z: Wound macrophages express TGFa and other growth factors in vivo: analysis by mRNA phenotyping. Science 241:708–712, 1988

60. Massague J: Transforming growth factor beta modulates the high affinity receptors for epidermal growth factor and transforming growth factor alpha. J Cell Biol 100:1500–1514, 1985

61. Stroobant P, Rice AP, Gullick WJ, Cheng DJ, Kerr IM, Waterfield MJ: Purification and characterization of vaccinia virus growth factor. Cell 42:383–393, 1985

62. Brown JP, Twardzik DR, Marquardt HJ, Todaro GJ: Vaccinia virus encodes a polypeptide homologous to epidermal growth factor and transforming growth factor. Nature 313:491–492, 1985

63. Johnson TM, Duvic M, Rapini RP, Ross A: AIDS Exacerbates Psoriasis. New Engl J Med 313(22):1415, 1985

64. Hunter T, Ling N, Cooper JA: Protein kinase C phosphorylation of the EGF receptor at a threonine residue close to the cytoplasmic face of the plasma membrane. Nature 311:480–483, 1984

65. Castagna M, Takai Y, Kaibuchi K, Sano S, Kikkawa U, Nishizuka Y: Direct activation of calcium-activated phospholipid-dependent protein kinase by tumor-producing phorbol esters. J Biol Chem 257:7847–7851, 1982

66. Livneh E, Dull TJ, Berent E, Prywes R, Ullrich A, Schlessinger J: Release of a phorbol ester-induced mitogenic block by mutation at Thr-654 of the epidermal growth factor receptor. Mol Cell Biol 8(6):2302–2308, 1988

67. Shoyab M, DeLarco JE, Todaro GT: Biologically active phorbol esters specifically alter affinity of epidermal growth factor membrane receptors. Nature 279:387–391, 1979

68. Lee LS, Weinstein IB: Tumor-promoting phorbol esters inhibit binding of epidermal growth factor to cellular receptors. Science 202:313–315, 1978

69. Davis RJ: Independent mechanisms account for the regulation by protein kinase C of the epidermal growth factor receptor affinity and tyrosine-protein kinase activity. J Biol Chem 263(19):9462–9469, 1988

70. Cochet C, Gill GN, Meisenhelder J, Cooper JA, Hunter T: C-kinase phosphorylates the epidermal growth factor receptor and reduces its epidermal growth factor-stimulated tyrosine protein kinase activity. J Biol Chem 259:2553–2558, 1984

71. Davis RJ, Girones N, Faucher M: Two alternative mechanisms control the interconversion of functional states of the epidermal growth factor receptor. J Biol Chem 263(11):5373–5379, 1988

72. Northwood IC, Davis RJ: Activation of the epidermal growth factor receptor tyrosine protein kinase in the absence of receptor oligomerization. J Biol Chem 263(16):7450–7453, 1988

73. Hanai N, Dohi T, Nores GA, Hakamori S: A novel ganglioside, De-N-acetyl-GM$_3$ (II3NeuNH2LacCer), acting as a strong promoter for epidermal growth factor receptor kinase and as a stimulator for cell growth. J Biol Chem 263(13):6296–6301, 1988

74. Igaarashi Y, Hakomori S, Toyokuni T, Dean B, Fujita S, Sugimoto M, Ogawa T, El-Ghendy K, Racker E: Effect of chemically well-defined spingosine and its n-methyl derivates on protein kinase C and src kinase activities. Biochemistry 28:6796–6800, 1989

75. Horn F, Marks F, Fisher GJ, Marcello CL, Voorhees JJ: Decreased

protein kinase C activity in psoriatic versus normal epidermis. J Invest Dermatol 88:220–222, 1987

76. Bartel RL, Marcello CL, Voorhees JJ: Partial characterization of phospholipase C activity in psoriatic, uninvolved and lesional epidermis. J Invest Dermatol 88:447–451, 1987

77. Gates RE, King LE Jr: Calcium facilitates endogenous proteolysis of the EGF receptor-kinase. Mol Cell Endo 27:263–276, 1982

78. Cassel D, Glaser L: Proteolytic cleavage of epidermal growth factor receptor. J Biol Chem 257:9845–9848, 1982

79. Sawyer ST, Cohen S: Enhancement of calcium uptake and phosphatidylinositol turnover by epidermal growth factor in A-431 cells. Biochemistry 20:6280–6286, 1981

80. Van der Kerkhof PCM, Van Erp PEJ: Calmodulin levels are grossly elevated in the psoriatic lesion. Br J Dermatol 108:217–218, 1983

81. Jetten AM, George MA, Nervi C, Boone LR, Rearick JI: Increased cholesterol sulfate and cholesterol sulfotransferase in relation to the multi-step process of differentiation in human epidermal keratinocytes. J Invest Dermatol 92:203–209, 1989

82. Gilfix BM, Green H: Bioassay of retinoids using cultured human conjunctival keratinocytes. J Cell Physiol 119:172–174, 1984

83. Varani J, Nickoloff BJ, Dixit VM, Mitra RS, Voorhees JJ: All trans retinoic acid stimulates growth of adult human keratinocytes cultured in growth factor-deficient medium, inhibits production of thrombospondin and fibronectin, and reduces adhesion. J Invest Dermatol 93(4):449–455, 1989

84. Jetten AM: Retinoids specifically enhance the number of epidermal growth factor receptors. Nature 284:626–629, 1980

85. Jetten AM: Effects of retinoic acid on the binding and mitogenic activity of epidermal growth factor. J Cell Physiol 110:235–240, 1982

86. Roberts AB, Anzano MA, Lamb JM, Sporn MB: Antagonistic actions of retinoic acid and dexamethasone on anchorage independent growth and epidermal growth factor binding of normal rat kidney cells. Can Res 44:1635–1641, 1984

87. Harper R: Specificity in the synergism between retinoic acid and EGF on the growth of adult human skin fibroblasts. Exp Cell Res 178:254–263, 1988

88. Liebow C, Reilly C, Serrano M, Schally AV: Somatostatin analogues inhibit growth of pancreatic cancer by stimulating tyrosine phosphatase. Proc Natl Acad Sci USA 86:2003–2007, 1989

89. Stoscheck CM, Friedman DB, LE King Jr: Identification of a phosphotyrosyl-protein phosphatase in mouse epidermis. J Invest Dermatol 92:12–19, 1989

90. Gentleman S, Martensen TM, Digiovanna JJ, Chader GJ: Protein tyrosine kinase and protein phosphotyrosine phosphatase in normal and psoriatic skin. Biochim Biophys Acta 798:53–59, 1984

91. Venier A: Treatment of severe psoriasis with somatostatin: four years of experience. Arch Dermatol Res (suppl) 280:51–54, 1988