Conclusions

Although many of the effects of retinoids on epidermal cells are now well studied, a great deal remains to be discovered about their biochemical mode of action. A better understanding of the role of vitamin A derivatives in skin would offer the possibility of designing clinically more effective and less toxic retinoids.

Note added in proof. While this paper was being prepared, two papers have appeared describing much larger retinoid binding proteins that probably contain DNA-binding zinc fingers (Petkovich et al., 1987) Nature (London) 330, 444–450; Giguere et al. (1987) Nature (London) 330, 624–629).

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Structure and activity of epidermal-growth-factor-like peptides: induction of basal cell proliferation by a poxvirus gene product?

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Epidermal growth factor (EGF) was initially described as a polypeptide hormone, isolated from the mouse submaxillary gland, that caused precocious eyelid opening and tooth eruption when injected into newborn mice. Both effects are a consequence of enhanced epidermal growth and keratinization. EGF also stimulates the proliferation of keratinocytes and fibroblasts in culture. For his discovery and subsequent work Stanley Cohen has recently been awarded the Nobel Prize (Cohen, 1987). Mouse EGF is a 53 amino acid polypeptide with three disulphide-bridged loops (Savage et al., 1972, 1973). The human homologue is the same size and is identical to the gastric anti-secretory hormone urogastrone (Gregory, 1975).

The biological responses to EGF are mediated by a membrane receptor, a 170 kDa protein with an extracellular domain, substrates for which include lipocortin and the receptor itself (Yarden & Schlessinger, 1987). Other responses to EGF include enhanced Na+/H+ antiport activity, enhanced release of Ca2+ from intra- and extracellular stores, enhanced turnover of phosphoinositides in A31 cells (but not in 3T3 cells) and a mitogenic response with induction of c-fos and c-myc expression, culminating in DNA synthesis (Carpenter & Cohen, 1979; Schlessinger et al., 1983). After binding, the ligand–receptor complex is internalized and proteolytically degraded; this processing is necessary for induction of the late response of DNA synthesis (Schäudies et al., 1987). The kinase domain is crucial to the function of the EGF receptor, since mutagenesis of its ATP binding site abolishes all early and late responses to EGF (Chen et al., 1987). The product of the oncogene v-erbB is homologous to part of the EGF receptor and thought to be a truncated version of the chicken homologue: it lacks the ligand binding and autophosphorylation domains and is constitutively active (Lax et al., 1985). Over-expression of the EGF receptor is associated with a high proportion of squamous cell carcinomas and has also been detected in other tumour types (Ozanne et al., 1986). In normal epidermis, EGF receptors occur predominantly in the basal cell layer (Nanney et al., 1984).

The major site of synthesis in the mouse is the submaxillary gland, where EGF is initially synthesized as a 128 kDa transmembrane precursor from which it is proteolytically cleaved (Gray et al., 1983). The precursor possesses...
seven other EGF-like domains. It is also made in the kidney cortex, but is not processed in this tissue (Rall et al., 1985). Based on homology to the receptor for low density lipoprotein, the precursor has been postulated to be a receptor for an as yet unidentified ligand; this may explain its presence in kidney (Pfeffer & Ullrich, 1985).

Following retroviral or chemical transformation, many cells lose their ability to bind EGF owing to the production by these cells of an EGF-related polypeptide, α-transforming growth factor (TGF) (De Larco & Todaro, 1978). In terms of its primary amino acid sequence and its ability to bind to the EGF receptor with a similar affinity and elicit a similar response, TGF is structurally and functionally homologous to EGF (Sporn & Todaro, 1980). TGF is a 50 amino acid polypeptide and is cleaved from a 17 kDa transmembrane precursor (Teixido et al., 1987). In many biological assay systems the response to EGF and TGF is identical; however, TGF is more potent at stimulating hair follicle development, bone resorption, angiogenesis and keratinocyte migration. It is synthesized early in mouse fetal development and is present throughout the epidermis of neonatal and adult human skin, where it is synthesized by keratinocytes (reviewed by Derynck, 1986; Barrandon & Green, 1987; Coffey et al., 1987). TGF is autoinduced and so must normally be held in check by some other control mechanism. It may play a role in vivo in response to wounding (Coffey et al., 1987). In transformed cells inappropriate expression of TGF may act to stimulate cell growth by an autocrine or paracrine mechanism (Rosenthal et al., 1986). In agreement with this hypothesis, TGF is synthesized in many solid tumours, such as squamous cell carcinomas, which over-express the EGF receptor (Derynck et al., 1987).

The orthopoxvirus vaccinia has been shown to synthesize a polypeptide (approximately 77 amino acids), present in the conditioned medium of infected cells, with structural and functional homology to EGF/TGF (Stroobant et al., 1987; Twardzik et al., 1985). This growth factor (VGF) is released from a putative 140 amino acid transmembrane precursor synthesized early in viral infection. In contrast to mature EGF and TGF, VGF is glycosylated. The role of VGF in vaccinia viral infection, which is cytolytic, is unknown. Saturation of EGF receptors causes only a small decrease in infectivity (Epstein et al., 1985) and the fact that vaccinia can infect a cell line lacking EGF receptors argues against a role for VGF in mediating viral attachment (Stroobant et al., 1985). It may be that VGF works to enhance the metabolic activity of infected and neighbouring cells and so create a more favourable environment for viral replication. However, the possibility of mitogenic stimulation by such a growth factor is of interest given the association of proliferative disease with other poxviruses. Recently, the poxvirus Shope fibroma virus, associated with a widespread proliferative disease in rabbits, and the poxvirus myxoma, the agent of myxomatosis, have been shown to encode analogous polypeptides (SFGF and MGF) (Chang et al., 1987; Upton et al., 1987). However, SFGF and MGF are apparently not synthesized as transmembrane precursors. Other poxviruses responsible for the induction of cell proliferation include Yaba monkey tumour virus, which causes fibromas in monkeys, and Molluscum contagiosum virus. The latter causes benign skin tumours in man: viral replication occurs in the squamous cell layer and the basal cell proliferation rate is enhanced (Postlethwaite, 1970).

Fig. 1 shows the aligned sequences of the members of the EGF-like growth factor family. In addition to the overall conservation of Cys and Gla residues there is a striking level of conservation of amino acids within the third disulphide loop (residues 33–42), implying an important functional role for this region of the molecule (see below). Although EGF, TGF and the poxviral growth factors are structurally and functionally homologous, there is no significant homology at the nucleotide level between the genes encoding them (Postlethwaite, 1987; Upton et al., 1985). In agreement with this hypothesis, TGF is synthesized in many solid tumours, such as squamous cell carcinomas, which over-express the EGF receptor (Derynck et al., 1987).

![Fig. 1. The EGF-like growth factor family](image)

The sequences of EGF, α-TGF and the poxvirus growth factors from vaccinia (VGF), Shope fibroma (SFGF) and myxoma (MGF) are aligned. Species designations are human (h), mouse (m), rat (r) and guinea-pig (gp). Numbering refers to amino acid positions in EGF and the positions of the disulphide bridges are indicated below. Residues boxed are conserved among all EGF species. The N-terminal portions of the viral growth factors are omitted. Data are taken from Simpson et al. (1985 and references therein), Chang et al. (1987) and Upton et al. (1987).
The sequences of clotting factors IX, X, XII, C and Z, tissue plasminogen activator and the developmental genes Notch (third repeat) and lin-12 (sixth repeat) from D. melanogaster and C. elegans, respectively, are aligned with human EGF. Species designations are human (h) and bovine (b). The conserved Cys and Gly residues are boxed and indicated below as two sequence motifs. Data are taken from Blomquist et al. (1984), McMullen & Fujikawa (1985), Greenwald (1985) and Wharton et al. (1985).

Fig. 2. Other EGF-like polypeptide sequences

The sequences of clotting factors IX, X, XII, C and Z, tissue plasminogen activator and the developmental genes Notch (third repeat) and lin-12 (sixth repeat) from D. melanogaster and C. elegans, respectively, are aligned with human EGF. Species designations are human (h) and bovine (b). The conserved Cys and Gly residues are boxed and indicated below as two sequence motifs. Data are taken from Blomquist et al. (1984), McMullen & Fujikawa (1985), Greenwald (1985) and Wharton et al. (1985).

Fig. 3. Schematic structure of human EGF as determined by solution n.m.r.

The disulphide bonds (bold lines) and hydrogen bonds (dashed lines) involved in β-sheet and β-turn formation are indicated. The residues identified are those highly conserved among members of the EGF-like growth factor family. Adapted from Cooke et al. (1987).

proteins thought to be involved in intercellular communication (products of the Drosophila melanogaster Notch gene and the Caenorhabditis elegans lin-12 gene) (Blomquist et al., 1984; Greenwald, 1985; Wharton et al., 1985). These sequences are aligned in Fig. 2. The two developmental proteins possess tandem repetitions of this EGF-like domain, reminiscent of the structure of the EGF precursor itself; one such repeat of the Notch product contains all of the conserved residues in the putative receptor-binding third disulphide loop of the EGF-like growth factors and so may
functionally interact with the *Drosophila* homologue of the EGF receptor. It is conceivable that the clotting factors may also bind to the EGF receptor; this could have a physiologically significant role to play in the induction of the wound response, particularly if they bound as their activated forms only.

The solution structure of mouse EGF and human EGF has been determined by proton n.m.r.; both form two regions of disulphide-bridged anti-parallel β-sheet which divide the molecule into two structural domains: residues 1–32 and 33–53 (Montelione et al., 1986; Cooke et al., 1987) [see Fig. 3]. Efforts to identify regions of the EGF molecule responsible for receptor binding have involved the use of short synthetic peptides. A peptide corresponding to human EGF residues 20–31 shows binding to the receptor with an affinity >10^4-fold lower than EGF itself and also elicits receptor autophosphorylation (Komoriya et al., 1984). A peptide corresponding to residues 34–43 of TGF (equivalent to residues 33–42 of EGF) showed an affinity 50-fold lower than EGF or TGF, but had no other activity (Nestor et al., 1985). These peptides occur in the two regions of β-sheet: both bind antibody raised to the whole molecule suggesting that they correspond to surface epitopes. As noted above, sequence comparison of these regions with the other EGF-like growth factors indicates a high level of conservation associated with the region corresponding to the latter peptide while there is no conservation in the former region with the exception of the cysteine residues. Assuming that sequence conservation reflects functional conservation this argues in favour of the third disulphide loop mediating binding to the EGF receptor. Additional evidence in favour of this is that EGF cleaved with cyanogen bromide at Met-21 still binds with 10% the affinity of EGF and induces some response, particularly if they bound as their activated forms.

It is conceivable that high levels of such a growth factor could be tumorigenic by over-riding the normal control mechanisms of the cell. DNA probes is a model by which basal cell replication might be enhanced, thus used as a probe a degenerate synthetic oligonucleotide has been determined by proton n.m.r.; both form two regions of disulphide loop of the EGF family which is thought to mediate receptor binding (see above). Such an approach was also used to identify the SFGF gene in Shope fibroma virus DNA. We have reported that MCV does indeed have the potential to encode such a peptide (Porter & Archard, 1987). Nucleoside sequencing of this region of the viral genome is in progress.


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