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COMMENTARY

Targeting the Epidermal Growth Factor Receptor for Therapy of Carcinomas

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ABSTRACT. As a group, the carcinomas represent a substantial proportion of all human malignancies, but, with relatively few exceptions, current treatments are ineffective. Modification of existing chemotherapeutic agents has not led to significant improvements in the survival of carcinoma patients, and development of new therapeutic strategies is imperative. It is now becoming apparent that activation of the epidermal growth factor receptor (EGF-R) has much wider implications than a straightforward stimulation of cell division. The pleiotropic effects of EGF-R signalling may influence tumour behaviour and the response of carcinomas to treatment; these are important considerations for the development of new therapies that aim to exploit the expression or modulate the function of the EGF-R in these tumours. BIOCHEM PHARMACOL 51;9:1101–1110, 1996.

KEY WORDS. epidermal growth factor receptor; growth factors; proliferation; cancer; therapy

The human EGF-R,‡ (HER, c-erbB1) is a transmembrane cell surface growth factor receptor that belongs to a family of structurally related receptors including c-erbB2 (HER2), c-erbB3 (HER3) and c-erbB4 (HER4) [see Refs. 1–4 for reviews]. EGF-R is expressed on cells derived from all three lineages, but is characteristically expressed on cells of epithelial origin, including malignant carcinomas. There are a number of homologous ligands that can bind to EGF-R. These include EGF, TGFα, AR, HB-EGF, and BTC. Extracellular binding of ligand activates the intracellular intrinsic protein tyrosine kinase activity of the receptor, thus initiating signal transduction and a biological response by the target cell.

The occurrence of the EGF-R in human malignancies has been studied extensively, and in several tumour types the EGF-R status is an important prognostic indicator [see Ref. 5 for review]. Most notably, in carcinoma of the breast, high level expression of the receptor is associated with disease recurrence, reduced survival, and the presence of metastases [6, 7]. Furthermore, there is an inverse correlation between oestrogen receptor levels and EGF-R levels [7]. Although not as comprehensively studied, similar associations occur for ovarian, bladder, gastric, and squamous lung carcinomas [see Refs. 2 and 5]. For example, in bladder carcinoma there is a much lower incidence of EGF-R expression in superficial tumours than in invasive tumours, and in gastric tumours expression of EGF-R occurs more frequently in advanced disease. Thus, it can be seen that EGF-R expression occurs in many common tumours, and its presence is indicative of a poor outcome for the patient.

CONTRIBUTION OF THE EGF-R TO THE MALIGNANT PHENOTYPE

Proliferative Effects of EGF-R Activation

Ligand binding to the EGF-R can elicit a variety of cellular responses. Of these, the mitogenic response has been studied most extensively, and stimulation of cell proliferation is one obvious way by which the presence of EGF-R can contribute to malignancy. It has been proposed that cells can become malignant by endogenous production of growth factors that act in an autocrine fashion [8]. However, autocrine stimulation and transformation are not synonymous. Synthesis of autocrine AR, TGFα, or HB-EGF is a phenomenon that occurs in normal cells, such as keratinocytes [9–11]. Likewise, fibroblasts transfected with EGF or TGFα genes reach high saturation density, but fail to exhibit a transformed phenotype (i.e., form colonies in soft agar and exhibit reduced serum requirements) [12, 13]. To achieve transformation, the EGF-R must be expressed at high levels *in addition to* the presence of activating ligand [14]. Consistent with this requirement, tumour cells that overexpress EGF-R frequently co-express ligand [15, 16]. In such cells, blocking EGF-R activation by use of an antibody against the ligand binding site prevents proliferation [16, 17].

* This review is dedicated to the memory of Dr. Peter Alexander. The authors were privileged to work with Peter for several years before his death in December 1993. His insight, optimism, and enthusiasm remain an inspiration.

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‡ *Abbreviations:* EGF, epidermal growth factor; EGF-R, epidermal growth factor receptor; TGFα, transforming growth factor-alpha; AR, amphiregulin; HB-EGF, heparin-binding EGF-like growth factor; BTC, betacellulin; EMT, epithelial-mesenchymal transition; TCC, transitional cell carcinoma; MDR, multidrug resistance; Pgp, P-glycoprotein; and HSPG, heparan sulphate proteoglycans.

While cells expressing normal levels of EGF-R do not undergo ligand-dependent transformation, continuous exposure to ligand may lead to hyperproliferation. This is an important early event in multi-step carcinogenesis, and one obvious mechanism for hyperproliferation is unscheduled autocrine ligand synthesis by pre-malignant cells. Alternatively, abnormal distribution (not an increase in number) of EGF-Rs may lead to their stimulation by secretory EGF. For example, EGF-Rs of epithelial cells that line the bladder [18], lung [19], or gut [20] are localized basolaterally; thus, they do not proliferate even though they are bathed in secretory fluid containing EGF. Presumably, exposure of the EGF-Rs to ligand only occurs if the epithelial layer is damaged, whereupon repair can be initiated. Any perturbation causing presentation of the receptor on the luminal surface implies unscheduled signalling and proliferation. Altered localization of EGF-Rs has been observed in TCCs [18]. Moreover, in these patients, the "malignant" EGF-R distribution was also found on endoscopically normal urothelium, indicating that pre-malignant and malignant TCCs expose EGF-Rs to urinary EGF. This difference has been exploited therapeutically (see below).

Morphological Effects of EGF-R Activation

Cellular proliferation is only one of many consequences of EGF-R activation. Two other well documented responses are morphogenesis and chemotaxis. Together, these two phenomena are likely to have physiological roles in tissue remodelling during embryogenesis or, in the adult, during wound healing. The mechanisms whereby EGF-R activation leads to divergent cellular responses are not understood completely; however, it has been shown that EGF-Rs lacking all five autophosphorylation sites cannot cause morphological changes in response to EGF, even though mitogenesis is unaffected [21]. This suggests that at least some of the SH2 domain interactions that occur between EGF-Rs and second messenger signalling enzymes are involved with alterations in the cytoskeleton. One such enzyme is phospholipase C-γ [22].

EGF/TGFα treatment of several epithelial cell lines induces an EMT that results in the cells becoming motile and fibroblastic in appearance [23–25]. This change in phenotype is reversible upon removal of the growth factor and is associated with redistribution and/or upregulation of distinct types of adhesion molecules involved in both cell–cell and cell–matrix interactions. Overall, EGF has been shown to decrease cell–cell contact by affecting components of the adherens [26] and desmosomal [23] junctions and to increase cell–substratum interactions by enhancing integrin expression [27]. These effects on adhesion are probably a component part of the known ability of EGF to enhance the motility of keratinocytes, fibroblasts, and endothelial cells.

In tumours, these effects on cellular adhesion mechanisms probably play an important role in the dissemination of cancer. In clinical observations of breast cancer, tumours that exhibit EGF-R expression are often those that are disseminated [5, 6]. Similarly, in bladder cancers, high EGF-R expression occurs predominantly in invasive, but not superficial tumours [5, 28]. These observations may be due, in part, to the upregulation of cell matrix degrading proteases via EGF-R [29–32]. They may also be the consequence of EGF-R-mediated effects on cell adhesion and motility. In *in vitro* studies with two colonic tumour cell lines that express only low levels of EGF-R, we have observed that EGF or TGFα treatment causes a marked reduction in intercellular junctional staining for E-cadherin while expression of α_2-integrin, carcinoembryonic antigen (CEA) and CD44 is increased [33]. In a tumour, such a reduction of E-cadherin could lead to decreased cell–cell adhesion enabling initial detachment of cells from the primary tumour; increased integrin and CEA expression could enhance attachment and spreading of cells through the extracellular matrix while increased expression of CD44 could enable attachment of the tumour cells to endothelial cells and facilitate access to the circulation. Most importantly, the effects of EGF or TGFα on individual tumour cells are dynamic and highly dependent on the local environment (e.g. growth factor supply and extracellular matrix components). Therefore, once tumour cells have lodged at distant sites in the body, a change in the microenvironment can result in a switch back to an epithelial phenotype capable of forming a new tumour nodule. Thus, EGF-R activation may be synonymous with the invasive phenotype.

EGF-R and Drug Resistance

Development of MDR in cancer patients is a frequently encountered problem resulting in treatment failure. In these patients, MDR is associated with increased expression of Pgp, a plasma membrane channel responsible for the active efflux of cytotoxic drugs from the cell [34]. *In vitro*, multidrug-resistant cells expressing elevated levels of Pgp can be selected by exposure to sublethal doses of anticancer agents such as vincristine or actinomycin D [34]. EGF treatment has been shown to protect breast cancer cells from antinomycin D cytotoxicity [35], and in other studies drug-resistant cell lines have been shown to have enhanced expression of the EGF-R [36–38], suggesting a role for EGF-R signalling in Pgp-mediated drug resistance. This may be at the transcriptional level (e.g. by regulation of transcription factors such as AP1) or at the protein level (e.g. by affecting phosphorylation of Pgp, through activation of protein phosphatase-1 and -2A [39]). In view of the clinical data linking EGF-R expression with a failure to respond to therapy, further investigation of the role of the EGF-R in drug resistance is warranted.

Paracrine Effects of EGF-R Ligands

Many carcinomas synthesize EGF-R ligands (particularly TGFα and AR), which can act in an autocrine fashion on the producer cells as detailed above. Moreover, these li-

gands can also have important effects on surrounding supporting tissue. For example, a major factor that limits tumour growth is its requirement for an adequate blood supply [40]; as TGFα is a potent angiogenic factor (more so than EGF) [41], it may aid tumour growth through promotion of vascularization. Stromal proliferation, extracellular matrix deposition, and induction of cytokine release are also affected by EGF-R ligands [42–44], and these may also influence the local environment of the tumour.

CONTRIBUTION OF HOST-DERIVED EGF-R LIGANDS

Host-derived growth factors also influence tumour development [45]. It has been shown that blood-borne cancer cells exhibit organ-selective tumour growth [46], and tumour outgrowth occurs preferentially at sites of wound healing [47]. In the latter study, it was postulated that factors released by host cells into the inflammatory wound environment to promote healing also facilitated tumour growth. Several lines of evidence have indicated an *in vivo* role for EGF in tumour development and progression. Thus, EGF has been found to enhance spontaneous mammary tumorigenesis [48] and to promote implantation and/or growth of spontaneous mammary tumours in mice [49, 50]. In these studies, direct evidence for a role of host-derived EGF was obtained by use of mice in which the amount of EGF in blood had been reduced to low levels by surgical removal of the salivary gland (sialoadenectomy). In such mice, the growth of the tumour was reduced, and the effects of sialoadenectomy could be reversed by the administration of EGF. Similarly, we have inhibited intraperitoneal growth of a murine mammary tumour, MT1, using anti-EGF antibodies, even though the tumour did not synthesize any detectable EGF [51]. Our results suggested that host-derived EGF contributed to the establishment of microcolonies either directly, by supplementing growth factors produced by the tumour, or indirectly, by affecting the production of other growth stimulatory factors or cytokines. Alternatively, the EGF may have had a non-proliferative effect, e.g. by affecting adhesion molecule expression and promoting initial attachment of the tumour cells to the mesentery or peritoneal wall. Interfering with the establishment of micrometastases by host-derived EGF may identify another potential site for EGF-R-directed therapeutic intervention.

EGF-R LIGANDS

Ligand Structure

Members of the EGF-R ligand family are characterized by a three-looped structure that is imposed by three conserved intramolecular disulphide bonds [52]. In addition to forming the framework for the presentation of EGF-R interacting residues, this structural motif may also be responsible for conferring resistance to proteolytic degradation. This may explain how these growth factors can operate in chemically hostile environments such as the gut, the bladder, and at sites of infection or wounding. The similarity of EGF-R ligands with the protease inhibitor hirudin (which is itself highly resistant to degradation) [53] supports this conjecture. The individual EGF-R ligands have significantly different isoelectric points as a result of their differing amino acid compositions: the pI of EGF is 4.6 [54] compared with 6.8 for TGFα [55], 7.2 to 7.8 for HB-EGF [56], and about 7.8 for AR [57]. Thus, the local environment will play an important part in establishing the ability of cells to respond to a particular ligand.

AR, HB-EGF and BTC have, in addition to the EGF-motif, extended N-termini that confer on them further specificity for target cells. AR and HB-EGF both possess a highly basic N-terminus that enables these growth factors to bind to heparin or HSPGs expressed on the cell surface [58, 59]. This interaction can increase the apparent binding affinity for EGF-R by as much as two orders or magnitude by localizing the growth factor at the cell surface. The influence of HSPGs on the binding of AR and HB-EGF to cells clearly has potential for selectively targeting EGF-R on certain cell types and has already been exploited in the form of a chimeric heparin-binding TGFα toxin (see below). BTC possesses a relatively neutral N-terminal extension [60] that might also mediate a secondary interaction with a cell surface component, but, to date, its function has not been investigated.

Ligand Specificity

Of the five cognate ligands for EGF-R, amphiregulin is unusual in that the biological response evoked by its binding to EGF-R is not always the same as that of EGF or TGFα. Most notably, it cannot synergize with TGFβ to promote anchorage-independent growth [61]. We have also found that it is unable to promote an EMT in two colon carcinoma cell lines that are highly responsive to EGF and TGFα [62]. While some of the differences in cellular responsiveness to amphiregulin can be attributed to its truncated C-terminal tail and consequent lowered affinity for EGF-R [63], this does not appear to be the cause of its inability to induce a transformed phenotype or to promote an EMT. Distinct cellular responses to amphiregulin have obvious relevance to tumour biology as AR expression has been reported in breast [64, 65], colon [66], gastric [67], ovarian [68], and pancreatic [69] carcinomas. To date, no clear correlation exists between AR expression and tumour stage or grade, although in colon cancer it has been reported to be expressed in well differentiated tumours rather than in poorly differentiated ones [66].

TARGETING THE EGF-R

Expression of the EGF-R is correlated with poorly manageable tumours, and several mechanisms have been discussed above whereby the presence of the EGF-R can contribute to the malignant behaviour of solid tumours. The EGF-R is therefore an obvious target for therapeutic intervention.

Two basic approaches have been developed for targeting EGF-R: the first exploits the presence of EGF-R to direct cytotoxic agents to the tumour cells, while the second attempts to modulate EGF-R responses. While each has particular advantages and disadvantages, a common problem for any of the strategies is that the EGF-R expressed by most cancer cells is the normal non-mutated form. Thus, *selective* targeting of tumour cells may be difficult. Furthermore, the ability of liver EGF-Rs to clear large quantities of EGF from the circulation [70] represents a potential problem for any EGF-R targeting reagent. This problem indicates the need for low molecular weight therapeutic molecules that can extravasate rapidly to minimize liver clearance. Small molecules also benefit from improved penetration into solid tumours, a significant problem for many targeted therapies.

Although the majority of common tumours express normal EGF-Rs, certain brain tumours, e.g. glioblastomas, express a mutant form of the receptor that has a deletion in the extracellular ligand binding domain [71, 72]. In this case, selective targeting is possible by use of antibodies to the junctions of the deletion coupled with anti-cancer drugs or toxins. Similarly, such a therapy may also be effective in non-small cell lung cancer where there is some evidence that mutant EGF-Rs also occur [73].

Exploiting the Presence of the Receptor on the Surface of the Cell for Targeting of Toxins or Drugs to the Tumour Cells

LIGAND TOXINS. Considerable effort is being directed towards targeting growth factor-conjugated toxins to tumour cells [see Ref. 74 for review]. This approach utilizes receptor-mediated endocytosis as a mechanism for selectively targeting toxin into tumour cells. In the case of EGF-R, it is possible to take advantage of the high levels of EGF-R exposed on the surface of certain tumour cells, resulting in their binding proportionately more ligand than normal cells. One group has achieved further selectivity by incorporation of a heparin-binding domain to the N-terminal residue of TGFα: this chimeric growth factor-toxin was up to 10 times more potent against cells expressing HSPGs in conjunction with low numbers of EGF-R [75]. *In vitro*, the efficacy of EGF-R ligand toxins has been shown to be dependent on the level of EGF-R expression [76]; however, even cancer cells with low numbers of EGF-R appear more susceptible to the cytotoxic effects than normal cells [77]. *In vivo*, the toxins have also shown promising results when tested in experimental animals, although early studies clearly showed evidence of liver toxicity [78]; these problems were overcome by metering the dose of toxin with a controlled delivery system [78, 79]. The potential of an EGF-R ligand toxin in the treatment of bladder cancer is also being evaluated by intravesicular administration of the toxin [80]. This route not only enables local administration of the toxin (thus minimizing liver clearance and subsequent toxicity), but also takes advantage of the differential distribution of EGF-R in normal and malignant bladder epithelia, i.e. only malignant cells present EGF-R on their luminal surfaces.

ANTI-EGF-R ANTIBODY-DIRECTED THERAPIES. A number of studies have explored the use of anti-EGF-R antibodies to inhibit growth of cancer cells *in vitro* and *in vivo* [see Ref. 81 for a recent review]. Anti-EGF-R antibodies that block ligand binding also inhibit *in vitro* growth of cells that overexpress EGF-R; this demonstrates that such carcinoma cells have not escaped the requirement for ligand activation. *In vivo*, studies of the growth of xenografts in athymic mice also clearly demonstrate the ability of EGF-R antibodies to prevent tumour growth [82–84]. In these studies, blockade of ligand binding was not an essential requirement if the antibody was able to recruit the host immune effector system. However, in their studies with rat monoclonal anti-EGF-R antibodies, Modjtahedi *et al.* [85] have observed that the most efficacious antibody prevented ligand binding as well as promoting a host immune response.

Efforts to increase the anti-tumour activity of anti-EGF-R antibodies have focused on incorporation of a cytotoxic compound into the treatment regimen. Thus, anti-EGF-R antibodies have been shown to cause regression of established xenografts when used in conjunction with conventional chemotherapeutic compounds such as *cis*-diamminedichloroplatinum (*cis*-DPP) [86] or doxorubicin [87]. The effectiveness of this combination therapy may be linked to the possible effects of EGF-R on the regulation of Pgp. Anti-EGF-R antibodies conjugated to toxins have also been evaluated and shown to exhibit increased cytotoxicity, which is dependent on EGF-R number and drug concentration [88]. More recently, bispecific antibodies that recognize EGF-R and the cytotoxic drug doxorubicin have been developed and shown to deliver drug to the tumour cells [89]. These antibodies also appear to offer an additional advantage as they modify doxorubicin distribution and decrease its concentration in the intestine, the major site of early toxicity.

A problem frequently encountered in immunotherapeutic approaches using mouse or rat antibodies is generation of an immune response to these proteins (the human anti-mouse/rat antibody, HAMA/HARA, response) [see Refs. 81 and 90]. Similarly, although TGFα is not highly immunogenic, once conjugated to a toxin, the resultant molecule will be. Thus, upon repeated administration, the host antibody response will affect the circulation time of the EGF-R-directed reagent. For EGF-R antibody-based therapies, use of humanized antibodies or genetically engineered fragments of the antibody [see Ref. 90] may circumvent some of these problems. For EGF toxins, a panel of different toxins conjugated to TGFα may suffice.

Antibodies directed against the EGF-R are now being tested in clinical trials for their ability to localize to human tumours [81, 91, 92]. Preliminary reports appear encouraging, and no undue toxicity has been noted. However, the long-term effects of antibodies against EGF-R have yet to be evaluated. For example, immune damage to the liver caused by binding of the antibodies to liver EGF-Rs may

present a problem in humans: this would not have emerged in animal studies because the antibodies used were specific to the human EGF-R.

Modulating or Interfering with EGF-R Function

Alternative approaches to EGF-R-directed therapy attempt to interfere with EGF-R function, either by preventing activation (e.g. ligand antagonists and dimerization inhibitors) or by preventing signal transduction (e.g. tyrosine kinase inhibitors, SH2 domain inhibitors, and protein kinase C inhibitors) [93]. In the case of the latter approach, the web of interactions arising from receptor activation complicates attempts to block signal transduction pathways within the cell. This problem can be illustrated by the finding that simultaneous point mutation of all five major tyrosine phosphorylation sites in the cytoplasmic domain of EGF-R has no effect on DNA synthesis [21, 94], even though activation of $p21^{ras}$ is coupled to EGF-R stimulation via these phosphotyrosine residues and SH2 domains interactions involving Grb and Sos [95]. This failure to affect DNA synthesis may be due to exposure of cryptic tyrosines on EGF-R to direct or indirect phosphorylation of Shc (enabling coupling to Grb2) [96] or to receptor cross-talk [e.g. interaction of the EGF-R with other members of the c-erbB receptor family (see below) or with other unrelated receptors, such as that for platelet-derived growth factor]. Lateral signal transduction may pose a significant problem for inhibitors of second messenger signalling; thus, it is essential that much more information is acquired about the interrelationships of pathways leading from receptor activation and how their perturbation might influence cell behaviour. Furthermore, as common pathways are activated for both proliferation and differentiation (see below), blocking intracellular signalling pathways may be a double-edged sword.

In contrast to the approaches that depend on the presence of EGF-R to target poisons into the cells to kill them, reagents that interfere with EGF-R function may be cytostatic rather than cytotoxic. For most carcinomas, conventional chemotherapy reduces tumour mass, but eventually the patient relapses; even after more cycles of therapy, the outcome is poor. Cytostatic therapies aim to stabilize the disease by slowing growth and limiting further dissemination. Therefore, reagents that modulate EGF-R function may find a use in conjunction with conventional chemotherapy by delaying tumour regrowth and prolonging the period before relapse. This may improve significantly the quality of life of the cancer patient. Furthermore, by blocking EGF-R activation, any EGF-R-related effects on drug resistance should be inhibited, and this may improve the probability of survival of patients who undergo repeated cycles of chemotherapy. Cytostatic reagents will, by necessity, require long-term administration, and compliance, to maintain their anti-tumour effect. Thus, they must also be easy to administer and well tolerated. Such a situation already exists in the clinic where luteinizing hormone-releasing hormone and somatostatin analogues are used successfully for long-term treatment of certain endocrine tumours, and these drugs have few side effects [97]. For the EGF-R, it might be expected that long-term blockade of its activity may have some adverse effects on normal tissues; however, it has been shown that in mice removal of the salivary glands (which are the major source of EGF) has little effect on the animals, as judged by body-weight gain, uptake of food and water, and gross appearance [48]. Moreover, no gross pathological defects have been observed in TGFα knock-out mice [98].

TYROSINE KINASE INHIBITORS. Tyrosine kinase inhibitors differ from other inhibitors of signal transduction pathways because they block the initiating intracellular signal. As most tyrosine kinase inhibitors act by competing with the ATP binding site within the enzyme [for a review see Ref. 99], it is perhaps a little surprising that compounds have been developed with specificity towards individual tyrosine kinases. However, recent compounds such as 4,5-dianilinophthalimide (DHAP1) do exhibit selective inhibitory activity towards EGF-R and potent *in vivo* anti-tumour activity [100]. Even more selective is the quinazoline PD 153035, which has a K_i for the EGF-R tyrosine kinase of 5 pM, whereas other tyrosine kinases are inhibited at micromolar concentrations [101]. These types of compounds represent an exciting new class of potential therapeutic reagents.

LIGAND ANTAGONISTS. Ligand antagonists offer an attractive approach for preventing EGF-R activation because they block the signal at the source, thus limiting receptor cross-talk or signal transmodulation. Furthermore, there is no requirement for the drug to cross the plasma membrane and enter the cell; thus, they are not susceptible to MDR mechanisms. There is also the exciting possibility of selectively blocking binding of TGFα in a manner similar to the mode of action of a monoclonal antibody, 13A9 [102]. This might offer some advantage as TGFα is the ligand most commonly expressed by tumour cells.

In recent years, considerable effort has been invested in understanding the ligand receptor interaction with a view to developing antagonists. However, because there is still no detailed structural information concerning the receptor or the ligand:receptor complex, progress has been slow and directed primarily towards ligand structure–activity relationships [see Ref. 103 for a recent review]. It is clear that the three-looped structure of the growth factor is important for activity, and that ligand:receptor interaction involves discontinuous ligand epitopes [104]. Of the functional residues identified, Arg-41 [105] and Leu-47 (EGF numbering) [106], which both lie in the C-domain, are particularly important and together contribute over half of the binding energy of the ligand to the receptor. It also appears that the B-loop β-sheet in the N-domain of the growth factor is also an important determinant in ligand recognition, enabling the receptor to distinguish between EGF and TGFα [107]. While almost every residue in EGF has been studied, to date no EGF-R antagonists have been generated by mutagenesis, i.e. loss of biological activity has always been paralleled by a loss of receptor binding activity.

In view of the complex nature of the ligand:receptor interaction, generation of a small molecule ligand antagonist for EGF-R may seem a daunting prospect. However, the recent demonstration that growth hormone binds to its receptor through a much smaller functional epitope may suggest that designing small inhibitors for the EGF-R will be feasible [108]. Although many groups have attempted to synthesize fragments of EGF or TGFα, that retain the ability to interact with EGF-R, results from studies with short cognate peptide fragments [e.g. 109–113] have been disappointing and may reflect loss of conformational information. By taking structural considerations into account, we recently designed and synthesized a series of short modified TGFα peptides with activity towards EGF-R [114]. The modifications affected the propensity of the peptide to adopt a particular conformation, and this correlated with EGF-R activity. This type of approach, or exploitation of the enormous diversity offered by combinatorial libraries [115], may enable development of novel EGF-R interacting peptides.

DIMERIZATION INHIBITORS. Activation of the EGF-R is believed to be through induction of receptor dimerization, which enables cross-phosphorylation to occur between the two receptor molecules (i.e. receptor phosphorylation is not strictly "autophosphorylation") [4]. The precise molecular mechanism that leads to dimerization has not been elucidated. Although c-*neu*, the oncogenic form of c-erbB2 (the second member of the EGF-R family), contains a single point mutation in its transmembrane region which promotes dimerization and constitutive activation of the receptor [116], introduction of this point mutation in the EGF-R transmembrane region is not transforming [117]. Roles for both the extracellular and transmembrane domains of EGF-R in receptor dimerization are indicated from studies of EGF-R deletion mutants [118]. These showed that truncated EGF-Rs, which lacked the cytoplasmic domain, were able to form heterodimers with full-length EGF-R and exhibit a dominant negative effect on growth by reducing EGF-R tyrosine phosphorylation. More work to identify the surface(s) of EGF-R involved in dimerization may facilitate development of dimerization inhibitors.

The ability of EGF-R to form heterodimers with other members of the Type 1 receptor tyrosine kinase family (i.e. c-erbB2, c-erbB3, or c-erbB4) is a new and intriguing aspect of EGF-R function [119, 120]. Heterodimerization has already been shown to affect the affinity of EGF-R for ligand [121, 122], and, in the case of c-erbB2, heterodimerization modulates EGF-R function [121]. In view of the pleiotropic effects of EGF, it is essential that future studies address the possibility that these effects may be due to differing complements of c-erbB family members (and so different proportions of heterodimers) on the surface of a given cell. The potential of heterodimers to affect cellular signalling and EGF-R-mediated responses represents a potential new approach to therapy; it might be speculated that modified or chimeric ligands designed to promote formation of specific heterodimers could be used to direct cellular responses.

INDUCTION OF DIFFERENTIATION. The ability of EGF to induce differentiation is another property of this growth factor. In normal tissue, it accelerates proliferation and differentiation in skin, corneal epithelium, and mammary epithelium. For tumour cell lines that overexpress EGF-R, dual effects of EGF are also observed. At low (pM) concentrations it stimulates growth, but at higher (nM) concentrations it inhibits growth [e.g. 17], and there is some evidence that the latter effect may be related to induction of differentiation [123]. Recent studies have shown that common components are activated in the pathways that lead to cell proliferation or differentiation: the choice of pathway appears to be governed by altered subcellular localization of key enzymes such as mitogen-activated protein (MAP) kinase, and this appears to be due to temporal differences in phosphorylation [124]. Thus, in cells that overexpress EGF-R, low doses of EGF may give only transient stimulation of the MAP kinase pathway leading to cellular proliferation, whereas higher doses of EGF cause sustained stimulation of the MAP kinase pathway causing differentiation. If we fully understood the processes that caused the switch from growth to differentiation, then it might be possible to develop a therapeutic strategy that exploited the occurrence of the EGF-R on tumour cells to promote differentiation. In this context, it is of considerable interest that a monoclonal anti-EGF-R antibody caused dramatic tumour regression when administered to experimental animals bearing EGF-R overexpressing tumours and that the major effect of the antibody appeared to be through induction of differentiation of cells within the tumour [125].

CONCLUDING REMARKS

With the advent of molecular genetics and the identification of tumour susceptibility genes, earlier identification of patients at risk of developing cancer is now possible. These advances must be coupled with the availability of new and effective treatments. Elevated expression of the EGF-R by carcinoma cells is associated with a poor clinical outcome. Of the diverse cellular responses initiated by EGF-R activation, several of these may be exploited by cancer cells and contribute to the malignant behaviour of the tumour. The correlation of EGF-R expression with invasive or disseminated tumours and the connection with drug insensitivity provide ready justification for therapeutic intervention aimed at modulating the activity of this receptor.

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