Role of Epidermal Growth Factor in Carcinogenesis

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Abstract

For cell growth and division to occur, a large variety of metabolic processes must be carefully coordinated in the cell. Through evolutionary pressures, specific hormones and growth factors have acquired the ability to trigger a complex coordinated "pleiotropic growth response" in their target cells. This complex response is mediated by specific cellular receptors and intracellular messengers. Teleologically then, it makes sense that in oncogenesis this growth regulating network is utilized by the production of proteins which mimic growth factors, the activated form of their receptors or, the messengers themselves. Several lines of evidence indicate that the epidermal growth factor-stimulated growth regulatory system is involved in cellular proliferation, both normal and neoplastic. Some of the effects of epidermal growth factor in carcinogenesis are separable from its direct, growth stimulatory effects. Thus, the role of epidermal growth factor in carcinogenesis is more complex than is its role in stimulating growth.

Introduction

EGF³ is a M, 6045 polypeptide which can stimulate or inhibit proliferation or differentiation of a wide variety of cells (1–3). While EGF is thought to play a role in almost every tissue in the body both during development and in the adult, the exact nature of this role is not clear. The inability to experimentally lower EGF levels in mice by removing the salivary glands (4), the major site of EGF synthesis in the mouse (5), has hampered clarification of the role of EGF. This inability to alter EGF levels surgically is probably due to EGF being synthesized at several anatomical sites in the body (6–9), where EGF functions more in a paracrine rather than an endocrine manner. Additionally, the use of antibodies against EGF are unlikely to lower EGF levels because of the very fast turnover rate (half-life, 1.5 min) of EGF in vivo (10). Since a specific receptor located at the cell surface is required in order for EGF to interact with the cell, the availability of antisera against the EGF receptor which blocks EGF binding (11) offers a new approach in which to deplete cells of an EGF signal.

The EGF receptor is an integral membrane protein with many complex functions which have recently been reviewed in detail elsewhere (12). Briefly, these functions fall into two categories; signal transmission and autoregulation. (a) Signal transmission may involve an intrinsic EGF-stimulated kinase which phosphorylates certain proteins on their tyrosine residues. Another mechanism may involve receptor clustering or a combination of clustering and kinase activity which, in turn, produces an intracellular signal. (b) Modulation of EGF-stimulated receptor activity (auto-regulation) occurs by at least three modes. First, binding and kinase activities may be inhibited by phosphorylation of certain regulatory sites of the receptor. Second, receptor activity may be reduced by receptor degradation following its interaction with EGF. Third, sequestration of the EGF receptor in intracellular compartments may prevent EGF activation of this pool of receptors.

Several lines of evidence indicate that the EGF-stimulated cell regulatory system may play a role in carcinogenesis. This will be discussed in detail below. In brief, the first line of evidence indicates that EGF directly elicits transformation-associated phenotypes in certain target cells. Many of these effects are reversible upon the removal of EGF, but in a small but highly significant number of already partially transformed cells, they are not reversible; e.g., EGF has been observed to potentiate chemical transformation in vivo and viral transformation in vitro. The second line of evidence that EGF plays a role in carcinogenesis is that many cancer cells produce a peptide, termed α-TGF, which is structurally related to EGF and activates the EGF receptor. However, to date, it is not definitively determined whether α-TGF is essential for the transformed properties of its parent cell. Another, albeit indirect, line of evidence is that EGF depresses the immune system whose function is to destroy newly arising tumors (immune surveillance). The EGF-stimulated cell regulatory system could conceivably also be activated by a mutation of the receptor eliminating the requirement for elevated EGF (or TGF levels). This may be the mechanism by which erythroblastosis or lymphoid virus causes a variety of cancers including erythroblastosis, sarcomas, and carcinomas. Also discussed are data which contradict the hypothesis that the EGF-stimulated cell regulatory system plays a role in carcinogenesis.

EGF Elicits Transformation-associated Phenotypes in Normal Cells

The addition of EGF to normal cells elicits certain responses which are associated with neoplastic transformation. For example, EGF induces a partial loss of density dependent inhibition of growth and the dependency on serum for growth (2, 13, 14), an increase in the level of phosphotyrosine in proteins (15–18), and an increase in cellular metabolism including sugar and amino acid transport, ATP turnover, and ornithine decarboxylase activity (19–20, 52, 53). EGF induces the expression the mRNA of c-fos and c-myc genes, cellular proto-oncogenes (22). Cellular protooncogenes are the normal cellular homologs of viral oncogenes. Oncogenic viruses are thought to have acquired the oncogene from normal cellular RNA. Through uncontrolled
expression (plus, in some cases, mutation as well) they induce uncontrolled growth in the host cell (23). EGF also elicits certain responses which are associated with cancer such as the loss of fibronectin, and an augmentation of the secretion of plasminogen activator (24–27). Growth of cells in soft agar, considered by many (28–30) to be one of the best assays for showing the tumorigenicity of a cell, is potentiated by EGF or EGF-like factors (31–33). This potentiation by EGF is even greater for partially tumorigenicity of a cell, is potentiated by EGF or EGF-like factors activator (24–27). Growth of cells in soft agar, considered by fibronectin, and an augmentation of the secretion of plasminogen chemical, and spontaneously transformed cells may be respon

dehomology, respectively (43–46). The DNA sequence encoding α-TGF was observed in normal cells (46), indicating that it is an unmodified normal protein which is expressed in cancer cells in abnormal quantities and/or at the wrong stage of the cells’ development. α-TGF is not an EGF-like peptide from precursor TGF, indicating that both forms are derived from the same molecule. One of the other molecules exhibiting α-TGF activity was identified to be urogastrone, and the identity of the other factors is not known but may be precursor forms of urogas

tation of cell growth.

EGF Promotion of Viral Carcinogenesis

EGF enhances viral transformation of cells (87). Rat embryo cells infected by adenovirus formed almost 5 times as many colonies in soft agar when EGF was present. Additionally, the colonies appeared sooner, grew faster, and had a more diffuse morphology than did non-EGF treated cells. These effects were very similar to those produced by 12-O-tetradecanoylphorbol-13-acetate, a classic tumor promoter. Classic tumor promoters have no or very weak tumorigenicity by themselves but can markedly enhance the carcinogenicity of low doses of chemical carcinogens. The mechanism by which tumor promoters and EGF may enhance carcinogenesis may in part be due to stimulation of cell growth.

EGF plays even a more active role in the transformation of cultured granulosa cells by the Kirsten murine sarcoma virus (88). In the absence of EGF, infected cells formed few transformation foci, whereas in its presence, the cells formed many foci of rounded cells. It should be noted that EGF does not produce these effects in noninfected cells. Removal of EGF resulted in the reversion of the majority of the infected granulosa cells to a normal phenotype, indicating that the presence of EGF is essential for the transformed phenotype. The presence of the remaining persistent transformed foci was dependent on their prior
exposure to EGF. Thus EGF has the capacity to elicit a transformed phenotype in partially transformed cells. This phenotype may be “locked” into place allowing cells to become EGF independent.

EGF did not appear to be acting as a classic tumor promoter in the granulosa cells since its transforming activity was separable from its growth promoting activity. This was shown by the following results: (a) EGF did not stimulate the growth of either normal or virally infected granulosa cells, and (b) the time required for formation of transformation foci was not accelerated.

Transformation of normal cells, in contrast to certain established cell lines, requires the presence of at least two complementary oncogenes (89–92), thus in this case, EGF is apparently substituting for the second oncogene. Since the Kirsten sarcoma virus expresses the ras oncogene (which creates the traits of focus formation and tumorigenesis) is complemented by oncogenes responsible for immortalization (92), EGF may partially substitute for oncogenes in this second group. Alternatively, EGF may be an enhancer of ras function. EGF enhances the guanine nucleotide binding activity of ras transforming proteins as well as enhancing the phosphorylation state of at least one of them (28).

**EGF Promotion of Chemical Carcinogenesis**

One of the first observations that indicated that EGF may play a role in carcinogenesis is that EGF enhances the carcinogenic potential of methylcholanthrene in skin (93, 94). A specific interaction of chemical tumor promoters with the EGF receptor was shown in cultured cells where TPA and benzopyrene inhibited the binding of EGF but not insulin, multiplication stimulating activity, concanavalin A, nerve growth factor, low density lipoprotein, or murine type C ectopic viral glycoprotein. Decreased EGF binding was found to be caused by lowered receptor affinity rather than decreased receptor number (95–101).

This effect of these chemical tumor promoters on EGF binding is thought to be mediated by intracellular processes since they have no effect on the binding of EGF to broken cell preparations (100). Protein kinase C fulfills many of the requirements of such an intracellular mediator: it is activated by a variety of chemical tumor promoters and decreases EGF receptor affinity for EGF when added to EGF receptor preparations in vitro (102). The mechanism by which protein kinase C inhibits EGF receptor activity may be via direct phosphorylation of a threonine residue which is near the membrane spanning region of the EGF receptor (103). It should be emphasized that this is not an autophosphorylation site. EGF stimulates the phosphorylation of its own receptor on tyrosine residues near the cytoplasmic end of the molecule.

In addition to decreasing EGF receptor affinity, chemical tumor promoters also decrease tyrosyl kinase activity of the EGF receptor (49, 95, 102). Since an increase in tyrosyl kinase activity, rather than a decrease, is usually associated with transformation, it is unclear how these effects promote carcinogenesis. In fact, EGF receptor activities recover within hours in the continued presence of TPA (96, 98). Thus, since TPA promotion of carcinogenesis requires several days, it is not likely that phorbol ester action is mediated through decreasing EGF receptor binding and kinase activities. Perhaps chemical carcinogens promote other functions of the receptor such as protein recognition and binding properties of the kinase site or clustering, which in turn may have important roles in growth regulation. Alternatively, these alterations of EGF receptor activity by chemical carcinogens may simply be circumstantial. In this light it should be noted that phorbol esters have the capacity to stimulate cell growth in cells that do not respond to EGF and, depending on cell type, may either inhibit or be synergistic with EGF-induced mitogenesis (104–106).

**Immunosuppressive Activity of EGF**

Although immunosuppression may not directly produce cancers, it may prevent the elimination of spontaneously arising tumors by the immune system. Thus, immunosuppression usually is considered to permit cancer growth rather than to actually cause it. An example of the role of an impaired immune system in neoplastic disease is the increased incidence of cancer observed after the destruction of T-cells by the human T-cell leukemia virus type III which causes the acquired immune deficiency syndrome (107).

Injection of EGF 1 day before the injection of an antigen (sheep RBC) suppressed the production of antibodies to this antigen. Since the IgG response was affected more greatly than the IgM response, and since the IgM response is more T-cell dependent than is the IgM response, helper T-cells were suggested to be the target of EGF induced immunosuppression. Recently, it has been shown that the antigen-presenting function of lymphocytes affecting the growth of T-cells is potentiated by EGF in vitro (108). It should be noted that EGF does not have a general toxic effect on lymphocytes. This was shown by the observation that EGF was somewhat stimulatory for immunoglobulin production if injected 3 days after the injection of the antigen, and that it did not prevent the development of immunological memory (109, 110). Suppression of T-cell function by EGF was also implicated by studies showing that EGF depressed the delayed type hypersensitivity response to 2,4-dinitro-1-fluorobenzene (111). Salivary gland extract (containing high levels of EGF) delays the rejection of skin allografts (112), a process also predominantly mediated by T-cells.

In addition to these relatively short term immunosuppressive effects, EGF may also have a long term effect. The possibility that EGF may cause long term effects on the immune system was first shown by Takeda and Grollman (113), who injected crude salivary gland extract into mice, causing the thymus and lymph nodes to atrophy. In a subsequent study, purified EGF was observed to cause a decrease in DNA synthesis in the thymus, spleen, and bone marrow, although an increase in DNA synthesis was observed in other tissues (114).

Although EGF stimulates cortisol secretion by the adrenal glands (99), it appears unlikely that EGF induction of cortisol synthesis would mediate the immunosuppressive activity of EGF. Thymic and lymphoid atrophy induced by injections of crude submandibular extracts was not prevented by prior extirpation of the adrenal glands (113). The ability of thymus and spleen membranes to bind EGF (115) is another indication that EGF may have a direct effect on the immune system, since growth and development of lymphocytes occurs in these tissues.
Relationship of the EGF Receptor to Transforming Proteins

The EGF receptor itself is related to certain transforming proteins both structurally and functionally. Transforming proteins are produced by an oncogene in a viral genome. Deletion of this gene renders the virus incapable of inducing carcinogenesis. Functionally, both the EGF receptor and several transforming proteins, which are also protein kinases, have the unusual property of being able to phosphorylate proteins on tyrosine residues. Of all transforming proteins known, 30% have tyrosyl kinase activity. These include src, erbB, abl, yes, fgr, ros, fes(fps), and fms (116, 117). Phosphotyrosine represents usually less than 0.03% of the total phosphoaminoacids in nontransformed cells and increases approximately 30-fold in transformed cells (118). This property is also shared with other receptors for growth promoting factors such as PDGF, insulin, and somatomedin C (116). Thus it is thought that tyrosyl kinase activity may play a role in growth control. The pathways which tyrosyl kinases control have not been identified at this time.

Protein substrate specificities may be similar between the various tyrosyl kinases. Both the EGF receptor and pp60-src, the protein encoded by the transforming gene of the Rous sarcoma virus, phosphorylate a M, 36,000 normal cell protein at the same site (119). These kinases also phosphorylate synthetic peptide substrates related to the tyrosine autophosphorylation site with similar specificities (120). pp60-src phosphorylates certain antibodies directed against it; these antibodies also serve as substrates for the EGF receptor, although they do not have the capacity to precipitate it (121, 122). Thus, the EGF receptor and pp60-src may both stimulate cell growth through the same pathway, i.e., by phosphorylation of the same regulatory proteins due to similar substrate specificities.

Structurally, the cytoplasmic portion of the receptor has an average of 25% homology to the kinase domain of several transforming proteins. Even greater homology (95%) has been observed with the erbB-transforming protein of the avian erythroblastosis virus (123, 124). This extent of homology suggests that the erythroblastosis virus incorporated the genome for the cytoplasmic portion of the EGF receptor from its host. Since the erythroblastosis virus infects chickens, whereas the EGF receptor is from its host, the extent of homology suggests that the EGF receptor, although they do not have the capacity to precipitate it (121, 122). Thus, the EGF receptor and pp60-src may both stimulate cell growth through the same pathway, i.e., by phosphorylation of the same regulatory proteins due to similar substrate specificities.

The EGF receptor itself can become an oncogene if a viral promotor or activator is inserted into the cellular gene. This appears to be one mechanism by which the avian leukosis virus induces tumors, a slowly oncogenic retrovirus, causes erythroblastosis (128). A slowly oncogenic retrovirus is a retrovirus that does not carry a transforming gene yet still induces tumors albeit with a latency period of weeks. The mechanism by which the avian leukosis virus induces tumors seems to be through promotor insertion into the host genome. If the promotor is placed in front of a cellular gene whose unrestricted transcription can cause uncontrolled growth, the host organism will develop cancer. In a chicken with erythroblastosis, the viral promotor was mapped in front of the c-erbB gene which is thought to be the gene for the EGF receptor. Additionally, increased levels of RNA which hybridize to probes against v-erbA and v-erbB, were detected in the erythroblasts. Thus the inserted promotor appears to successfully promote the transcription of c-erb proteins, most likely the EGF receptor. It is not clear in this study whether the whole or truncated form (as in v-erbB) of the receptor is produced in the transformed cells and whether the receptor is appropriately membrane bound.

It is not entirely clear whether increased kinase activity necessarily leads to cell proliferation. For example, infection of cells by the avian erythroblastosis virus does not appreciably increase phosphotyrosyl levels in vivo (118, 126, 129). EGF, which presumably increases tyrosine kinase activity in target cells, inhibits the growth of several lines of mammary, liver, and pituitary cancer cells, as well as several other tumor cell lines (35, 51, 130–132). EGF inhibits A-431 cell proliferation (16, 133), although it increases intracellular phosphotyrosyl levels 3-fold (17). In fact, monoclonal antibodies acting as EGF agonists (but also those which have no effect on receptors) inhibit the growth of A-431 tumors in nude mice (134). Stimulation of EGF receptor kinase activity without concurrent stimulation of growth can also occur when cells are exposed to cyanogen bromide-treated EGF (135). Also, cells containing 10 times the normal concentration of the tyrosyl-kinase c-src, the cellular homologue of the transforming protein v-src do not express the transformed phenotype (136). These examples show that increased tyrosyl kinase activity is not necessarily correlated with increased cell proliferation. Thus, qualitative as well as quantitative changes may contribute toward the transforming capacity of a tyrosyl kinase. Such qualitative rather than quantitative changes have previously been observed for proteins encoded by ras genes. These examples also indicate that, in certain cases, EGF may be antagonistic toward cancer cell growth. Since EGF is relatively nontoxic to the use of EGF as well as monoclonal antibodies to the EGF receptor (134) should be considered in cancer therapy.

Measurement of EGF Receptor Levels in Cancer Cells

These is no simple correlation of EGF receptor levels to cancer. In many transformed cells, including those transformed by viruses, the levels of EGF receptors decreases (34, 54, 55, 68, 69, 137, 146). This decline is thought to be caused by the production of α-TGFs, which can down regulate the receptor (32), by the transformed cells. Some preliminary evidence indicates that this relationship may not necessarily hold true. No correlation was observed in variants of a mammary carcinoma cell line and in various lung cancer cells between EGF-receptor levels and the capacity of the conditioned medium to stimulate cells to grow in soft agar (137, 146). On the other hand, this assay is relatively nonspecific because β-TGF and PDGF, which are also produced by many transformed cells, also potentiate cellular growth in soft agar (31, 37, 139). Thus altered production of these latter factors
and possibly others by the cells can independently modulate the capacity of conditioned medium to support cell growth in soft agar. Another confounding aspect of measuring α-TGF levels is that simple competitive binding assays on live cells may not produce valid results. Several substances, including PDGF, rapidly lower the affinity of the EGF receptor (96, 97, 140–143), which could make it appear as if the substance was competing with EGF. More accurate assessment of α-TGF levels should involve high performance liquid chromatography to partially characterize the TGF and a binding assay utilizing cellular membranes rather than live cells (115). The absence of α-TGF from the medium may not necessarily mean that the cell is not producing it; interaction of TGF with its receptor may occur intracellularly, i.e., within the endoplasmic reticulum, Golgi or exocytotic vesicles (144). Thus, intracellular levels of specific messenger RNA for TGFs may more accurately quantitate TGF production. Another reason for the lack of a correlation of TGF production and receptor levels is that different cell types may have varying capacities to recycle EGF receptors rather than necessarily degrading them in response to ligand binding (145).

In some cases EGF receptor levels are higher in malignant as compared to normal tissues. In several different squamous cell lung carcinomas EGF receptor levels were highly elevated as compared to the normal lung. In contrast, other lung carcinomas (in particular, small cell carcinoma) had undetectable EGF receptor levels (146–148). Increased levels of EGF receptors were also observed in some breast cancer cells as compared to normal cells, but in others the levels of EGF receptors were lower (149). Another instance in which increased levels of EGF receptors were detected are in brain tumors (150). This finding may at least partially be explained by the presence of different ratios of nonneuronal versus neuronal cells in the tumors, since neurons have a particularly low EGF binding capacity as compared to other cells. In some tumors the number of EGF receptor gene copies per cell was increased, which could also explain high EGF receptor expression (150). An increased number of EGF receptor gene copies has also been reported for A-431 epidermoid carcinoma cells (124, 151, 152).

In conclusion, EGF receptor levels may be decreased, increased, or not changed in cancer cells as compared to their normal counterpart. In many cases a decrease in EGF receptor levels is associated with the production of α-TGF. In some cases an increase in EGF receptor levels is associated with gene amplification.

**Overall Perspective**

It has been clear for quite some time that hormonal systems are involved in cancer growth. The identification of oncogenes and their products is shedding new light on the involvement of hormones in cancer. In addition to the previously discussed homology of the EGF receptor to v-erbB, homology of PDGF to the putative transforming protein p28amide and homology of G-proteins (GTP-binding proteins which transduce a hormone binding signal to adenyl cyclase) to p21trans have been observed (153–155). Functional similarities, such as tyrosyl kinase activity, between these oncogene products and their homologous cellular products have also been observed (12, 116, 125–127, 156). If a simple substitution by an oncogene product for its cellular homologue occurs, a hormone stimulus should substitute for the oncogene product as EGF must in granulosa cells (88).

The complexity of hormonal interactions with cells makes the precise elucidation of their role in carcinogenesis difficult. For example, the same hormone may stimulate, inhibit, or have no effect on proliferation, depending on the cell type and its state of differentiation. Thus, the choice of target cells can highly influence the results of an experiment where a hormone may have no effect, enhance, or inhibit carcinogenesis.

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**References**


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