Review Article

Epidermal Growth Factor Receptor and Transformation

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Abstract
The action of transforming growth factor peptides is mediated by distinct membrane receptors, which in turn activate a postreceptor signaling mechanism, eventually resulting in a mitogenic response. Such a signaling pathway may be modified by oncogene expression at the receptor or postreceptor levels, as well as by alterations in the levels of expression of the growth factor itself. One of the most extensively studied group of receptors is the type I epidermal growth factor receptor family. Activation of this receptor triggers the induction of receptor dimerization, which enables cross-phosphorylation to occur between two receptor molecules. This dimerization model provides a universal mechanism to activate the type I receptor family for growth factors and subsequent transformation.

Key words Epidermal growth factor receptor · Oncogene · Transformation · Dimerization · Amplification

Introduction
The multiplication of normal cells is regulated by the interplay between various growth factors and hormone-like factors which are present in body and tissue fluids. In contrast, malignant cells are no longer subject to such endocrine controls. In most cases, tumorigenic cells require less of these exogenous mitogens for proliferation than the normal cells from which they are derived. For example, chick fibroblasts display insulin-like growth factor-dependent growth, whereas these cells when transformed by the Rous sarcoma virus require much less of this growth factor. A model designed to explain autonomy of tumor cell growth would have to predict that growth factors produced by the tumor cell itself would render cells independent of the exogenous supply of required mitogens.

Epidermal Growth Factor Receptor Expression in Human Cancer
The idea that tumor cells synthesize, release, and respond to their own growth factors is not novel. A large variety of transformed cells have been reported to produce mitogenic factors, suggesting that this ability of self-stimulation might be important in the establishment and maintenance of transformation. The action of the transforming growth factor peptides is mediated by their distinct membrane receptors, which in turn activates a postreceptor signaling mechanism eventually leading to a mitogenic response. Such a signaling pathway may be modified by oncogene expression at the receptor or postreceptor levels, as well as by changes in the level of expression of the growth factor itself. Indeed, the ability of many oncogenes to confer growth factor autonomy on cancer cells seems to be related to how they alter a receptor at a postreceptor signaling pathway, rather than to a primary alteration in the synthesis and release of a specific growth factor. The autocrine action of the effector peptide may be amplified by mechanisms other than an increase in concentration. For example, enhanced cellular responsiveness to a growth factor may also result from a change in the number or affinity of receptors of the growth factor.

Thus, very high numbers of epidermal growth factor receptors (EGFr) are found in squamous carcinoma cells derived from human head and neck cancers, as well as in the intensively studied A431 cell line, also derived from a squamous carcinoma. In A431 cells, a
population of receptor molecules with extremely high affinity for epidermal growth factor (EGF) has been identified; and this subset of receptors may be involved in the mitogenic response of cells to EGF. Such receptors are important because monoclonal antibodies to them inhibit not only the proliferation of A431 cells in culture, but also their growth in vivo in athymic mice.

Structure and Regulation of the Epidermal Growth Factor Receptor

The amino terminal portion of 621 amino acids of the EGFr faces the outside of the cell and contains the recognition site for EGF and transforming growth factor-alpha (TGF-α). The 170 kDa holoreceptor contains about 40 kDa of carbohydrate side chains. In the extracellular portion, there are 12 potential sites for amino-linked glycosylation and at least 11 of these appear to be used. Digestion with endoglucosidase F, which removes amino-linked sugars, was found to render the EGFr Schiff-negative, suggesting that most, if not all carbohydrate is amino-linked. A striking feature of this portion of the molecule is its high cysteine content. The extracellular domain of the EGFr contains 51 cysteine residues compared with nine cysteine residues in the carboxyterminal intracellular part of the molecule. These cysteines are located primarily in two extracellular regions, residues 134-313 and 446-612. A hydrophobic region of 23 amino acids provides a membrane-spanning segment that separates the extracellular region from the 542-amino-acid carboxyterminal intracellular domain. The structure of this intracellular domain can be closely aligned with retroviral protein tyrosine kinases, and with the catalytic subunit of cyclic adenosine monophosphate (cAMP)-dependent protein kinase. There is a strong homology in the region of lysine-721 in the EGFr, which corresponds to the ATP binding site in the cAMP-dependent protein kinase (A kinase). However, by comparing these sequences, it was found that although there is close homology between the tyrosine kinases, this region is not closely homologous to A kinase, which phosphorylates serine and threonine residues in proteins. Differences in this region may determine substrate specificity (Fig. 1).

The EGFr is a phosphoprotein containing 2.5–3.5 mol of phosphate per mole of receptor in vivo. Tryptic phosphopeptide mapping reveals that these phosphates are largely distributed in serine- and threonine-containing phosphopeptides. Tyrosine-1173 is the major site detected with in vivo labeling, but with careful protein isolation in the presence of phosphatase inhibitors, the phosphorylation of tyrosine-1148 and -1068 is also evident, and there are likely to be additional sites of self-phosphorylation. The major sites of self-phosphorylation are located in the extreme carboxyl terminus of the protein. Studies by Cohen in 1982 indicated that conversion of the 170 kDa holoreceptor to a 150 kDa form by a Ca²⁺-sensitive neutral protease resulted in a kinase that was kinetically active against exogenous substrates, but had reduced ability to self-phosphorylate. This was most likely attributable to removal of the carboxyterminal 20 kDa of protein which contains the major self-phosphorylation sites. The effect of the self-phosphorylation of EGFr kinase activity was investigated by first incubating the EGFr with adenosine triphosphate for varying periods then measuring its activity using exogenous peptide substrates. Maximum kinase activity was observed when about 1.5 mol of phosphate was incorporated in each mole of an EGFr molecule. When ligand binds to the receptor there is a change in the conformation of the extracellular domain. In the unoccupied state the receptor is largely monomeric, but ligand binding seems to stabilize the receptor dimers. The dimeric receptor has a higher rate of catalysis than the monomeric receptor and rapidly becomes phosphorylated on tyrosine residues at three to four sites in its C-terminal autophosphorylation.