Epidermal growth factor receptor inhibitors in cancer treatment: advances, challenges and opportunities

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Aberrant expression of the epidermal growth factor receptor (EGFR) system has been reported in a wide range of epithelial cancers. In some studies, this has also been associated with a poor prognosis and resistance to the conventional forms of therapies. These discoveries have led to the strategic development of several kinds of EGFR inhibitors, five of which have gained US Food and Drug Administration approval for the treatment of patients with non-small-cell lung cancer (gefitinib and erlotinib), metastatic colorectal cancer (cetuximab and panitumumab), head and neck (cetuximab), pancreatic cancer (erlotinib) and breast (lapatinib) cancer. Despite these advances and recent studies on the predictive value of activating EGFR mutation and KRAS mutations with response in non-small-cell lung cancer and colon cancer patients, there is currently no reliable predictive marker for response to therapy with the anti-EGFR monoclonal antibodies cetuximab and panitumumab or the small molecule EGFR tyrosine kinase inhibitors gefitinib and erlotinib. In particular, there has been no clear association between the expression of EGFR, determined by the US Food and Drug Administration-approved EGFR PharmDX kit, and response to the EGFR inhibitors. Here, we discuss some of the controversial data and explanatory factors as well as future studies for the establishment of more reliable markers for response to therapy with EGFR inhibitors. Such investigations should lead to the selection of a more specific subpopulation of cancer patients who benefit from therapy with EGFR inhibitors, but equally to spare those who will receive no benefit or a detrimental effect from such biological agents. *Anti-Cancer Drugs* 20:851–855 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Advances in tumour targeting using the EGFR inhibitors

In 1962 during the examination of a crude extract from mouse submaxillary glands, Cohen [1] discovered epidermal growth factor (EGF), the first member of the EGF family of ligands. The discovery of this growth factor led to research for and the discovery of its cellular receptor (EGFR) and then other members of the EGF family ligands and other EGFR family members [2–5]. Currently, the EGFR system is one of the most well-characterized growth factor receptor systems. In the past four decades, in addition to their role in normal development, aberrant expression of the EGFR system has also been associated with a wide range of pathological conditions and, in particular, human cancer [4–7].

EGFR is the prototype of the type-I growth factor receptor (*erb*B of EGFR) subfamily, which currently includes three additional known members: HER-2 (Neu, c-erbB-2), HER-3 (c-erbB-3) and HER-4 (c-erbB-4) [7–9]. The binding of growth factors (e.g. EGF, tumour growth factor-α, amphiregulin, HB-EGF, β-cellulin, epirgulin) to the external domain of the EGFR leads to the formation of homodimers or heterodimers with the EGFR and other members of this family, autophosphorylation of several tyrosine residues in its intracellular domain which in turn leads to activation of several downstream signalling pathways such as the ras/raf/MAPK, JAK-STAT and the PI-3/Akt pathways (Fig. 1) [7–14]. The biological consequences of EGFR activation in human malignancies include the following: increased cell proliferation, reduced apoptosis, increased angiogenesis, increased motility, invasion and metastasis and these are the hallmarks of human malignancies (Fig. 1) [7,15].

Since the early 1980s, aberrant expression of the EGFR system has been reported in a wide range of human epithelial malignancies and in some studies it has been associated with a poor prognosis and resistance to conventional therapies [6,16]. These discoveries have led to the strategic development and approval of several EGFR inhibitors such as anti-EGFR monoclonal antibodies (mAbs) cetuximab and panitumumab, which bind to the external domain of the EGFR, and small molecule EGFR tyrosine kinase inhibitors (TKIs) [e.g. gefitinib, erlotinib and lapatinib (a dual EGFR/HER-2 inhibitor)], which target the intracellular tyrosine kinase domain of the receptor, for the treatment of human malignancies [12,13,17–21].

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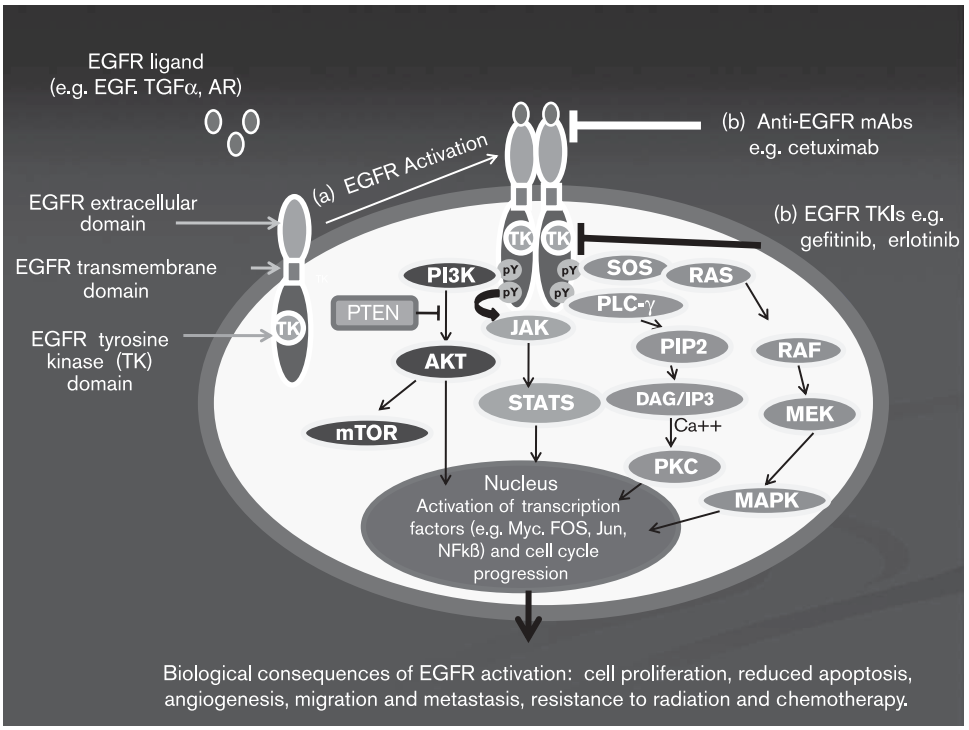
Fig. 1



Epidermal growth factor receptor (EGFR) activation and EGFR targeting in human cancers. (a) Aberrant activation/tyrosine phosphorylation of the EGFR in human cancers can occur through several mechanisms: (i) increased production and binding of autocrine, paracrine and/or juxtacrine ligands to the extracellular domain of the EGFR, (ii) EGFR mutation (e.g. EGFRvIII, T790M), (iii) EGFR overexpression, (iv) EGFR transphosphorylation through homodimerization or heterodimerization or (v) low phosphotase activity (e.g. mutation of PTEN). (b) Anti-EGFR monoclonal antibodies (mAbs) inhibit growth of EGFR positive tumour cells by blocking the binding of ligands to the external domain of EGFR and tyrosine phosphorylation of the EGFR (pY) which ultimately inhibit several downstream cell signalling pathways. *In vivo*, anti-EGFR monoclonal antibodies (mAbs) (e.g. cetuximab) can also aid tumour destruction by inducing antibody-dependent cellular cytotoxcity and complement-mediated cytotoxicity. (c) Small molecule EGFR tyrosine kinase inhibitors (EGFR TKIs) inhibit EGFR phosphorylation by competing with ATP for binding to the intracellular tyrosine kinase domain of the EGFR [7,10,12–14]. AR, amphiregulin; DAG, diacylglycerol; IP3, inositol triphosphate; JAK, Janus-associated kinase; NFkB nuclear factor-kappaB; PI-3K, phosphatidylinositol-3 kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PKC, protein kinase C; PLC-γ, phospholipase C gamma; PTEN, phosphotase and tensin homologue, pY, phosphorylated tyrosine; STAT, signal transducer and activator of transcription.

Challenges associated with tumour targeting with EGFR inhibitors

There are currently several challenges associated with the routine use of the EGFR inhibitors in the treatment of cancer patients. For example, although the EGFR inhibitors improve survival in cancer patients, the duration of response to the EGFR inhibitors is often limited in these patients. In addition, there is currently no reliable predictive factor for response to therapy with the EGFR inhibitors [7,22,23]. With few exceptions (e.g. [24]), it is also unclear whether a predictive marker for response to therapy with one type of EGFR inhibitor (e.g. EGFR TKI gefitinib) could play an important role as a predictive marker for response to therapy with another type of EGFR inhibitor (e.g. anti-EGFR monoclonal antibody cetuximab) or even similar type of EGFR inhibitors (e.g. EGFR TKI eroltinib).

In some studies, the expression of other members of the EGFR family (e.g. HER-2, HER-3) or heterologous growth factor receptors (e.g. IGF-IR and c-Met), the presence of somatic EGFR mutations (exon 19 deletions or L858R), EGFR gene amplifications, mutated KRAS, mutated PTEN, expression of autocrine EGFR ligands (e.g. tumour growth factor-α, epiregulin), or development of skin rash were suggested as indicators of response to treatment with the EGFR inhibitors [14,25–40]. For example, in several randomized clinical trials, the presence of activating KRAS mutation in patients with metastatic colorectal cancer has been associated with resistance to therapy with anti-EGFR mAbs cetuximab and panitumumab [26,29,30,40–42]. On the basis of these results, the provisional clinical opinion of the American Society of Clinical Oncology is that all patients with metastatic colorectal carcinoma who are candidates for therapy with anti-EGFR antibodies should have their tumour tested for KRAS mutations. If KRAS mutation in codon 12 or 13 is present then such patients should be spared therapy with anti-EGFR antibodies as part of their treatment [43].

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In relation to the EGFR itself, it is still unclear whether EGFR expression detected by immunohistochemistry, EGFR gene copy number detected by fluorescence in situ hybridization, EGFR mutations or a combination of these three tests should be used in the selection of patients for therapy with EGFR inhibitors [7,44–46]. In addition, patients with metastatic colorectal cancer are selected for therapy with anti-EGFR antibodies based on EGFR expression in the primary tumours by immunohistochemistry. However, there has been conflicting data on EGFR expression in primary colorectal tumours and related metastatic sites [47–49]. There has also been a report of cetuximab activity in colorectal cancer patients whose tumours were EGFR negative [50]. These studies highlight the need for measurement of EGFR expression and KRAS mutations in primary tumours and related metastasis from cancer patients. Interestingly, both EGFR and phosphorylated EGFR have been detected on tumour-associated endothelial cells, which could form a primary target for EGFR inhibitors [51,52]. It is therefore important that future studies include a detailed examination of the expression pattern, prognostic significance and predictive value of the EGFR in the primary tumours and corresponding metastatic lesions as well as tumour associated endothelial cells from cancer patients. Such studies may illuminate why some patients with EGFR negative tumours have responded to treatment with the EGFR inhibitor cetuximab [50].

It is also noteworthy that there has been no study on the expression level of wild-type EGFR protein and its role as a predictive marker for response to therapy with the EGFR inhibitors. The reason is that the mouse anti-EGFR antibody, clone 2-18C9 in the EGFR pharmDx Kit (DakoCytomation, Dako, Capinteria, California, USA) which gained US Food and Drug Administration approval for immunohistochemical detection of the EGFR, can bind to both EGFR (i.e. wild type) and type III deletion mutant form of EGFR (i.e. EGFRvIII) [53–55]. It is therefore very important that future work includes investigations on the role of wild-type EGFR as a predictive factor for response to therapy with the EGFR inhibitor. Such studies should be performed with another anti-EGFR body, such as mAb ICR10 which, unlike the anti-EGFR mAb clone 2-18c9, does not cross-react with EGFRvIII ([56], Modjtahedi *et al.* in preparation). We believe that future work on the relative expression of various forms of EGFR (i.e. wild type, mutant, phosphorylated, membranous, cytoplasmic or nuclear) could result in the identification of more reliable predictive markers for response to therapy with EGFR inhibitors. In addition, such measurements should be conducted in primary tumours, related metastatic sites as well as tumour-associated endothelial cells from cancer patients and could ultimately aid in the selection of a more specific subpopulation of cancer patients who could benefit from therapy with EGFR inhibitors.

Interestingly, in this issue of anticancer drugs, Rukazenkov and colleagues [57] compare the pharmacology and pharmacokinetics of the two small molecule EGFR TKIs, gefitinib which has been approved for the treatment of non-small-lung cancer, and erlotinib which has been approved for the treatment of both non-small-lung cancer and pancreatic cancers. In this important study, Rukazenkov and colleagues discuss how these properties may affect the clinical efficacy and optimum dose of each drug and whether there is a relationship between skin rash and the clinical outcome with each of these drugs. They highlight that, although gefitinib and erlotinib have similar modes of action and pharmacological profiles, the different molecular structures of the two drugs confer different pharmacodynamics with important clinical implications. They hypothesized that since gefitinib is effective at a dose below its maximum tolerated dose, it accumulates in tumour tissues providing the concentration for inhibition of the EGFR in the tumour whilst producing less skin toxicity than erlotinib. Such results suggested that skin rash may not be a useful marker for efficacy with gefitinib compared with the other EGFR inhibitors [58]. Unlike erlotinib, gefitinib has been shown to inhibit the growth of HER-2 overexpressing breast tumour cells [58,59], which again could be attributed to the structural differences between gefitinib and erlotinib. Consequently, because of structural differences and differences in the modes of action of gefitinib and erlotinib, each EGFR TKI inhibitor may have a unique set of predictive markers. The challenge is the discovery of a unique set of predictive markers for each EGFR inhibitor. Recent studies also suggest that a predictive marker for response to therapy with an EGFR inhibitor in one type of cancer may lose its importance as a predictive marker in another type of cancer [24,40,57].

Future opportunities in tumour targeting with EGFR inhibitors

It is clear that there are major differences between the mode of actions of anti-EGFR mAbs and the EGFR TKIs, which target the extracellular domain and intracellular domain of the EGFR respectively (Fig. 1), and between the anti-EGFR mAbs cetuximab, a mouse-human chimeric IgG1 antibody, and panitumumab, a fully human IgG2 antibody [12,13,18–20,22,25]. For example, cetuximab inhibits the growth of EGFR overexpressing tumour cells by blocking the binding of growth factors to the EGFR and downstream cell signalling molecules, and by inducing antibody-dependent cellular cytotoxcity and complement-mediated cytotoxicity (Fig. 1). Therefore, the level of soluble extracellular EGFR (sEGFR) in the sera of cancer patients can reduce the effective dose of anti-EGFR mAbs (i.e. by trapping therapeutic antibodies) reaching the tumour sites [60,61]. Although the level of sEGFR may also play an important role as a prognostic indicator in cancer patients, it does not have

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any effect on the therapeutic dose of the EGFR TKIs reaching the tumour sites. Therefore, an ideal group of patients who should benefit from therapy with anti-EGFR mAbs are those with overexpression of cell surface (i.e. membranous) EGFR in their tumours and with no or limited expression of sEGFR in their sera. In contrast, patients well placed for therapy with the EGFR TKIs are those whose tumours are dependent on EGFR phosphorylation for proliferation and metastasis.

It is time to return to the accurate measurement of the target antigen recognized by each type of EGFR inhibitor and selective targeting of such antigens in cancer patients [22,61]. Thanks to recent advances in our understanding of cancer biology and tumour biology, as well as recent technological advances in drug design and testing, we should be able to identify and validate the role of various markers as prognostic indicators or predictive factors for response to therapy with an EGFR inhibitor and any other type of EGFR inhibitor including pan-EGFR inhibitors. The results of future work in these areas should aid the selection of a more specific subpopulation of cancer patients who are most likely to benefit from treatment with the EGFR inhibitors, but equally to spare those who will receive no benefit, or a detrimental effect from such biological agents [61–63]. This is particularly applicable to patients in most countries where health-economic constraints preclude the general use of biological agents and the identification of a subgroup of cancer patients who have a significantly higher chance of response to the EGFR inhibitors is paramount [64]. Accurate assessment of various forms of EGFR protein, which contains the target antigen recognised by the anti-EGFR mAbs and the EGFR TKIs, should therefore be conducted in primary tumours, metastatic sites and tumour-associated endothelial cells from cancer patients. Such studies should help us to accomplish our goal, in identifying potential 'responders' to EGFR inhibitors, sooner rather than later [44,47,50,65,66].

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