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The Role of the Epidermal Growth Factor Receptor in Breast Cancer

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Abstract Recent trials of drug therapy targeting the erbB receptor HER2 have met with success in breast cancer. The epidermal growth factor receptor or EGFR is a closely related receptor from this same family that is involved in cellular signal transduction and tumor cell growth and survival. Emerging evidence indicates that EGFR is implicated in the development of hormone-resistant breast cancer, and that its activity is intertwined with estrogen receptor. Here, the role of EGFR in breast cancer is reviewed, and data from selected clinical trials of signal transduction inhibition of this cellular target are summarized.

Keywords Breast · ErbB · EGFR · Signal transduction · Hormone resistance · Gefitinib · Erlotinib

Abbreviations

HER	human epidermal growth factor receptor
EGFR	epidermal growth factor receptor
ER	estrogen receptor
PR	progesterone receptor
pMAPK	phosphorylated mitogen activated protein kinase
EGFRvIII	type 3 variant of EGFR
ATP	adenosine triphosphate
IGF1-R	insulin growth factor 1 receptor

Introduction—The EGFR and Cancer

The erbB (HER, human epidermal growth factor receptor) family comprises four homologous transmembrane receptors (HER1-HER4 or erbB1-erbB4) which form a complex system involved in growth factor cellular signaling [1]. EGFR (epidermal growth factor receptor, HER1 or erbB1), the first of these discovered in 1978 [2], is composed of an extracellular ligand-binding domain and an intracellular tyrosine kinase domain joined and anchored by a transmembrane portion. The other members of the family (HER2–HER4) share this same configuration, with notable differences. The extracellular domain of HER2 is unable to bind extracellular ligand and relies instead on heterodimerizing with other ligand-activated members. In contrast, HER3 is largely deficient of tyrosine kinase activity and requires other receptors of this family for enzyme function [3].

The effects of several different ErbB ligands [4] are transduced by their binding to specific receptors within this family. Ligand binding leads to conformational changes in the ectodomain which facilitate the creation of homo- and heterodimers and oligomers triggering tyrosine kinase phosphorylation. As a consequence, second-messenger pathway cascades are set in motion, including mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K), ultimately influencing cellular proliferation, angiogenesis and cell survival [5].

Since the identification of the link between EGFR and a transforming viral oncogene in 1984 [6], it has been well established that EGFR is involved in malignant transformation and cancer progression [7]. Indeed, overexpression of this cellular receptor has been demonstrated in several human tumor types and often portends a more aggressive phenotype and accordingly worse survival [8]. This scenario makes the erbB protein family an ideal target to be exploited for cancer therapeutics [9, 10].

The role of HER2 is perhaps the best known of this family in breast cancer [11]. Furthermore, HER2 has been successfully exploited in the therapy of both advanced [12] and early-stage disease [13, 14]. HER2 is further reviewed in this issue of the journal; in this article we shall review the role of EGFR and cancer of the breast.

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EGFR as a Prognostic Indicator in Breast Cancer

A plethora of studies reporting EGFR positivity in breast cancer have been published. A review of 5,232 patients with breast cancer across 40 different studies report that 45% (range 14–91%) are positive for EGFR [15]. Similarly in a second review of 370 patients, 47% were reported to be positive [16]. The wide range of 14–91% in the former studies is likely to be due to the several different techniques employed for analysis, including autoradiography, immunocytohistochemistry, immunoenyzmatic assay, and the detection of gene transcripts and phosphotyrosine kinase activity.

With regard to EGFR as a marker of prognosis, there is no consensus; a search for correlations with well-established markers shows that EGFR expression is approximately twice as frequent in estrogen receptor / progesterone receptor (ER/PR) negative cancers as in ER/PR positive tumors [15]. The majority of studies [15–18] show no association between EGFR and tumor size, except for one recent abstract in which significant association was observed in 150 cases examined by immunohistochemistry [19]. There may be higher EGFR positivity in those with nodal involvement and poorly differentiated histology and higher grade, although this has not been consistent in all studies [15–21]. There is no apparent association between EGFR status and long-term overall survival, although those who are EGFR positive and ER negative have a significantly worse prognosis [15, 16].

With little doubt, many human breast cancers do express EGFR. As a factor for disease prognosis and / or a predictive factor for therapy, there is at present much conflicting data which is difficult to summarize in light of differing laboratory methodologies and nonuniform thresholds for positivity. Furthermore, the patient groups differ among the above studies in terms of case selection and the use of adjuvant treatment. To date, there are no widely agreed criteria for EGFR analysis for status or as a predictive or prognostic marker for this disease. This situation is in contrast to HER2 in breast cancer, in which similar issues have now been largely resolved [22, 23]. In conclusion, the prognostic significance of EGFR status and its clinical importance remain unclear in breast cancer for the present time [20]. Despite this, EGFR status as an inverse event related to ER status seems to be a consistent finding across many studies [15–17, 21, 24], which fuels the notion of targeting the EGFR, particularly in those patients with ER negative or hormone-therapy resistant breast cancer.

EGFR and Hormone-Therapy Resistant Breast Cancer

Anti-hormone drug therapy for breast cancer has assumed several guises: the traditional selective estrogen receptor modulator tamoxifen, the more modern aromatase inhibitors, such as anastrazole and letrozole, and the pure antiestrogen, namely fulvestrant [25]. Such therapy has proved invaluable for hormone responsive breast cancer, as predicted by positive ER status, and negates or delays the need for more toxic chemotherapy. However, tumor resistance to antihormone therapy is inevitable in many of these patients; furthermore, there is also the group whose breast cancer is endocrine-resistant *de novo*. Recent evidence infers that EGFR contributes to this process, increased EGFR activity provides a means for "tumor escape" from therapy. As mentioned previously, there is an inverse relationship between EGFR expression and ER expression–it is conceivable that since both pathways are capable of driving growth of malignant breast disease, breast cancer cells might evolve in a way that relies on one or the other of these signaling ligands. Indeed, there is evidence that the two signaling pathways are intertwined [26, 27]. For instance, activation of the EGFR tyrosine kinase phosphorylates and activates nuclear estrogen receptor and its coregulator proteins. Furthermore, plasma membrane-associated estrogen receptor alpha is able to utilize the EGFR cascade for its own signal transduction [28], thereby providing a means for bidirectional crosstalk [27]. Upon treatment with antihormonal agents, a tumor might "escape" by upregulation of the erbB pathway. This mechanism is exemplified in preclinical studies in which the breast cancer cell line MCF-7 with acquired tamoxifen resistance shows increased EGFR expression and HER2 transcripts and protein compared to its parent cell line [29–31]. Immunoprecipitation studies show increased receptor dimerization and phosphorylation; furthermore, there is an increase in autocrine and paracrine ligand production, all signifying increased activity of erbBs in these cells [29, 30]. Accordingly, untreated breast cancers with increased expression of EGFR and HER2 or of the downstream signaling protein pMAPK are more likely to be tamoxifen-resistant [32].

Targeting the EGFR in Breast Cancer

Molecular profiling by gene expression of breast cancer cases has shown that HER2 positivity or overexpression, rather than EGFR, forms a major subtype in this group of heterogeneous diseases [33]. HER2 overexpression does not seem to be common in other solid tumors; instead, mutations in the EGFR have been a greater focus of recent interest. EGFR deletion mutant variant III (EGFRvIII) is a mutant receptor with constitutive activity in glioblastoma mutiforme [34]; various other mutations within the tyrosine kinase domain have been found in some lung cancers and confer higher sensitivity to kinase-inhibiting drugs [35].

Although rarely, mutations of the HER2 kinase domain have been observed [36]; such mutations have not been found within this domain of the EGFR in breast cancer [37]. Furthermore, EGFRvIII has not been detected in 170 cases of primary breast cancer [38]. This result suggests that such phenomena are unlikely to be significant in this disease. Nonetheless, due to its intimate links with hormone signaling pathways and its role as a coreceptor for HER2, EGFR remains a potential therapeutic target. For instance, it has been suggested that EGFR-HER2 heterodimerization is essential for tumor growth in some breast cancers and that the attenuation of one or the other of these receptors can inhibit growth. This mechanism is demonstrated by growth inhibition after infection with retroviruses carrying single-chain antibody fragments targeting either the EGFR or HER2 in certain breast cancer cell lines [39]. The modulation of EGFR thus might be exploited either sequentially or concurrently with other receptors and pathways, all of which merit investigation.

To date, two therapeutic strategies for signal transduction inhibition against EGFR have reached the clinical arena: 1) small molecule inhibitors such as gefitinib [40] and erlotinib [41] which target the intracellular ATP-binding pocket of the tyrosine kinase moiety and 2) the extracellular monoclonal antibody cetuximab [42], which sterically hinders ligand-binding and activation [43]. To date, the greater part of research in this area has been the study of gefitinib. Gefitinib has been shown to inhibit the growth of a variety of human tumor-derived cell lines, alone or in combination with other drugs or irradiation [44]. Dose dependent tumor growth inhibition has also been demonstrated in xenograft tumors in mouse models [40, 44]. Notably, there is no apparent correlation between EGFR expression and tumor sensitivity to gefitinib, which is in stark contrast to HER2 overexpression and its targeted therapy using the monoclonal antibody trastuzumab.

As mentioned previously, there is considerable interest in the utilization of drugs against EGFR within the setting of hormone-therapy resistant breast cancer, i.e., estrogen receptor positive disease with acquired resistance to anti-hormone therapy or estrogen negative disease with *de novo* resistance. In experimental studies, tamoxifen-resistant cell lines have been produced to which the addition of gefitinib decreases EGFR activation and MAPK phosphorylation, resulting in prolonged growth inhibition [30, 31, 45]. Similar growth curtailment has also been observed in *de novo* tamoxifen-resistant cells [46]. However, it is noted that this effect is circumvented by the upregulation of alternative signaling pathways, such as ER and insulin growth factor 1 receptor (IGF1-R) [47]. Accordingly, studies are in progress whereby ER and EGFR pathways are targeted synchronously. The antiestrogen fulvestrant in combination with gefitinib induces additive inhibition of cell growth [48]. Fulvestrant with MAPK and PI3K inhibitors also show enhanced inhibitory effects in breast cancer cells [49]. Likewise when AG1024, an inhibitor of IGF1-R is added to gefitinib, a synergistic response is observed in some breast cancer cell lines [50]. Notably, it has also been reported that gefitinib reverses BCRP (breast cancer resistance protein)-mediated cytotoxic drug resistance in several cell lines and in a mouse model [51, 52] Interestingly, this phenomenon of cytotoxic drug resensitization is also apparent when cetuximab is administered to patients with irinotecan-resistant colorectal cancer [53]. To our knowledge, little work has been published in the evaluation of cetuximab monotherapy in breast cancer. On the other hand the monoclonal antibody, trastuzumab, against HER2 has been given in combination with gefitinib to attempt dual inhibition in cancer cells expressing both HER2 and EGFR. One study confirmed decreased receptor activation and additive inhibition of *in vitro* tumor growth [54]. In another study, although the combination led to effective growth inhibition, this effect was not significantly greater than when trastuzumab was utilized alone. Further analysis within this study revealed that the successful dimunition of viable hypoxic cells induced by gefitinib in a mouse xenograft was unfortunately reversed by the coadministration of trastuzumab [55]. It is established that gefitinib enhances the efficacy of ionizing radiation, as evidenced by growth inhibition of several different tumor cell lines [56].

Clinical Trials of EGFR Directed Therapies in Breast Cancer

Relative to HER2 targeted therapy with trastuzumab, clinical trials of drugs against EGFR in breast cancer are in their infancy. No randomized controlled phase III trials have been reported involving EGFR signal transduction inhibitors, but data from phase I and II studies are accumulating (Table 1), mainly for gefitinib at the single daily dose of 500 mg. Selected published data from phase II studies [57–59] show that gefitinib monotherapy in heavily pre-treated metastatic breast cancer has a modest disease control rate (complete or partial response or stable disease) of between 10% and 38%, the upper limit representing that observed at 3 months in one study. The majority of responders achieved the status of stable disease. Phosphorylation of EGFR was noted to be successfully inhibited by gefitinib in both skin and tumor biopsies in all patients; it is concluded that lack of clinical response is due to lack of tumor dependence upon EGFR rather than incomplete receptor blockade [57]. Furthermore, no correlation between receptor expression and response was observed, and, paradoxically, one responder was classified as EGFR

Table 1 Selected trials of EGFR inhibitor therapy in breast cancer.

Author/year/ reference	*n*	Clinical trial phase	Target group	Treatment	Outcome/notes
Albain et al. [59]	63	II	MBC, pre-treated	Gefitinib	CR: 0 PR: 1.6% SD: 12.7% PD: 85.7%
Baselga et al. [57]	31	II	MBC, pre-treated	Gefitinib	CR+PR: 0 SD: 38.7% PD: 61.3% mTTP: 55 days mOS: 503 days Inhibition of pEGFR in all patients
von Minckwitz 2005 [58]	58	II	MBC, pre-treated	Gefitinib	CR: 0 PR: 1.7% SD: 8.6% PD: 89.7% mTTP: 61 days mOS: 357 days
Francis et al. [60]		II	MBC, pre-treated	Gefitinib	
	i) 40		i) ER+ve HRBC		i) CR+PR: 0 SD: 2.5% PD: 97.5%
	ii) 25		ii) ER-ve		ii) CR+PR: 0 SD: 8% PD: 92% Study prematurely closed due to low benefit
Robertson 2003 [61]		II	MBC, pre-treated	Gefitinib	
	i) 13		i) ER+ve HRBC		i) CR: 0 PR: 33% (1 of 3 evaluable) SD: 67% (2 of 3 evaluable) PD: 0
	ii) 20		ii) ER-ve		ii) CR: 0 PR: 6% SD: 6% PD: 88%
ECOG E1100 [62]		II	MBC, HER2+ve	Trastuzumab Gefitinib	
	i) 26		i) 1st line		i) CR: 3.8% PR: 0 SD: 23% PD: 73.2% mTTP: 2.9 months
	ii) 8		ii) Pre-treated		ii) PD: 100% mTTP: 2.2 months
Mita et al. [63]	15	II	MBC, ER+ve, pre-treated	Anastrazole Gefitinib	CR+PR: 0 SD: 20% PD: 80% Study termination likely due to low benefit
Ciardiello et al. [65]	41	II	MBC, 1st line	Taxotere Gefitinib	CR: 13.2% PR: 44.7% SD: 15.8% PD: 18.4%

Table 1 (continued)

Author/year/ reference	*n*	Clinical trial phase	Target group	Treatment	Outcome/notes
Fountzilas et al. [66]	68	II	MBC, 1st line	Paclitaxel Carboplatin Gefitinib	CR: 13.2% PR: 44.1% SD: 30.9% PD: 4.4% Efficacy similar to paclitaxel with carboplatin alone
Polychronis et al. [64]	56	II	PBC, ER+ve, EGFR+ve	RCT of neoadjuvant: i) Anastrazole Gefitinib vs ii) Placebo Gefitinib	i) CR: 0 PR: 44% SD: 37% PD: 0 ii) CR: 0 PR: 48% SD: 48% PD: 0
Blackwell et al. [75]	44	II	MBC, Trastuzumab refractory	Lapatinib	CR: 0 PR: 8% SD: 14% PD: 78%
Gomez et al. [74]	29	II	MBC, HER2+ve 1st line	Lapatinib	CR: 0 PR: 38% SD: 46% PD: 15%
Miller et al. [76]	46	II	MBC, pre-treated	ZD6474	CR+PR: 0 SD: 2.3% PD: 97.7%

MBC Metastatic breast cancer, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *RCT* randomised controlled trial, *HRBC* hormone-therapy refractory breast cancer.

negative on immunohistochemical staining [58]. Two trials have investigated the efficacy of single agent gefitinib in hormone-therapy resistant breast cancer in i) ER positive disease with acquired resistance and ii) ER negative disease [60, 61]. One study terminated prematurely due to low clinical benefit rates of 2.5 and 8% disease control in the two groups, respectively, while the second trial has observed one patient with partial response and two with stable disease of only three evaluable patients in the ER positive group. Clearly, patient numbers were too low to be significantly meaningful at the time of interim reporting.

Dual blockade of HER2 and EGFR using concomitant trastuzumab and gefitinib has been tried in 34 patients, three quarters of whom received the therapy as first line treatment [62]. Disappointingly, there was neither an additive nor synergistic effect on response rates, and, alarmingly, the observed time to progression was less than that of standard trastuzumab monotherapy. These data infer that the combination is antagonistic; further *in vitro* tests are warranted to delineate this adverse interaction. Combined therapy against both the hormonal and EGFR pathways may hold more promise. In one study, anastrazole with gefitinib induced disease stabilization in 20% of patients [63]. In a larger study, patients with both ER and EGFR positive primary breast cancers were randomized to gefitinib and anastrazole versus gefitinib with placebo. Both groups experienced similar primary tumor size diminution after four to six weeks of treatment, and there was a greater reduction in the Ki-67 tumor proliferation index in the combined therapy group [64]. In combination chemotherapy, gefitinib plus taxotere is well tolerated and shows encouraging antitumor activity [65]. However, when given with paclitaxel and carboplatin, efficacy is similar to that reported for the doublet chemotherapy alone [66]. This study, taken in combination with the INTACT 1 and 2 trials of gefitinib with chemotherapy in NSCLC [67, 68], are cautious reminders of our lack of sufficient understanding of how signal transduction inhibitors and conventional cytotoxics might interact in these diseases.

There are fewer published studies of the other commercially available tyrosine kinase inhibitor, erlotinib [69]. A phase II study of erlotinib with gemcitabine in pre-treated metastatic breast cancer of 59 patients shows a response rate of 14%, which was lower than the expected antitumor activity for this trial [70]. A second study pairing erlotinib with bevacizumab, a monoclonal antibody against vascular

endothelial growth factor, allowed 33% of patients to achieve disease control in the same setting [71]. One other trial to be noted is a phase I evaluation of an "all-out multi-faceted" approach employing simultaneous erlotinib, trastuzumab and paclitaxel. In this trial, 4 of 14 (29%) patients with advanced breast cancer responded to this combination, which seemed to be well-tolerated [72]. Other studies are underway using lapatinib [73], an oral small molecule inhibitor of both EGFR and HER2 kinase activity. In a phase II study of first-line lapatinib monotherapy in patients with advanced HER2-amplified breast cancer, 38 and 46% derived partial response and stable disease, respectively [74]. This degree of disease control, although anticipated to be higher in the first line setting than in heavily pre-treated patients, is highly impressive, and more comprehensive data are eagerly awaited. Additional studies have focused on patients with metastatic disease refractory to trastuzumab; lapatinib monotherapy has a reported disease control rate of 22% [75] while lapatinib and capecitabine in combination significantly prolongs median time to progression (HR 0.51, 95% CI 0.35-0.74) when compared to capecitabine alone [77].

Conclusion

Extensive investigations of HER2 in breast cancer has successfully led to the bench-to-bedside use of trastuzumab and provides proof of principle for the targeting of the erbB family of receptors in breast cancer. Despite this, there is still a proportion of patients who do not respond despite harboring disease that overexpresses HER2. With regard to hormonal therapy, ER negative and eventually many ER positive breast cancers are or will become resistant to antihormone drugs. Hypothetically, abrogation of the EGFR may improve outcome in both of these situations. Besides this, EGFR is an attractive and valid target in its own right, given its established role in cancer cell survival and proliferation. Thus far, pure EGFR expression in breast cancer bears no prognostic value and is not a useful predictive factor for therapy in breast cancer. Standardized methods for its measurement and interpretation are required for further evaluation and for its inclusion in future clinical trials. Indeed, it may be that a more comprehensive profile of all the erbB receptors and their ligands will be more informative in this regard. There exists robust evidence of an interplay between the different members of the erbB family of receptors and their role in cancer. Furthermore it is becoming apparent that an additional level of complexity exists whereby EGFR is intimately involved with ER and possibly other pathways, including IGF1-R. It may be that these pathways are utilized collectively in a dynamic fashion to aid tumor survival through aberrant signaling promoting acquired drug resistance. Targeting several of these pathways either sequentially or concurrently is a logical approach and under active clinical investigation based on highly encouraging *in vitro* studies. Other areas for exploration include defining the precise mechanisms of action of signal transduction inhibitors and their interaction with each other and with conventional chemotherapy. No phase III study results are available; data from phase II studies so far show limited efficacy for gefitinib as monotherapy or when combined with antihormone therapy. Studies conducted using gefitinib or erlotinib in partnership with conventional cytotoxics have shown good antitumor activity, but only phase III trials will prove if there is significant additional benefit. Dual targeting of EGFR and HER2 using small molecule tyrosine kinase inhibitors with trastuzumab may be detrimental according to the limited data currently available. Clearly, further study and understanding is required of how these different signaling pathways interact and how exactly their targeted therapies operate. These many different facets might explain why it has been difficult so far to identify the best treatment populations for erbB targeted compounds. Further knowledge would lead to improved selection of patients for targeted therapy and more successful application of combining drugs against multiple targets. Unmistakably, inhibition of signal transduction will continue to play a part in the therapy against breast cancer.

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