EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) TYROSINE KINASE INHIBITORS IN BREAST CANCER: CURRENT STATUS AND FUTURE DEVELOPMENT

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1. ABSTRACT

Evidence suggests that the epidermal growth factor receptor (EGFR) and its ligands are involved in the pathogenesis of different human carcinomas, including breast cancer. Results of phase II clinical trials of EGFR tyrosine kinase inhibitors (TKIs) have shown that these compounds have little activity in breast cancer patients when used as single agents. The potential pitfalls of these clinical trials, and the molecular mechanisms that might be involved in regulating the sensitivity/resistance of breast cancer cells to EGFR TKIs are discussed in this brief article. In particular, preclinical findings clearly demonstrate that breast cancer cells are able to activate different mechanisms to escape the anti-tumor effects of drugs directed against growth factor-driven pathways. Therefore, it is conceivable that significant blockade of tumor growth might be obtained only through contemporary blockade of different growth promoting pathways, at least in advanced disease. In addition, preclinical and clinical findings support the use of EGFR TKIs in specific subgroups of breast cancer patients, such as estrogen receptor positive (ER+), tamoxifen resistant patients. In this regard, we describe potential future applications of these compounds in combination with other agents in the treatment of breast carcinoma.

2. INTRODUCTION

It has long been hypothesized that the autonomous growth of cancer cells depends on their ability to produce high levels of peptide growth factors and/or growth factor receptors. In particular, tumor derived growth factors may function through intracrine, paracrine, juxtacrine and/or autocrine pathways to control cell proliferation and survival in tumor cells that express the cognate receptors for these peptides (1). Tumor growth factors are also involved in regulating angiogenesis and formation of metastasis through interaction with the surrounding stromal cells. Therefore, deregulated expression and/or activation of either peptide growth factors or growth factor receptors might be involved in tumor pathogenesis and progression.

Tumor cells are able to synthesize and to respond to a number of different peptide growth factors. Among these, the epidermal growth factor receptor (EGFR) and its cognate ligands have been shown to contribute to the growth of tumor cells in pre-clinical studies (2). Furthermore, expression of the EGFR and its ligands has been found in a majority of human carcinomas, including breast cancer (2). This evidence led to the development of different anti-EGFR agents that are currently in clinical development (3).

Despite the frequent expression of the target receptor in primary carcinomas, the results of clinical trials with anti-EGFR agents in human carcinomas when used as single agents have been disappointing in the majority of cancer types (4). In this regard, this short review will summarize the pre-clinical evidence that support the use of anti-EGFR agents in breast cancer and the results of
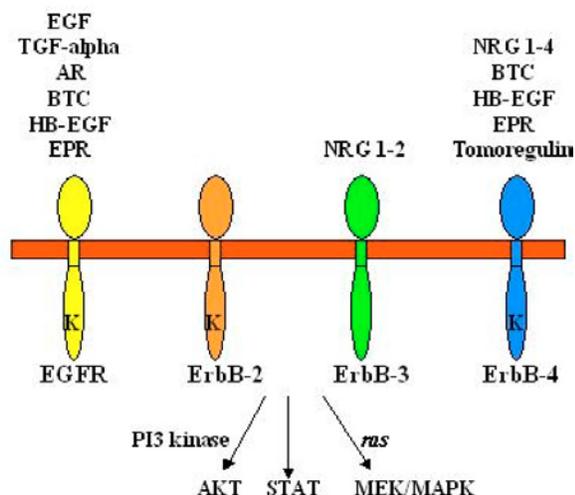
Figure 1. The ErbB family of receptors and their cognate ligands. The ErbB receptors possess an extracellular domain that is able to bind specific ligands, and an intracellular domain that has a tyrosine kinase activity (K). There are two exceptions within this family: ErbB-2 that does not bind any known ligand, and ErbB-3 that lacks kinase activity. Following activation, the ErbB receptors become able to activate different signaling pathways including the *ras/raf*/MEK/MAPK, the PI3K/AKT and the STAT pathways (see text). Abbreviations: EGF, epidermal growth factor; TGF-alpha, transforming growth factor-alpha; AR, amphiregulin; BTC, betacellulin, HB-EGF, heparin binding-EGF; EPR, epiregulin; NRG, neuregulin.

clinical trials with EGFR tyrosine kinase inhibitors (TKIs) in breast cancer patients. More importantly, we will try to discuss the potential pitfalls of these studies, and the future development of EGFR TKIs in breast carcinoma.

3. THE EGFR SYSTEM

The EGFR belongs to a family of tyrosine kinase receptors that includes ErbB-2, ErbB-3 and ErbB-4 (figure 1). Each of these proteins possesses three different domains: the extracellular domain that is involved in recognizing and binding the ligands that are able to activate the receptor; the transmembrane spanning sequence that regulates the interaction between receptors; and the intracellular domain in which resides the enzymatic activity of the tyrosine kinase that is able to phosphorylate tyrosine residues on different intracellular adaptor proteins (5). There are two exceptions within this family: ErbB-2 that binds no known ligand, and ErbB-3 that has no tyrosine kinase activity. Upon ligand binding, the ErbB receptors form either homo- or hetero-dimers. Formation of dimers is essential for receptor activation and subsequent transmission of intracellular signals (6). In fact, following dimerization, auto- and trans-phosphorylation in tyrosine residues of the ErbB receptors occur. The tyrosine phosphorylated receptors become able to interact with adaptor proteins that couple the receptors to intracellular signaling pathways (6). Indeed, ErbB receptors are able to activate different signaling cascades, including the phosphatidylinositol 3-kinase (PI3K)/Akt, the

ras/raf/MEK/mitogen-activated protein kinase (MAPK) and the signal transducer and activator of transcription (STAT) pathways.

4. EGFR SIGNALING IN BREAST CANCER

Different lines of evidence suggest that EGFR signaling is involved in the pathogenesis of breast carcinoma. In fact, triple knockout mice lacking expression of the EGFR ligands EGF, amphiregulin (AR) and transforming growth factor-alpha (TGF-alpha) showed aberrant mammary alveolar growth and reduced milk production suggesting an important role for EGFR signalling in alveolar development and lactogenesis (7). Co-expression of EGFR and its ligands has been frequently described to occur in breast cancer, suggesting that autocrine loops involving these proteins are operating in breast cancer cells (table 1) (2, 8). In particular, expression of EGFR has been reported in 14% to 91% of breast carcinomas with an average of approximately 50% (2, 3). There are several reasons that explain such a variability. In fact, different techniques have been employed to assess the levels of expression of the EGFR protein in primary tumors, i.e. radioimmunoassays, western blotting and immunohistochemistry. The cut off values to discriminate positive and negative samples differ in each study. Furthermore, different antibodies have been used to detect the expression of the EGFR in tumor tissue. Finally, some studies have measured the overexpression of the EGFR in the tumor as compared with normal mucosa, and they have assessed as negative the tumor samples that express levels of the EGFR similar to normal mucosa. Coexpression of different receptors of the ErbB family, such as EGFR, ErbB-2, ErbB-3 and ErbB-4 has also been found in breast cancer cells, implying that these proteins might cooperate in sustaining the growth of tumor cells (2, 3). ErbB receptors and EGF-like peptides are generally expressed at higher levels in estrogen receptor negative (ER-) breast carcinomas as compared with ER+ tumors, suggesting that overexpression of these proteins might lead to the acquisition of an estrogen independent phenotype (8). Finally, overexpression of either EGFR or TGF-alpha in the mammary gland of transgenic mice led to development of mammary carcinomas in multiparous female (9-11). These *in vivo* experiments represent a formal proof that activation of EGFR signalling is able to enhance breast tumorigenesis.

5. PHASE II STUDIES OF EGFR-TKIs IN BREAST CANCER

Several agents directed against the EGFR have been explored for their anti-tumor activity in the past two decades. The EGFR-TKIs are in advanced phase of clinical development in different tumor types including breast cancer. These compounds directly inhibit tyrosine kinase phosphorylation by physical interaction with either the ATP and/or the enzyme substrate binding sites (table 2). Some of these compounds are reversible inhibitors; others are irreversible inhibitors of the EGFR. Furthermore, both agents specific for the EGFR or agents that are able to block different ErbB receptors are in clinical development.

Table 1. Frequency of expression of ErbB receptors and cognate ligands in breast cancer

Receptors	Ligands
EGFR (14-91%) [1]	TGF-alpha (40-70%)
	AR (37-80%)
	HB-EGF (72%)
ErbB-2 (9-39%)	
ErbB-3 (22-90%)	HRG (25-30%)
ErbB-4 (82%)	HRG (25-30%)
	HB-EGF (72%)

[1]: range of expression

Table 2. EGFR TKIs that are in most advanced phase of clinical development

Drug	Target	Description	Source
Gefitinib (Iressa; ZD1839)	EGFR	Quinazoline reversible	AstraZeneca
Erlotinib (Tarceva; OSI-774)	EGFR	Quinazoline reversible	Genentech/Roche
CI-1033	ErbB receptors	Quinazoline irreversible	Pfizer
PKI-166	EGFR/ErbB-2	Pyrrolo-pyrimidine reversible	Novartis
AEE-788	EGFR/ErbB-2	Pyrrolo-pyrimidine reversible	Novartis
EKB-569	EGFR	Quinazoline irreversible	Wyeth-Ayerst
GW-572016	EGFR/ErbB-2	Quinazoline reversible	GlaxoSmithKline

Table 3. Phase II studies of EGFR TKI in breast cancer

Drug	Dose (mg/d)	N° pts	RR[1](%)	SD[2] (%)	DC[3] (%)	Ref
Gefitinib	500	63	1 (1.6)	5 (7.9)	9.5	14
Gefitinib	500	46	1 (2.2)	3 (6.5)	8.7	13
Gefitinib	500	31	1 (3.2)	3 (9.7)	12.9	15
Gefitinib	500	18[4]	1 (5.5)	1 (5.5)	11	12
Gefitinib	500	9[5]	1 (11.1)	5 (55.5)	66.6	12
Erlotinib	150	69	4 (6.5)	15 (21.7)	28.2	20

[1]: response rate; [2]: stable disease; [3]: disease control rate (RR+SD); [4]: ER- patients; [5]: Tamoxifen-resistant ER+ patients

The results of clinical trials with EGFR TKIs as single agents in breast cancer patients are summarized in table 3. Albain and co-workers enrolled 63 patients with metastatic breast cancer in a trial of daily administration of gefitinib (500 mg/die). Of the 63 patients, 27 expressed ER (43%) and 17 (27%) had high levels of ErbB-2 expression (3+ at immunohistochemistry) (12). The majority of the patients were heavily pretreated with several lines of chemotherapy and/or hormonal therapy. All ErbB-2 positive patients had been also previously treated with the anti-ErbB-2 antibody herceptin. The treatment was well tolerated, with the most frequent side effect being grade 3 diarrhoea. However, the results of the trial were disappointing. Only one patient had partial remission (PR), whereas stabilization of the disease (SD) lasting at least 4 months was observed in 5 patients. In the study by von Minckwitz and co-workers, 46 patients with advanced breast cancer were enrolled. After 12 weeks of treatment, 1 patient (2.2%) had PR, 3 patients (6.5%) had SD and 42 patients had progressive disease (PD) (13). Similar findings were obtained in the studies by Baselga and collaborators (14) and Robertson and co-workers (15) (table 3). However, in the latter study, a disease control rate of 66.6% was observed in a small cohort of ER+ tamoxifen resistant patients. Finally, an approximately 28% disease control rate was obtained in a trial of erlotinib in patients with locally advanced or metastatic disease (16). Skin rashes and diarrhea were the most frequent adverse effects in these patients. The majority of events were mild to moderate in severity and reversible on cessation of treatment.

6. BIOLOGICAL CHARACTERISTICS AFFECTING THE RESPONSE OF BREAST CANCER CELLS TO EGFR-TKIs

Different mechanisms might be involved in regulating the sensitivity/resistance of breast cancer cells to EGFR TKIs. It has been recently demonstrated that in non-small-cell lung cancer (NSCLC) response to EGFR TKIs is directly related to the occurrence of specific mutations in the EGFR TK domain (17-19). However, preliminary findings suggest that such mutations do not occur in breast carcinoma cells. Therefore, it is unlikely that patients might be selected for treatment with these agents on the basis of EGFR mutations.

Pre-clinical and clinical studies have demonstrated that no correlation exists between the levels of expression of EGFR and the response to gefitinib in tumor cells. In this respect, we have shown that breast cancer cell lines with low levels of expression of the EGFR are extremely sensitive to the anti-tumor effects of gefitinib, if they co-express high levels of ErbB-2 (20, 21). Patients with ErbB-2 overexpression have been enrolled in clinical trials of gefitinib and erlotinib in breast cancer (12, 16). Although response to EGFR-TKIs has been described to occur in some patients with ErbB-2 overexpression, the majority of these patients did not respond to treatment with these compounds. However, all the patients enrolled in these trials had been previously treated with herceptin.

Resistance to herceptin has been suggested to occur by at least two different mechanisms. In fact, it has been shown that breast cancer cells with acquired resistance to herceptin express higher levels of EGFR-ligands as compared with parental, herceptin-sensitive cells (22). Interestingly, herceptin-resistant cells were found to have an higher sensitivity to EGFR TKIs such as gefitinib and erlotinib as compared with parental cells. These findings suggest that resistance to herceptin might be mediate by an EGFR-dependent autocrine loop, and that patients with this type of resistance might respond to treatment with anti-EGFR agents. In this respect, preliminary findings of a phase II trial of the dual EGFR/ErbB-2 inhibitor GW572016 in trastuzumab-refractory metastatic breast cancer patients might support this hypothesis (23). In fact, 3 PR and 5 SD were observed in the first 36 patients enrolled in this trial. These results might imply that an EGFR dependent pathway is involved in the resistance to trastuzumab in these patients, although it is not possible to exclude at this point the hypothesis that the anti-tumor activity of GW572016 is related to its ability to block ErbB-2 with a different mechanism as compared with herceptin. However, it has also been demonstrated that resistance to herceptin is mediated by increased activation of insulin-like growth factor I (IGF-I) receptor (24). In this respect, it is conceivable that patients that develop resistance to herceptin mediated by increased activation of growth promoting pathways independent from the EGFR, are also resistant to anti-EGFR drugs. A recent paper has indeed demonstrated that acquired resistance to gefitinib in breast cancer cells is mediated by the IGF-I receptor (25).

The above mentioned findings also suggest that response to EGFR TKIs might be correlated with the total levels of expression of the different ErbB receptors. In fact, low levels of EGFR might be sufficient to trans-activate other ErbB-receptors through the formation of ErbB heterodimers and through lateral signaling (6). In addition, activation of autocrine and paracrine loops involving the EGFR is strictly dependent on the levels of EGFR-ligands that are available within the tumor (26). Therefore, the total levels of expression of ErbB receptors and their ligands might affect the response of primary breast cancer to EGFR TKIs.

An high response rate to gefitinib has been described by Robertson and co-workers in breast cancer patients with ER+ tumors and acquired resistance to tamoxifen (15) (table 3). In this respect, it has been shown that breast cancer cells selected *in vitro* for resistance to tamoxifen express higher levels of EGFR and ErbB-2 as compared with parental, tamoxifen-sensitive cells (27). In agreement with these findings, tumor cells resistant to tamoxifen also show an increased sensitivity to both gefitinib and herceptin as compared with tamoxifen-sensitive cells. These findings support clinical studies of gefitinb in tamoxifen-resistant breast cancer patients. Furthermore, the observation that contemporary treatment of breast cancer cells with tamoxifen and gefitinib prevents the occurrence of resistance to tamoxifen, supports the exploration of combinations of anti-estrogenic drugs and anti-EGFR agents in ER+ breast cancer patients (28).

Finally, there is a general agreement on the hypothesis that the occurrence of molecular alterations that activate signal transduction pathways downstream the EGFR might affect the response to these drugs. This hypothesis has been confirmed by a pharmacodynamic study of Baselga and collaborators (29). These Authors found that treatment of advanced breast cancer patients for 28 days with gefitinib produced in tumor tissue a significant inhibition of both EGFR phosphorylation and MAPK activation, but no reduction of tumor proliferation as measured by Ki67 immunostaining. In this regard, preclinical studies have suggested that constitutive activation of the PI3K/Akt pathway might produce resistance to anti-EGFR agents (29-31). However, evidence suggests that the MEK/MAPK pathway might be also involved in the spontaneous and acquired resistance of breast cancer cells to EGFR TKIs (32). In this regard, preliminary results of a study with erlotinib in patients with untreated operable breast cancer support this hypothesis (33). In this study, after the initial diagnostic biopsy, patients were treated with 150mg/day erlotinib for 10-14 days until 12-24 h prior to surgery. A marked reduction of tumor cell proliferation in the surgical biopsy was observed in 4/13 patients. A significant reduction of MAPK phosphorylation was also observed in 8/13 patients, suggesting that the EGFR pathway was the major activator of MAPK in this subset of breast carcinomas and confirming biochemical activity of erlotinib *in situ*. The apparent discrepancy between these findings and the report by Baselga and collaborators (14) might be due to the different stage of the disease of patients enrolled in these trials. In fact, the Baselga study enrolled patients with advanced breast cancer. It is conceivable that cancer cells might accumulate different molecular alterations during tumor progression, and this phenomenon might reduce their response to blockade of EGFR.

7. PERSPECTIVES

The above described results clearly demonstrate that a minority of breast cancer patients can benefit of treatment with EGFR TKIs as single agents. In this regard, pharmacodynamic studies aimed to identify molecular markers of response to EGFR TKIs are definitely required. However, cancer is the result of several, different genetic and epigenetic alterations. In addition, evidence suggests that cancer cells might activate different mechanisms to escape the anti-tumor activity of drugs directed against growth factor-driven pathways. Therefore, it is conceivable that significant blockade of tumor growth might be obtained only through contemporary blockade of different growth promoting pathways, at least in advanced disease. In this respect, pre-clinical studies have demonstrated that treatment of cancer cells with combinations of target-based agents results in a more significant inhibition of tumor growth as compared with treatment with a single agent. For example, we and others have demonstrated that a combination of gefitinib and herceptin has a synergistic anti-tumor activity in breast cancer cell lines that co-express the two receptors (34, 35). In particular, this combination induces high levels of apoptosis in breast cancer cells as compared with treatment with a single

agent. A phase I/II clinical trial based on the use of this combination is ongoing (ECOG 1100 trial). Phase II clinical trials aimed to explore the efficacy of combinations of aromatase inhibitors and EGFR TKIs in ER+ metastatic breast cancer patients are also ongoing. The results of these trials will clear out the possibility to use such combinations in breast cancer patients. Furthermore, the activity of EGFR TKIs in early phases of the disease, and therefore in the neo-adjuvant and adjuvant setting needs to be explored.

Finally, completely different approaches of molecular therapy in breast cancer patients might be hypothesized. For example, a new molecular mechanism involved in the transcriptional repression of ER-alpha gene expression in breast cancer has been recently described. In particular, it has been demonstrated that multimolecular complexes containing pRb2/p130, HDAC1 and DNMT1 negatively regulate ER-alpha gene transcription in ER- breast cancer cells (36). Therefore, both DNA methylation and histone acetylation/deacetylation seem to be involved in the silencing of ER-alpha in breast carcinoma. Drugs potentially able to interfere with this phenomenon, such as HDAC inhibitors or demethylating agents, are currently in clinical trials. In this respect, studies in our laboratories are ongoing to assess whether induction of expression of ER-alpha in breast cancer cells might at least in part restore an estrogen-dependent phenotype. If this is the case, combinations of agents capable of inducing expression of the ER-alpha in ER- breast cancer cells, anti-estrogenic drugs and EGFR TKIs might represent a novel therapeutic approach in breast cancer patients. Such a combination of target based agents might prove efficient in blocking tumor growth, allowing at least a delay for the need of chemotherapy in selected patients. This is just an example of the potential use of EGFR TKIs and other target based agent in the management of breast cancer patients in advanced phase of the disease.

8. ACKNOWLEDGEMENTS

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