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Modulation of epidermal growth factor receptor in endocrine-resistant, oestrogen receptor-positive breast cancer

R I Nicholson, I R Hutcheson, M E Harper, J M Knowlden, D Barrow, R A McClelland, H E Jones, A E Wakeling[1] *and J M W Gee*

Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff, UK
[1]AstraZeneca, Macclesfield, Cheshire, UK

(Requests for offprints should be addressed to R I Nicholson; nicholsonri@cardiff.ac.uk)

Abstract

There is an increasing body of evidence demonstrating that growth factor networks are highly interactive with oestrogen receptor (ER) signalling in the control of breast cancer growth. As such, tumour responses to anti-hormones are likely to be a composite of the ER and growth factor inhibitory activity of these agents. The current article examines the modulation of growth factor networks during endocrine response, and presents *in vitro* and clinical evidence that epidermal growth factor receptor signalling, maintained in either an ER-dependent or -independent manner, is critical to anti-hormonal-resistant breast cancer cell growth. The considerable potential of the epidermal growth factor receptor-selective tyrosine kinase inhibitor, ZD 1839 (Iressa; AstraZeneca) to efficiently treat, and perhaps even prevent, endocrine-resistant breast cancer is highlighted.

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Introduction

Until relatively recently endocrine response pathways in breast cancer were described solely in terms of the intracellular pathways used by oestrogens and the subsequent disruptive effects exerted by anti-hormonal treatments on oestrogen receptor (ER) signalling (Seery *et al.* 1999). Thus, it was frequently proposed that oestrogens promoted tumour growth by binding to ERs, which then acted as nuclear transcription factors regulating the expression of genes involved in proliferation and survival mechanisms. In contrast, anti-hormones, acting either to reduce the amount of oestrogens available to the tumour cells or by binding the ER to antagonise the cellular actions of oestrogens, prevented this flow of information to promote tumour remission (Nicholson *et al.* 1993*a*, Seery *et al.* 1999).

However, a more modern view of endocrine response pathways retains the concept that oestrogens acting through ERs are central to the development of breast cancer, but also recognises that it is naive to consider ER signalling in isolation from the remainder of the cancer cell biology (Nicholson & Gee 2000). Indeed, an increasing number of elements within the breast cancer phenotype, notably including peptide growth factors, have now been identified which modify and can be modified by ER signalling (Nicholson & Gee 2000). As such, they have the capacity to significantly influence the sensitivity of breast cancer cells to oestrogens. Importantly, however, these factors are also likely to be critical in the mechanism of response to anti-hormonal drugs, and moreover may be integral in the escape from anti-hormone control of growth that occurs on disease relapse.

Within this context, the current article outlines a number of concepts regarding the interplay between ER and growth factor signalling in hormone-sensitive breast cancer. In particular, it is now known that while anti-hormones suppress both ER and insulin-like growth factor (IGF) signalling during response (Freiss *et al.* 1990, Guvakova & Surmacz 1997, Surmacz 2000), paradoxically they promote the expression of epidermal growth factor receptor (EGFR) and c-erbB2, receptors employed by EGF-like ligands (Dati *et al.* 1990, Chrysogelos *et al.* 1994, Yarden *et al.* 1996, deFazio *et al.* 1997). Our recent experimental data demonstrate that increased expression of EGFR and c-erbB2 can occur *in vitro* following challenge with several anti-hormonal drugs (HE Jones, JMW Gee, ME Harper, AE Wakeling & RI Nicholson, unpublished observations; JM Knowlden, I Hutcheson, JMW Gee & RI Nicholson, unpublished observations; McClelland *et al.* 2001). Importantly, while such increases are redundant in the anti-hormonal response, we have shown that the

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EGFR/c-erbB2 signalling network is ultimately harnessed by the cells, enabling re-establishment of their growth in an ER-dependent or -independent manner. These events thus appear to be critical in the generation of several forms of acquired endocrine resistance and insensitivity. Excitingly, the phenotypic characteristics of breast tumours from patients with hormone-resistant disease in many ways parallel these experimental data (Nicholson *et al.* 1993*a*, 1994*a*,*b*, 1997*a*,*b*, Gee *et al.* 2001*a*). Furthermore, our *in vitro* data also show that such endocrine-resistant or -insensitive cells are highly sensitive to the EGFR-selective tyrosine kinase inhibitor, ZD 1839 (Iressa; Astra Zeneca). The compound obliterates EGFR signalling and effectively blocks anti-hormonal-resistant tumour cell growth in the presence or absence of exogenous ligands for the EGFR. Since the anti-tumour effects are long lasting and synergistic with anti-hormones, the data highlight the considerable potential of such inhibition as a means of efficiently treating endocrine-resistant and -insensitive breast cancer.

ER signalling and growth factors in hormone-sensitive breast cancer

The concept that peptide growth factors can act as mediating factors in the growth of hormone-sensitive breast cancer is not a new one. It has its origins in the late 1980s, when it was first recognised that oestrogens were able to stimulate expression of a number of growth factor regulatory elements (e.g. transforming growth factor-alpha (TGF-α) and IGF-II) in hormone-sensitive human breast cancer cell lines (Bates *et al.* 1988, Lee *et al.* 1994). Such actions significantly supplemented the cellular mitogenic responses and gene expression directly primed by oestrogens (Cho & Katzenellenbogen 1993, Smith 1998). Importantly, this concept, although not repudiated in more recent years, has now been substantially modified to incorporate a further fascinating dimension. The intracellular signalling pathways associated with oestrogen and growth factor action are known to be more highly networked and interactive than was originally thought. Indeed, it is unlikely that mitogenic signalling arising from either of the pathways can operate efficiently in the absence of the other (Nicholson & Gee 2000). Moreover, this is perceived to be not just a function of their ability to coregulate the expression of genes involved in proliferation and cell survival (Musgrove *et al.* 1993, Lukas *et al.* 1996, Huang *et al.* 1997, Wang *et al.* 1998), but is, in part, due to a physical overlapping and common use of their signalling elements (Nicholson & Gee 2000). For example, numerous studies have now shown that key receptors in such pathways (for example ER and IGF-I receptor (IGF-IR)) are subject to activation by both oestrogens and peptide growth factors (Aronica & Katzenellenbogen 1993, Bunone *et al.* 1996, Richards *et al.* 1996). The important pharmacological significance of such convergence in hormone-sensitive breast cancer cells is that anti-hormonal drugs not only possess anti-oestrogenic activity through their ability to block ER signalling, but also have anti-growth factor actions by virtue of their ability to disrupt the intimate cross-talk between oestrogen and growth factor signalling (Freiss *et al.* 1990, Guvakova & Surmacz 1997). Indeed, the increasing body of experimental data, supplemented by recent clinical studies examining the phenotypic profile during the tamoxifen-responsive phase of the disease, indicates it is most likely a combination of these anti-oestrogenic and anti-growth factor actions that is responsible for tumour remissions following anti-hormonal challenge of breast cancer patients (Gee *et al.* 2001*b*).

At this juncture, it is noteworthy that the experimental and clinical data imply that not all growth factors are used equally to drive the growth of oestrogen-sensitive breast cancer cells, a phenomenon governed, at least in part, by the cellular availability of growth factor receptors. Thus, oestrogens appear to 'favour' synergistic growth interactions with IGFs (Dupont *et al.* 2000), with oestrogens inducing the expression of the IGF-IR. Not surprisingly, therefore, many ER-positive breast cancer cells *in vitro* and *in vivo* coexpress considerable levels of IGF-IR, with a strong correlation apparent between IGF-IR and ER levels in the clinic (Railo *et al.* 1994, Happerfield *et al.* 1997, Surmacz 2000).

In marked contrast, oestrogens appear to 'disfavour' growth interactions with EGF and TGF-α. Expression of the EGFR protein (and mRNA), as well as its favoured heterodimerisation partner c-erbB2 (Martinez-Lacaci *et al.* 1999), is suppressed by long-term therapy with oestrogens *in vitro* (Dati *et al.* 1990, Chrysogelos *et al.* 1994, Yarden *et al.* 1996, deFazio *et al.* 1997). In contrast to IGFs, EGFR ligands are poor inducers of the growth of hormone-sensitive cells, where at best they promote growth responses which are additive (but not synergistic) with oestradiol. Finally, there are obvious inverse associations between these receptor tyrosine kinase receptors and ER expression in clinical and experimental samples (Nicholson *et al.* 1993*a*, 1994*a*, 1997*a*,*b*, Sharma *et al.* 1994*a*,*b*). In parallel, there is merely low expression of the EGFR ligand TGF-α (Nicholson *et al.* 1994*b*), with activation of the important downstream signalling target for EGFR, mitogen-activated protein (MAP) kinase, also minimal in ER-positive disease both in the clinic (Gee *et al.* 2001*a*) and *in vitro* (McClelland *et al.* 2001). In total, these data convincingly demonstrate that hormone-sensitive breast cancer cells possess potent mechanisms to limit EGFR/c-erbB2-mediated signalling (Yarden *et al.* 1996).

This concept has significant clinical implications. Several studies have now demonstrated that while anti-hormones disrupt favoured ER–growth factor interactions to inhibit breast cancer cell growth (e.g. via diminishing activation/expression of IGF-IR (Freiss *et al.* 1990, Guvakova & Surmacz 1997, Surmacz 2000)), there is parallel

de-repression of disfavoured pathways. Indeed, we and others (Warri *et al.* 1991) have observed time-dependent increases in expression of EGFR/c-erbB2 during anti-hormonal challenge of MCF-7 human breast cancer cells *in vitro* and within clinical material obtained during therapy. The existence of such cellular mechanisms may offer breast cancer cells the option of using these pathways to (i) initially survive oestrogen deprivation (Yarden *et al.* 1997) and (ii) eventually re-instigate endocrine-resistant or -insensitive tumour cell growth (McClelland *et al.* 2001).

Long-term effects of anti-hormones on the growth of MCF-7 breast cancer cells

In order to further monitor the inductive effects of anti-hormonal drugs on EGFR and c-erbB2 signalling pathways, interplay with ER signalling, and tumour regrowth during therapy (i.e. endocrine-resistant or -insensitive growth), we have cultured MCF-7 breast cancer cells with various anti-oestrogens in long-term monolayer culture (McClelland *et al.* 2001).

Anti-oestrogens induce EGFR and c-erbB2 signalling and instigate an EGFR-primed autocrine growth regulatory loop in tamoxifen- and Faslodex-resistant breast cancer cells

MCF-7 cells are oestrogen-responsive for their growth and are growth inhibited by many anti-oestrogenic drugs (Nicholson *et al.* 1995, 1996). However, their continuous culture in the presence of tamoxifen or Faslodex eventually generates sublines which tolerate the presence of the anti-oestrogens, regrowing at rates equivalent to the original hormone-responsive parental cells (McClelland *et al.* 2001). This closely mirrors the clinical scenario, where development of resistance is almost inevitable for patients demonstrating an initial endocrine therapeutic sensitivity (Cheung *et al.* 1997).

In our own studies, such anti-hormonal-resistant MCF-7 sublines uniformly express increased amounts of EGFR mRNA and protein (McClelland *et al.* 2001). Thus, for example, while EGFR immunostaining of the parental MCF-7 cells demonstrates that they express only extremely modest levels of EGFR, both tamoxifen- and Faslodex-resistant cells contain up to 10-fold higher levels of EGFR membrane staining. We have also noted parallel increases in c-erbB2 immunostaining in the anti-oestrogen-resistant cells. Complementary data have previously been reported for the EGFR by Yarden *et al.* (1997), who showed that in the absence of oestrogen EGF had a much stronger proliferative effect, indicating an increased potential of such cells to use the EGFR for growth. Indeed, treatment of the cells with ICI 164384, a pure anti-oestrogen that similarly increases EGFR levels, also increased EGF growth responses, again indicating that therapies depriving cells of their oestrogenic input increase sensitivity to EGFR ligands. Our phenotypic data monitoring EGFR and c-erbB2 in the cell lines are further supported by a battery of *in vitro* gene transfer studies and the expression profiles observed in several additional acquired tamoxifen-resistance models (Vickers *et al.* 1988, Clarke *et al.* 1989, Valverius *et al.* 1990, Van Agthoven *et al.* 1992, 1994, Benz *et al.* 1993, Miller *et al.* 1994, Pietras *et al.* 1995, Van den Berg *et al.* 1996, Kurokawa *et al.* 2000).

Consistent with the concept that overexpressed EGFR and c-erbB2 may play a role in the development of anti-oestrogen resistance, we have been able to demonstrate by immunoprecipitation studies that these receptors are heterodimerised and fully active in such cells (JM Knowlden, I Hutcheson, JMW Gee & RI Nicholson, unpublished observations). Since the tamoxifen-resistant variants also express numerous EGFR ligands, each of which is able to further increase the levels of activated EGFR and c-erbB2 and induce additional growth responses, it appears likely that the new growth signal originates from an EGFR-primed autocrine regulatory loop. Significantly, Yarden *et al.* (1997) demonstrated that increased EGFR acts as a survival factor, since blocking this receptor with an EGFR-neutralising antibody caused a 2-fold induction of apoptosis.

Further assessment of the importance of EGFR/c-erbB2 signalling in the resistant cells was made following our development of an immunohistochemical procedure for localising the activated (i.e. phosphorylated) forms of erk 1/2 MAP kinases (actMAPK) using a phosphorylation state-specific antibody (Gee *et al.* 2001*a*). These enzymes are pivotal components of the intracellular phosphorylation cascade from the plasma membrane to the nucleus recruited for EGFR/c-erbB2 signal transduction (English *et al.* 1999). Using this technique, actMAPK was found to be considerably higher in the anti-hormonal-resistant sublines than in the parental MCF-7 cells (McClelland *et al.* 2001) and to be further inducible by various ligands for the EGFR. Interestingly, complementary associations have previously been reported *in vitro* between acquisition of steroid hormone independence (Coutts & Murphy 1998) or tamoxifen resistance (Kurokawa *et al.* 2000) by ER-positive breast cancer cells and increased erk 1/2 MAPK phosphorylation. We confirmed staining specificity in the resistant cells following its reduction by PD 098059, a MEK1 inhibitor previously shown to inhibit the phosphorylation and activation of erk 1/2 MAPK (Alessi *et al.* 1995). Importantly, PD 098059 was also found to be a highly effective inhibitor of the growth of the anti-hormonal-resistant cells, producing an arrest of cell proliferation (McClelland *et al.* 2001). These data in total confirm that this signalling pathway has been harnessed by the resistant cells and is of critical importance

in their escape from the growth restraints imposed by anti-hormonal challenge.

Tamoxifen-resistant breast cancer cells express and use ER as part of the EGFR-regulated growth pathway

The tamoxifen-resistant variants, like their clinical counterparts (Robertson *et al.* 1992, Nicholson & Gee 1996, Robertson 1996, Johnston *et al.* 1997), continue to express ER at a level equivalent to that observed in the parental cell line. Significantly, the ER can be demonstrated to be involved in maintaining the new EGFR-driven growth regulatory loop. Exposure of tamoxifen-resistant cells to the pure anti-oestrogen Faslodex at a dose which obliterates the ER protein by increasing the sensitivity of the receptor to proteolytic attack and disrupting its nucleocytoplasmic shuttling (Gibson *et al.* 1991, Dauvois *et al.* 1992, Seery *et al.* 1999) interestingly leads to a concomitant loss of activation of EGFR and c-erbB2. There is an equivalent reduction in activation of the EGFR/c-erbB2 downstream signalling components erk 1/2 MAPK. Importantly, the parallel loss of ER and EGFR/c-erbB2 signalling following Faslodex treatment is associated with an effective inhibition of the growth of the cells (JM Knowlden, I Hutcheson, JMW Gee & RI Nicholson, unpublished observations). Since Faslodex does not decrease the total cellular levels of the EGFR, c-erbB2 or erk 1/2 MAPK proteins in such cells, it appears likely that this anti-oestrogen influences the activity of the growth factor signalling pathway by limiting the availability of one or more of its ligands. Interestingly, our preliminary studies indicate that a ligand targeted by Faslodex in tamoxifen-resistant cells may be TGF-α. Such a concept is reinforced by 'add-back' experiments, where exogenous TGF-α or EGF not only activates EGFR, c-erbB2 and erk 1/2 MAPK but also supports substantial tumour cell growth in the presence of Faslodex. Strengthening the EGFR pathway thus appears able to entirely circumvent the catastrophic effects of this anti-oestrogen on the ER protein in such cells. EGFR ligand-treated cells are thus refractory to the growth inhibitory effects of both tamoxifen and Faslodex (i.e. complete endocrine insensitivity), data certainly implying that the primary growth regulatory role for ER in the tamoxifen-resistant cells is to maintain the efficiency of EGFR signalling.

Faslodex-resistant cell growth is EGFR regulated independently of ER

In marked contrast to the tamoxifen-resistant subline, we have observed that cells actively growing in the presence of Faslodex (i.e. Faslodex-resistant) show only very low basal expression of the ER protein. Indeed, using our standard H222-ERICA assay, only 2% of such cells can be shown to be weakly or very weakly stained for ER, and also with lower ER mRNA levels than the parental cell line (McClelland *et al.* 2001). Faslodex-resistant cells also fail to express the classically oestrogen-regulated gene progesterone receptor and show no oestrogen-response element (ERE) activity as judged through transient transfection of an ERE-bearing reporter gene plasmid construct into the cells (McClelland *et al.* 2001). Results in many aspects comparable with these data have been published by Larsen *et al.* (1997). These data indicate that the enhanced EGFR signalling observed in Faslodex-resistant cells provides their primary mitogenic stimulus that is not supplemented by an ER-mediated input.

EGFR/c-erbB2 signalling and endocrine response in clinical breast cancer

Almost two decades have now elapsed since the first report describing the presence of EGFR in some human breast tumours (Sainsbury *et al.* 1985). Of particular interest was the observation that predominance of the protein was associated with elevated proliferative capacity, disease progression and extremely poor patient prognosis (Nicholson *et al.* 1993*a*, 1994*a*, 1997*a*,*b*). Since that time, a universal finding has been that expression of the EGFR protein is highly variable within the breast cancer population. For example, ~50% of operable cases showing EGFR membrane immunostaining and several studies have recorded that EGFR positivity is associated with an increased likelihood of failure to respond to endocrine measures *de novo* (Nicholson *et al.* 1993*a*, 1994*a*, 1997*a*,*b*). A parallel relationship between c-erbB2 overexpression, poorer prognosis and anti-hormonal resistance has also been observed, although these associations as yet remain controversial (Nicholson *et al.* 1993*a*, 1997*a*,*b*, Elledge *et al.* 1998, Houston *et al.* 1999).

Similarly, we have observed that elevated TGF-α expression is correlated with *de novo* endocrine failure in ER-positive disease, where there is also a prominent association with proliferation (Nicholson *et al.* 1994*b*). In addition, we have observed a highly significant association between elevated actMAPK, shortened survival, and poorer quality and shortened duration of anti-hormonal response (Gee *et al.* 2001*a*). Enhanced actMAPK was observed in ~80% of ER-positive, tamoxifen-resistant tumours that also demonstrated evidence of elevated TGF-α/EGFR signalling (Gee *et al.* 2001*a*), with multivariate analysis demonstrating actMAPK to be a significant independent predictor for response duration and patient survival in such patients. In total, these data certainly indicate the existence of an EGFR-driven autocrine growth regulatory loop capable of maintaining tumour cell growth in the presence of anti-hormonal drugs. Indeed, although few data exist monitoring EGFR/c-erbB2/TGF-α/actMAPK levels in breast cancer specimens obtained during endocrine response and at

the time of relapse, our early clinical data employing highly sensitive immunocytochemical procedures have demonstrated small but significant increases in these elements at the time of acquisition of tamoxifen resistance. Moreover, it is feasible that there is cross-talk of such signalling with ER in acquired resistant disease, since second-line anti-hormonal responses (Cheung *et al.* 1997) and substantial ER expression (Robertson *et al.* 1992, Nicholson & Gee 1996, Robertson 1996, Johnston *et al.* 1997) are commonly noted in such patients.

Studies with the EGFR-selective tyrosine kinase inhibitor Iressa

Inhibition of tumour cell growth and EGFR signalling

The observation that our tamoxifen- and Faslodex-resistant cells express high levels of EGFR, c-erbB2 and actMAPK (McClelland *et al.* 2001) and a profile of EGFR ligands led us to evaluate the anti-tumour effects of ZD 1839. This is a small molecule EGFR-selective tyrosine kinase inhibitor, which we previously demonstrated to be highly effective in blocking growth of EGFR-positive DU145 and LnCAP prostate carcinoma cells *in vitro* (Jones *et al.* 1997, 2001). The compound is a non-peptide anilinoquinazoline currently demonstrating considerable promise in pre-clinical and clinical studies examining cancer types enriched for EGFR positivity (Baselga & Averbuch 2000, Ciardiello *et al.* 2000, Meric *et al.* 2000). It inhibits EGFR tyrosine kinase at concentrations at least 100-fold lower than for many other kinases tested, notably including c-erbB2 (Wakeling *et al.* 1994, 1996). In line with its action as a competitive inhibitor of ATP binding to EGFR, ZD 1839 has been shown to prevent autophosphorylation of EGFR in a number of cultured tumour cell lines, resulting in an inhibition of the activation of key downstream signalling molecules (Baselga & Averbuch 2000).

Significantly, in our breast cancer models of tamoxifen and Faslodex resistance, 1 μM ZD 1839 efficiently blocks the EGFR autophosphorylation and the activation of erk 1/2 MAPK under both basal and EGFR-primed conditions (JM Knowlden, I Hutcheson, JMW Gee & RI Nicholson, unpublished obsevations). In each instance, cell growth was markedly inhibited, contrasting the relative lack of effect of this drug on the growth of the parental endocrine-responsive MCF-7 cells (McClelland *et al.* 2001). Importantly, the growth inhibitory effects of ZD 1839 were long-lasting, indicating that the autocrine EGFR loop is critical to the growth of these anti-hormonal-resistant cells and that no other mitogenic network is readily available when EGFR signalling is blocked. The increase in cellular expression of EGFR generated by anti-hormones thus appears to provide a promising molecular target for effective treatment of endocrine-resistant and -insensitive phases of the disease. Since parallel analysis of EGFR expression profiles in resistant breast tumour specimens indicates this concept could prove applicable to clinical disease (Nicholson *et al.* 1993*a*, 1994*a*, 1997*a*,*b*), trial data with ZD 1839 in such breast cancer patients are eagerly awaited.

Combination of anti-oestrogen and anti-EGFR treatments efficiently blocks development of resistance in parental anti-hormone-sensitive breast cancer cells

As described above, in our breast cancer cell lines the upregulation of EGFR proved to be consistently critical in the development of anti-hormone resistance. In anticipation of their switch to this essential EGFR pathway used in resistance, we undertook experiments in which the parental anti-hormone-responsive MCF-7 cells were treated with ZD 1839 alone or in combination with tamoxifen or Faslodex. Importantly, while ZD 1839 was largely without an additive growth inhibitory effect with the anti-hormones during the first month of therapy, thereafter the agents showed synergistic growth inhibitory activity, fully blocking the development of anti-hormone resistance (JMW Gee & RI Nicholson, unpublished observations). Indeed, during this period not only did ZD 1839 substantially suppress proliferative activity within the dual-treated cells, but its presence led to a massive loss of cell numbers due to marked increases in the rate of apoptosis. This finding is highly supportive of the concept that combination therapies that simultaneously target oestrogen and growth factor signalling may be more effective than the sequential use of such drugs. Moreover, these exciting experimental studies indicate that ZD 1839 may prevent development of the endocrine-resistant state.

Herceptin challenge reveals an important role for c-erbB2 in directing the growth of tamoxifen-resistant cells

Although the data we have presented with ZD 1839 clearly demonstrate a central role for EGFR in the development of anti-hormone resistance, it is equally evident that phosphorylation of c-erbB2, the favoured heterodimerisation partner of the EGFR (Martinez-Lacaci *et al.* 1999), is also relevant. We have thus examined the role of c-erbB2 in anti-hormone-resistant cell growth using Herceptin, a c-erbB2-directed antibody therapy which inhibits the growth of many c-erbB2-positive cancer cell lines (Sliwkowski *et al.* 1999) and promotes tumour remissions in breast cancers overexpressing c-erbB2 by gene amplification (Baselga 2001, Slamon *et al.* 2001). We have noted that Herceptin is highly effective at inhibiting the growth of the tamoxifen-resistant variants, in marked contrast with its lack of effect on the

parental hormone-responsive cell line. Complementary data have been obtained by Kurokawa *et al.* (2000), who report efficacy of a small molecule inhibitor of this receptor in their MCF-7 model of tamoxifen resistance derived by stable transfection of c-erbB2 cDNA. Our data indicate that (i) the role of c-erbB2 in growth regulation is extremely limited in the parental anti-hormone-sensitive cells and (ii) autocrine activation of EGFR in the tamoxifen-resistant cells recruits the c-erbB2 receptor protein as an essential partner directing cell growth.

Summary and conclusions

Anti-oestrogen therapy is considered by many as the first-line therapeutic option for the management of ER-positive breast cancer. Unfortunately, clinical application of such endocrine measures has revealed that responses are remarkably variable and often short-lived. An understanding of the complex mechanisms contributory towards loss of anti-oestrogen response is an important research goal since it should allow a rational approach to be taken in the effective treatment, delay or even prevention of the development of resistance, thereby severely compromising the disease process and improving patient survival.

Significantly, in our current studies we have demonstrated that increases in EGFR/c-erbB2/actMAPK signalling can promote tamoxifen and Faslodex resistance in a human breast cancer cell line *in vitro*, and that resistant growth can be inhibited in a sustained manner by blocking of EGFR signalling using ZD 1839. Moreover, if ZD 1839 is used to treat hormone-responsive cells in combination with either of the anti-oestrogens, it increases tumour cell kill to such a degree that resistance to these agents cannot occur. Clinical trials of ZD 1839 are now obviously required to determine if such responses apply as fully to human breast cancer exposed to anti-hormones *in vivo* as they apparently do *in vitro*. Finally, our model indicates that in order for breast cancer cells to escape the cellular actions of anti-hormones, they must possess compensatory survival pathways that ultimately allow the development of drug resistance. The strategic targeting of such survival factors could potentially prove a highly complementary addition to the existing pharmacological armoury appropriate to the cancer patient. The identification and exploitation of such pathways in cancer cells treated with anti-hormones or chemotherapeutic agents is now the primary research goal within the Tenovus Centre for Cancer Research.

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