Growth factor-driven mechanisms associated with resistance to estrogen deprivation in breast cancer: new opportunities for therapy

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Abstract

There is an increasing body of evidence demonstrating that elevated growth signaling in breast cancer cells can promote forms of endocrine resistance in either an estrogen receptor-dependent or -independent manner. The current article reviews what is known about such growth factor signaling networks and resistance to estrogen withdrawal and considers the many novel therapeutic opportunities that stem from this knowledge.

Introduction

Within the last 5 years, clinical interest in estrogen deprivation strategies for the therapy of breast cancer has gained considerable momentum. Estrogen deprivation is achieved clinically through the use of ovarian ablation or suppression (e.g. luteinizing hormone-releasing hormone superagonists) in premenopausal women, and through aromatase inhibitors in postmenopausal women (Johnston & Dowsett 2003, Pritchard 2003). The ‘third generation aromatase inhibitors’ (AIs), exemplified by the non-steroidal competitive inhibitors of aromatase, anastrazole and letrozole, and the steroidal aromatase inactivator, exemestane, are potent and highly selective in their inhibition of the cytochrome P450 enzyme, aromatase. As a consequence, they efficiently block conversion of androgens (testosterone and androstenedione) to estrogens (estradiol and estrone respectively) in peripheral tissues and breast tumors (Santen 2003). Their induction of profound estrogen deprivation provides an effective therapeutic second line strategy in advanced postmenopausal breast cancer (Santen 2003). Their induction of profound estrogen deprivation provides an effective therapeutic second line strategy in advanced postmenopausal breast cancer (Johnston & Dowsett 2003). Moreover, emerging trial data indicate that AIs may be superior to the antiestrogen tamoxifen as first line agents in advanced ERα+ breast cancer (T. Baum et al. 2003, Wong & Ellis 2004). Clearly, longer follow-up for AI adjuvant trials such as ATAC remains essential if we are to judiciously assess whether these agents could be of routine value in the postmenopausal adjuvant setting (Winer et al. 2003). However, the clinical data are encouraging to date and there is hope that AIs may, in the future, replace tamoxifen as the ‘gold standard’ therapy for ERα+ disease and perhaps further improve breast cancer survival rates (Santen 2003). Our emerging knowledge of the molecular biology of ERα signaling sheds light on these promising observations. While the AF-2 activity of ERα is silenced, it is notable that weak AF-1-mediated agonistic effects are retained following tamoxifen binding to ERα. In contrast, under conditions of near-complete estrogen deprivation, it is predicted that ERα activity and hence ERα-mediated transcription would be fully abrogated (Johnston & Dowsett 2003).

Clearly, exciting advances in the endocrine therapy of breast cancer continue to be made. Importantly, however, as promising as the above prospects are, it is already
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evident that a switch to estrogen deprivation as the preferred endocrine strategy will not provide the whole answer to effective treatment of breast cancer. Thus, to date, improvement in relapse-free survival in the adjuvant setting observed with AIs versus tamoxifen remains modest. Moreover, not all patients who are treated with AIs respond equally well, while relapse rates in advanced disease are still unfortunately often measured in months rather than years (Pritchard 2003). So where should we look for the next advance in the therapy of breast cancer? Clearly, accurate identification of the causative elements for primary (de novo) and acquired endocrine resistance is essential if we are to develop new therapeutic approaches to delay, prevent or potentially even reverse these undesirable conditions (Nicholson et al. 2003). The biology of endocrine resistance is highly complex and poorly understood. As such, diverse mechanisms have been implicated that are at various stages of experimentation and exploitation. Several groups have focussed on whether alterations occurring in the structure/function of ERz contribute to resistance. For example, studies by Fuqua et al. (2000) demonstrate that ERz mutation (Lys303Arg) enables increased co-activator recruitment to the receptor, conferring hypersensitivity in the presence of reduced estrogens. Moreover, in some model systems, aromatase is increased in response to estrogen deprivation in parallel with acquisition of estrogen hypersensitivity, while aromatase-transfected MCF-7 cells are also hypersensitive (Yue et al. 2001, 2003). However, the role of such events in invasive breast cancer and clinical endocrine resistance remains obscure (Karnick et al. 1994, de Jong et al. 2003). In contrast, there is considerable evidence that growth factor signaling pathways should be of high priority in our quest for new therapeutic targets in endocrine resistance (Nicholson et al. 2003). Thus, the current article reviews the growth factor signaling-driven mechanisms that have been implicated to date in resistance to estrogen withdrawal primarily from breast cancer model systems. Where possible, parallels will be drawn with resistance to antiestrogens. Based on these concepts, the article will suggest how we might in the future effectively and rationally target growth factor signaling pathways to enhance response to estrogen deprivation and improve breast cancer survival.

Growth factor signaling associates with resistance to endocrine therapies, including estrogen deprivation

Developing breast cancer cells are bathed in a rich milieu of peptide growth factors that are produced locally or distally. Additionally, many neoplastic cells are able to produce growth factors by autocrine means and frequently express several classes of growth factor receptors and their down-stream signaling elements (Salomon et al. 1995, Nicholson et al. 2001b, Sachdev & Yee 2001, Gross & Yee 2003). Cancer cell responses to growth factors include enhancement of proliferation, cell survival, motility, invasiveness and angiogenesis (Wells 1999, Ciardiello & Tortora 2001, Sachdev & Yee 2001, Gross & Yee 2003). Thus, it is not surprising that inappropriate activation of growth factor signaling cascades, either through the enhanced supply of growth factor ligands or via up-regulation/increased activation of their target receptors or their recruited downstream signaling elements can associate with aggressive tumor biology and poor patient prognosis (Ciardiello & Tortora 2001, Nicholson et al. 2001a, 2004). Moreover, there is now considerable evidence that increased signaling through such pathways promotes in vitro/in vivo resistance to various cytotoxics (Dickstein et al. 1995; Gooch et al. 1999, Chen et al. 2000), radiotherapy (Dunn et al. 1997, Akimoto et al. 1999, Gee & Nicholson 2003), and notably endocrine strategies in breast cancer cells (Nicholson et al. 1994, 2004).

As an example, increased epidermal growth factor (EGF) receptor (EGFR) signaling is a relatively common feature in established cell models that have become refractory to antiestrogens (Vickers et al. 1988, Long et al. 1992, van Agthoven et al. 1994, van den Berg et al. 1996, McClelland et al. 2001, Knowlden et al. 2003b). Thus, our established MCF-7, and more recently T47D, human breast cancer sub-lines that have acquired resistance to tamoxifen or fulvestrant in vitro clearly demonstrate dominance of EGFR/HER2 signaling, with increased activation of the downstream growth factor signaling elements erk1/2 MAP kinase and Akt that are pivotal in driving antiestrogen resistant growth (McClelland et al. 2001, Knowlden et al. 2003b, Jordan et al. 2004). Extensive gene transfer studies further highlight the considerable potential for increased expression/activation of growth factor pathway elements to perturb endocrine response, notably to estrogen deprivation. These studies include overexpression of the growth factors heregulin-β2 (Tang et al. 1996), fibroblast growth factor (FGF)-4 (McLeskey et al. 1993), insulin-like growth factor (IGF)-II (Daly et al. 1991, Abdul-Wahab et al. 1999) and transforming growth factor (TGFβ)-1 (Arteaga et al. 1993), and also growth factor receptors such as EGFR and IGF-1R (Van Agthoven et al. 1992, Miller et al. 1994, Guvakova & Surracaz 1997, Abdul-Wahab et al. 1999). The receptor HER2 also appears to contribute to resistance either directly when over-expressed (Benz et al. 1993, Liu et al. 1995, Pietras et al. 1995) or indirectly as the preferred heterodimerization partner of liganded erbB receptor family members (Knowlden et al. 2003b). Furthermore, gene transfer of constitutively active growth factor signaling enzymes
including Ras family members (Yu & Feig 2002), Raf-1 (El-Ashry et al. 1997), MAP kinase (Donovan et al. 2001, Oh et al. 2001), Akt (Campbell et al. 2001, Kurokawa & Arteaga 2003), and protein kinase (PK) C (Tonetti et al. 2000) have all been associated with resistance, as has aberrant expression of nuclear transcription factors such as AP-1 (Smith et al. 1999).

Several mechanisms appear able to contribute to the increases in growth factor signaling in breast cancer cells that can promote endocrine resistance. These include genetic alterations in their key components (gene amplification, mutation etc.), best illustrated by HER2, where gene amplification is a feature of 30% breast cancers de novo (Tsuda 2001). However, adaptive events can also contribute, known to be instigated by diverse therapeutic strategies. Since growth factor pathways have been demonstrated to influence, and be influenced by, ERα signaling (Nicholson & Gee 2000, Nicholson et al. 2001b, 2003, 2004, Hutcheson et al. 2003, Johnston et al. 2003), it is perhaps not surprising that adaptive events in growth factor pathways also occur when breast cancer cells are subjected to therapies that suppress ERα signaling. Thus, in vitro data have demonstrated that upregulation of EGFR (Chrysogelos et al. 1994, de Fazio et al. 1997, Yarden et al. 2001, Wilson & Chrysogelos 2002, Gee et al. 2003) and HER2 (Russell & Hung 1992, Bates & Hurst 1997) occurs in several ERα+ endocrine responsive breast cancer cells when they are subjected to estrogen deprivation, while in clinical disease increases in HER2 have been detected in serum during ovarian ablation (Luftner et al. 2003). A particularly common adaptive feature during response to long-term estrogen deprivation of in vitro and in vivo models appears to be increased MAP kinase activation (Coultts & Murphy 1998, Jeng et al. 2000, Martin et al. 2003, Santen et al. 2004). Parallel adaptive data have been described for antiestrogens, again exemplified by our MCF-7 cell studies demonstrating that the initial response to tamoxifen (or fulvestrant) is associated with de-repression of the inhibitory effect of estrogen exposure on EGFR and HER2, with resultant increases in MAP kinase and also Akt signaling occurring during treatment (McClelland et al. 2001, Gee et al. 2003). Our extensive observations of these cells have clearly demonstrated that these adaptive events facilitate cell survival in the presence of anti-hormones (explaining incomplete initial anti-tumor activity) and ultimately promote proliferation and hence development of resistance (Gee et al. 2003, Nicholson et al. 2004). Similarly, emerging clinical studies are confirming up-regulation of various growth factor signaling elements, including HER2, that are apparent at the time of tamoxifen relapse (Gee et al. 1999, 2002, Johnston et al. 1999, Dowsett et al. 2003).

Growth factor signaling clearly has considerable potential to promote endocrine resistance. In the following sections, data will be presented demonstrating that altered growth factor signaling (either apparent de novo, or adapting during estrogen depletion) can contribute to development and growth of phenotypes resistant to estrogen deprivation. This appears to occur by a number of mechanisms that are also likely to be relevant when considering resistance to antiestrogens. The first of these resistance mechanisms is ERα-dependent, where increased growth factor signaling elements act to hypersensitize breast cancer cells to residual estrogens (and in the case of challenge with the anti-estrogen tamoxifen, enhance its agonistic capability). The second mechanism, proposed by our own studies, indicates again that growth factor signaling elements can promote resistance to estrogen deprivation in an ERα-dependent manner, but not by obviously increasing sensitivity to residual estrogens. Subsequent responses to anti-hormonal measures that further deplete ERα signaling (e.g. fulvestrant) are observed in both these forms of resistance, since interplay between ERα and growth factor signaling elements is a unifying feature. Thirdly, however, an ERα-independent mechanism can be employed where growth factor signaling promotes proliferation in the presence of endocrine agents under conditions of markedly reduced ERα expression and/or signaling. Indeed, at its extremes, there is tantalizing evidence to suggest that growth factor signaling may even allow progression to an ERα negative (ERα–) phenotype.

Increased growth factor signaling elements contribute to adaptive hypersensitivity in breast cancer cells by interplay with ERα

Adaptive estrogen hypersensitivity during estrogen deprivation

Estrogen deprivation of various endocrine responsive breast cancer cell models (including MCF-7 and T47D) in vitro and in vivo is associated with an initial inhibitory response. However, studies from several laboratories have demonstrated that estrogen deprivation in the long-term is associated with development of an estrogen hypersensitive state, whereby the exquisitely low (non-physiological) amounts of estrogens remaining after estrogen deprivation appear sufficient to allow development and maintenance of tumor cell growth. Indeed, in one instance (Santen et al. 2004), dose–response data show that a 4-log lower concentration of estradiol (10^{-14} M) is able to stimulate the growth of the resistant cells in comparison with the parental MCF-7 cell line. Furthermore, since the estrogen hypersensitivity dose–response curve is bell-shaped,
physiological levels of estradiol (10^{-10} M) are growth inhibitory (an event mediated through estradiol-induced Fas/FasL and hence apoptosis; Fernandez et al. 1998, Song et al. 2001, Martin et al. 2003, Song & Santen 2003, Santen et al. 2004). Clinical observations imply that an adaptive shift in estrogen sensitivity also occurs in breast cancer patients when estrogen/ER\textsubscript{a} signaling is compromised and that this is important in the emergence of resistance. Thus, endocrine therapies that systematically reduce estrogen signaling can be sequentially effective in breast cancer patients after resistance to initial anti-hormonal challenge develops (i.e. ‘manipulating the estrostat’; Lonning 2001).

Based on various model system data, overlapping explanations involving the predominant interplay between growth factor elements and non-genomic or genomic ER\textsubscript{a} signaling pathways have been proposed for the phenomenon of adaptive estrogen hypersensitivity that is associated with acquisition of resistance to estrogen deprivation. Unifying features of these hypersensitivity mechanisms are increased ER\textsubscript{a} and enhanced activation of MAP kinase and phosphatidylinositol-3-OH kinase (PI3K) signaling, phenotypic changes that appear to very commonly arise during prolonged estrogen deprivation. Indeed, such elements appear critical since, as will be described below, all these models show second-line responses to agents depleting such signaling (i.e. the selective ER\textsubscript{a} down-regulator, fulvestrant, and signal transduction inhibitors respectively).

**Dominant ‘non-genomic’ ER\textsubscript{a}/growth factor signaling mechanism**

An MCF-7 model of long-term estrogen deprivation (LTED) has been derived by Richard Santen’s group by use of phenol red-free medium containing charcoal-stripped serum, where the estradiol concentration is reduced to 10^{-13} M. Following a growth inhibitory phase of 3–6 months, LTED cells acquired resistance and resumed substantial proliferation (Santen et al. 2004). Such cells are markedly hypersensitive when challenged with estradiol, as described above, and in vivo xenograft model system data from the group parallel these in vivo findings (Masamura et al. 1995). In these cells that have adapted to grow in the presence of residual estrogens, Santen has placed emphasis on increased utilization of ‘nongenomic’ cell membrane ER\textsubscript{a}. His group has shown that ER\textsubscript{a} translocated to the plasma membrane is markedly elevated in LTED cells in comparison with parental MCF-7 cells. On binding of estrogen, this membrane ER\textsubscript{a} is able rapidly to recruit classical growth factor signal transduction (Santen et al. 2004). The estrogen-primed membrane ER\textsubscript{a} is thus able efficiently to associate with, and phosphorylate, the adaptor protein Shc in LTED (Santen et al. 2004). It is notable that Shc is a key modulator of several tyrosine kinase receptors including EGFR and IGF-1R (Pellici et al. 1995), and such growth factor receptors may contribute to the increased translocation of ER\textsubscript{a} to the membrane and participate in its coupling to Shc on estrogen binding. Of note in the LTED cells is IGF-1R, where Santen and colleagues have shown that both Shc and ER\textsubscript{a} co-immunoprecipitate using an anti-IGF-1R antibody, and that binding of ER\textsubscript{a} to the IGF-1R occurs within minutes of the addition of estradiol (Santen et al. 2004, Song et al. 2004). Shc subsequently binds to the adaptor proteins Grb2 and Sos and rapidly activates the Ras/Raf/Mek/ MAP kinase signaling cascade (Santen et al. 2001, 2003, 2004, Song et al. 2002a,b). Estradiol was thus shown to stimulate Shc and marked increase MAP kinase phosphorylation in a time- and dose-dependent fashion in LTED. These events were blocked by the ER\textsubscript{a} down-regulator, fulvestrant, indicating that ER\textsubscript{a} is a key upstream component of such signaling rather than MAP kinase activation being derived from constitutive, ER\textsubscript{a}-independent growth factor receptor activity/growth factor secretion in LTED cells (Santen et al. 2001, 2003, 2004, Song et al. 2002a). Certainly there is no evidence of constitutive TGF\textsubscript{\textalpha} over-expression in LTED cells from several studies (Herman & Katzenellenbogen 1994, Jeng et al. 1998, 2000, Santen et al. 2001, 2004).

Similarly, the blockade of MAP kinase activation in LTED by PP-2, a specific c-Src kinase inhibitor, indicates that c-Src kinase is also an important regulator of such signaling (Song et al. 2002b, Santen et al. 2004). The pivotal role for increased activation of MAP kinase in determining the estrogen hypersensitive growth adopted by LTED has been confirmed by abrogation of their basal proliferation and hypersensitive responses to estrogen in vivo using the MAP kinase inhibitors, PD98059 or U0126 (Shim et al. 2000, Yue et al. 2002, Santen et al. 2004). As further proof, the addition of TGF\textsubscript{\textalpha} to parental MCF-7 cells in vitro in order to augment their MAP kinase activity promoted a 2-log increase in sensitivity to growth stimulation by estradiol (Yue et al. 2002, Santen et al. 2004). Associated growth end-points of the elevated non-genomic ER\textsubscript{a}/MAP kinase signaling pathway identified to date in LTED include phosphorylation of ELK-1, a regulator of proliferative activity, and cell membrane changes (Song et al. 2002a,b, Santen et al. 2003, 2004).

Interestingly, however, MAP kinase does not appear to be the only intracellular signaling pathway central to estrogen hypersensitive growth of LTED, since it was observed that MAP kinase inhibitors were unable fully to block this event. Hypersensitive cells in vitro also exhibited enhanced activation of Akt, p70 S6 kinase and eukaryotic initiation factor-4E binding protein (4E-BP1),
key effectors of the PI3K pathway (Yue et al. 2003, Santen et al. 2004). In other cell systems, estrogen has been shown to be capable of activating PI3K in a non-genomic manner, triggering association between ERz, c-Src and the p85 PI3K subunit, with this event converging on cell cycle progression (Castoria et al. 2001). In LTED, dual-inhibition of PI3K with LY294002 and MAP kinase with U0126 was able completely to reverse estrogen hypersensitivity (Yue et al. 2003, Santen et al. 2004). Thus, it appears that co-ordinated activity of PI3K and MAP kinase promotes adaptive hypersensitivity to estrogen deprivation and hence allows LTED growth. Interestingly, changes in MAP kinase and Akt have been associated with endocrine resistance and poorer prognosis in breast cancer samples from patients, suggesting clinical relevance of these signaling elements (Gee et al. 2001, Perez-Tenorio et al. 2002).

While rates of ERz-mediated gene transcription are certainly enhanced basally in LTED, global hypersensitivity to estradiol at the level of classically estrogen-regulated genes is not apparent (Jeng et al. 1998). Furthermore, MAP kinase inhibition is ineffective on such genes in LTED, an observation discordant with the growth inhibitory effects of this agent (Jeng et al. 2000). Thus, Santen’s data suggest that it is an enhanced non-genomic estrogen/ERz mechanism that drives elevated MAP kinase/Akt signaling and thereby promotes growth in the presence of very low estrogen levels (Santen et al. 2004), rather than a predominant genomic pathway, where estrogens would classically interact with nuclear ERz to elevate gene transcription (for example, expression of growth factor ligands such as TGFz that could subsequently prime MAP kinase/Akt (Hutcheson et al. 2003)). However, additional studies from Santen’s group suggest that some degree of undefined, synergistic interaction between non-genomic and genomic pathways does occur at the level of cell cycle regulators, such as E2F1, in the promotion of LTED growth (Yue et al. 2002, Santen et al. 2003, 2004).

Dominant ‘genomic’ ERz/growth factor signaling mechanism

By way of contrast, Johnston and Dowsett (2003) have suggested that enhanced ERz function and the estrogen hypersensitive state arises primarily as a result of increased cross-talk between various growth factor pathways at the level of nuclear ERz in long-term estrogen-deprived breast cancer cells. In their model, estrogen-deprived breast cancer cells initially pass through a stage of estrogen hypersensitivity (LTED-H; hypersensitivity profile similar to that described by Santen and colleagues). In accordance with many models (Katzenellenbogen et al. 1987, Welshons & Jordan 1987, Fernandez et al. 1998, Santen et al. 2004), these cells again have elevated nuclear ERz. Interestingly, the ERz in LTED-H is highly phosphorylated on Ser118 residue in its AF-1 domain in the absence of estrogen, suggesting a key contribution to hypersensitive growth (Chan et al. 2002).

Surprisingly, however, after 80 weeks of culture under estrogen-deprived conditions, ERz appears to function independently of exogenous estradiol (LTED-I). This ‘independent’ state has similarly been reported in other prolonged estrogen deprivation studies (Darbre & Daly 1990, Daly et al. 1990). Dowsett’s group have hypothesized that this phase of growth on long-term withdrawal has actually become ‘super-sensitive’ to residual estrogens (Chan et al. 2002, Martin et al. 2003). Critically, the steroid hormone-depleted culture conditions incorporated insulin into the growth medium for the development and maintenance of LTED-I. The group reported that exclusion of insulin resulted in an approximately 50% drop in basal growth rate of LTED-I cells and restoration of estrogen hypersensitivity (Martin et al. 2003). These data led the group to suggest that the supersensitive LTED-I cells are growing maximally in the insulin-supported media and therefore that exogenous estrogens can have no further growth-promoting action, explaining the apparent LTED-I state. Characterization of the LTED-I cells has revealed that they express elevated ERz with increased phosphorylation on Ser118. They also exhibit increased transcriptional activity at estrogen response elements (EREs) that are hypersensitive to estrogen stimulation, and their growth is inhibited by fulvestrant. Clearly in this model, therefore, cross-talk between ERz signaling pathways and insulin at the level of nuclear ERz/ERE-mediated transcription is likely to be pivotal to the adaptation of the cells to estrogen deprivation. Although the mechanics of the cross-talk leading to estrogen hypersensitivity and super-sensitivity are undoubtedly complex, comprehensive studies from the group have demonstrated, in addition to elevated ERz, a key involvement for increased IGF-1R and HER2 signaling in LTED-I. These promote activation of MAP kinase, ERz (and ERz co-activator) phosphorylation, ERE transcriptional regulation, LTED-I cell growth and supersensitivity to residual estrogens. Increases in IGF-1R have also been implicated in adaptation to long-term estrogen deprivation by Stephen et al. (2001), with some growth inhibitory effect of IGF-1R blockade using the IGF-1R specific antibody zIR3 noted in their model. Increases in HER2 signaling have also been noted on development of acquired resistance to estrogen deprivation in HER-2-transfected MCF-7 cells (Massarweh et al. 2003). Of note, the increased activation of MAP kinase in LTED-I cells was not triggered in a rapid manner by...
estrogen, indicating that non-genomic ER\(\alpha\) signaling is not a dominant contributor (Martin et al. 2003).

Interestingly, Dowsett's group showed that inhibition of MAP kinase activation only partially reduced basal ERE transcriptional activity and was ineffective in blocking Ser118 ER\(\alpha\) phosphorylation. These data imply not only an involvement for additional interactive signaling elements in LTED-1, but also that enhancement of ER\(\alpha\) transcriptional activity occurs via additional ER\(\alpha\) phosphorylation sites (e.g. Ser167; Campbell et al. 2001) or perhaps via augmented co-activator activity (e.g. AIB1; Font de Mora & Brown 2000). In this regard, p90\textsuperscript{RSK} and PI3K signaling were shown to be additional contributory elements, data in total suggesting that a network of kinases is recruited on estrogen withdrawal to enhance growth sensitivity to residual estrogens via improvement of ER\(\alpha\) transcriptional activation.

Adaptive hypersensitivity to the agonistic effects of tamoxifen and estrogen during antiestrogen treatment

The process of adaptive hypersensitivity, involving increases in growth factor signaling and its cross-talk with ER\(\alpha\), could feasibly be initiated not only by estrogen deprivation strategies, but also by interference with the estrogen/ER\(\alpha\) response pathway using antiestrogens. This would be manifested by enhanced growth sensitivity not only to estrogens, but also to the agonistic effects of selective ER modulators (SERMs) such as tamoxifen, thereby allowing the emergence of tamoxifen-resistant growth. In agreement with this concept, MCF-7 breast cancer xenografts, while initially inhibited by tamoxifen, are growth-stimulated by this antiestrogen in the long-term and are also estrogen hypersensitive (Gottardis & Jordan et al. 1988, Osborne et al. 1991). Furthermore, in several acquired tamoxifen-stimulated xenograft MCF-7 and T47D models, very prolonged tamoxifen exposure is associated not only with tamoxifen stimulation but also tumoricidal effects of estrogen, a feature described above with excessive estradiol exposure of long-term estrogen-deprived cells (Jordan et al. 2003). Growth-enhancing effects of tamoxifen appear also to be a feature of some breast cancers clinically, as evidenced by antiestrogen withdrawal responses reported in ~20% of patients (Clarke et al. 2003). Moreover, enhanced tumor sensitivity to tamoxifen agonism may explain the initial superiority of AIs in the clinic, since these agents more effectively eliminate estrogens while exerting no agonistic effects (Mouridsen et al. 2001). A growth contribution for tamoxifen-bound ER\(\alpha\) is similarly inferred by the frequency of second-line responses to either fulvestrant or AIs in the clinic (Howell et al. 1996, Buzdar et al. 1997).

Interestingly, the above described models for the development of adaptive estrogen hypersensitivity/supersensitivity do show many phenotypic similarities with those generated for the study of ER\(\alpha^+\) SERM resistance. Supportive data have been derived both from in vitro and in vivo models of acquired resistance (reviewed in Nicholson et al. 2004), and are now becoming to emerge from clinical sample profiling at relapse (Gee et al. 2002, Dowsett et al. 2003). Schiff et al. (2003, 2004) provide an overview of experimental tamoxifen resistant data that are particularly complementary to the above-described mechanisms for adaptive hypersensitivity during estrogen deprivation. The group describe that both non-genomic (membrane, predominantly at the caveolar domains) and genomic (nuclear) ER\(\alpha\) signaling influence, and are influenced by, growth factor signaling pathways resulting in enhanced agonistic activity of the tamoxifen–ER\(\alpha\) complex in tamoxifen-resistant cells. In such cells, membrane ER\(\alpha\) (perhaps even activated by tamoxifen; Figtree et al. 2000) rapidly activates cell surface tyrosine kinase receptors (such as EGFR (Razandi et al. 2003), HER2 (Chung et al. 2002) or IGF-1R (Kahlert et al. 2000)), leading to signaling through p21Ras/MAP kinase and PI3K/Akt. These protein kinases are able to phosphorylate nuclear ER\(\alpha\) in its AF-1 domain, as well as phosphorylate other key components of the transcriptional machinery, notably co-activators (Bunone et al. 1996, Font de Mora & Brown 2000, Campbell et al. 2001). This results in re-activation of transcription by the tamoxifen-occupied ER\(\alpha\) complex, increases in growth factor expression to re-enforce the signaling loop, and cell growth in the presence of antiestrogen (Schiff et al. 2003, 2004).

Clearly, this ER\(\alpha\)–growth factor signaling loop could be dominant under conditions where growth factor receptors were either elevated de novo or were promoted during antiestrogen challenge. HER2 overexpression could thus serve to augment this signaling loop de novo, markedly increasing MAP kinase activation and its target coactivator AIB1 to subsequently enhance nuclear ER\(\alpha\) signaling and exaggerate the agonistic activity of the tamoxifen–ER\(\alpha\) complex (Font de Mora & Brown 2000, Schiff et al. 2003, 2004). Certainly, in de novo tamoxifen-resistant MCF-7/HER2 cells, tamoxifen is agonistic yet these cells remain sensitive to estrogen deprivation (Massarweh et al. 2003). The concept is particularly exciting since clinical observations are indicating that HER2/AIB1-co-expressing tumors do fare poorly when treated with adjuvant tamoxifen (Osborne et al. 2003), while preliminary adjuvant and neoadjuvant studies have revealed that HER2+ or EGFR+ patients are relatively resistant to this antiestrogen de novo, yet retain sensitivity to the AIs, anastrozole or letrozole (Dowsett et al. 2001, 2003).
Ellis et al. (2001). Adaptive changes in growth factor signaling elements acquired during therapy may also promote tamoxifen agonistic activity. Adaptive increases in EGFR/HER2 signaling are certainly promoted in endocrine responsive cells during long-term tamoxifen challenge (Gee et al. 2003). Moreover, study of several in vivo and in vitro models has revealed that elevated growth factor signaling is subsequently maintained in the acquired resistant phenotype, is recruited to growth and, importantly, is highly interactive with ERα (reviewed in Nicholson et al. 2004). HER2 overexpression and its cross-talk with ERα appears to be a common feature of acquired tamoxifen-stimulated MCF-7 and T47D xenograft models (Schafer et al. 2003). In parallel, Berstein et al. (2003, 2004) have demonstrated that long-term tamoxifen treatment of MCF-7 breast cancer cells (LTTT) in a castrated in vivo xenograft model is associated with early adaptive increases in MAP kinase signaling. This event promotes hypersensitivity to the agonistic effects of this anti-hormone and subsequently to estradiol, where the critical role of such signaling is evidenced by marked growth inhibitory effects of a Ras antagonist.

In our own acquired tamoxifen-resistant in vitro model that emerged following prolonged exposure of MCF-7 to this antiestrogen, we have observed upwards of 20-fold increases in total and activated EGFR/HER2, with a corresponding increase in phosphorylation of MAP kinase, Akt and nuclear ERα on the AF-1 residues Ser118 and Ser167 (Nicholson et al. 2001b, 2003, 2004, Britton et al. 2002, 2003, Hutcheson et al. 2003, Knowlden et al. 2003a, Jordan et al. 2004). Importantly, we have established that phosphorylation of nuclear ERα and ERα transcription in these cells is under the control of EGFR, where EGF-like ligands further enhance these ERα events and exposure to the EGFR-selective tyrosine kinase inhibitor, gefitinib, antagonises basal and EGF-primed ERα phosphorylation and transcription (Britton et al. 2002, Hutcheson et al. 2003, Nicholson et al. 2003, 2004). This enhanced ERα activity appears to result in increased expression of the EGFR ligands TGFα and amphiregulin to generate a self-propagating autocrine growth regulatory loop in the tamoxifen-resistant cells, essentially converting a growth inhibitory drug into one that readily facilitates tumor cell growth (Hutcheson et al. 2003). Additionally, IGF-1R appears to contribute by activating the EGFR and thus enhancing its impact on ERα signaling. An IGF-II challenge not only leads to the expected increase in the phosphorylation of IGFR-1R, but also to a reproducible secondary increase in EGFR activation (Knowlden et al. 2003a, Hutcheson et al. 2004, Nicholson et al. 2004). These events, together with the phosphorylation of the ERα, can be blocked by inhibitors of IGF-1R signaling. The contribution of membrane-localized ERα to such signaling (if any) is currently being assessed by our group. Importantly, however, in this acquired tamoxifen-resistant model we have again noted that adaptive changes in growth factor pathways are associated both with tamoxifen agonism and enhanced sensitivity to estradiol. As may be seen from Fig. 1, following treatment of tamoxifen-resistant cells with fulvestrant to efficiently eliminate ERα, break the autocrine growth factor/ERα signaling loop and promote arrest of cells in Go/G1 (Fig. 1A), both estradiol and tamoxifen are able to induce substantial entry into the S-phase of the cell cycle (Fig. 1B) that translates out into

Figure 1 Regrowth of fulvestrant-treated tamoxifen-resistant cells in the presence of estradiol and tamoxifen. Tamoxifen-resistant MCF-7 cells were grown for 2 days in charcoal-stripped 5% FCS containing fulvestrant (10⁻⁷ M). The cells were then exposed to continuing fulvestrant (182), estradiol (E2; 10⁻⁷ M) or tamoxifen (10⁻⁷ M). At the time points indicated cells were subjected to FACS analysis and the proportion of cells in G0/G1 (A) and S-phase (B) of the cell cycle was recorded.
growth promotion of these cells. Our preliminary data indicate that activation of Akt and increases in cyclin E are components of this escape mechanism.

In total, the above data indicate that adaptive tamoxifen hypersensitivity, in common with adaptive estrogen hypersensitivity during estrogen deprivation, can result from multiple levels of interplay between growth factor signaling cascades and membrane and/or nuclear ERα. These interactions are likely to play a key synergistic role in the resultant acquired resistant growth. Frequent second-line responses to fulvestrant in these models, as well as to appropriate anti-growth-factor strategies, confirm the pivotal role played by ERα and growth factor signaling.

Increased growth factor signaling elements can interplay with ERα on estrogen deprivation of breast cancer cells in a manner not associated with adaptive hypersensitivity

It is clear from the above studies of Santen and Dowsett that their experimental conditions of estrogen deprivation are associated with substantial changes in sensitivity of the resultant resistant cells to estrogen, where the availability of exogenous growth factors appears to impact on the underlying molecular mechanism. In order to examine this further, our group has recently developed and characterized a further in vitro model of resistance to estrogen withdrawal (MCF-7X) where cells are routinely grown in charcoal-stripped, heat-inactivated serum and therefore subject to growth regulation primarily by autocrine growth factor pathways and residual steroid hormone. In our new model, although rapid tumor cell growth was established after a marked inhibitory phase in MCF-7 cells of approximately 6 months and the cells could be further stimulated by estrogens, we have failed to reveal any evidence of estrogen hypersensitivity. This is despite functional, phosphorylated ERα in MCF-7X cells and obvious growth inhibition by fulvestrant. Functional ERα and fulvestrant responses have similarly been reported in the MCF-7/S9 cell line that acquired resistance to serum-free culture conditions (Jensen et al. 2003). In addition, MCF-7X cells have readily detectable levels of activated MAP kinase and Akt. While we have failed, to date, to demonstrate a dominant role for MAP kinase signaling in these cells, we have observed that the PI3K inhibitor, LY294002, is effective, again suggesting an importance for the latter growth factor signaling element in promotion of estrogen deprivation.

Interestingly, in MCF-7X cells we have been unable to demonstrate a dominant growth factor-driven autocrine growth regulatory pathway, with for example, the cells being only weakly EGFR/HER2 positive and showing only extremely modest growth inhibition (~10%) with gefitinib or the humanized HER2-directed antibody, herceptin. The resistant cells are similarly only very weakly positive for IGF-1R and virtually unresponsive to IGF-1R ligands or inhibitors. We have now profiled the effects of multiple growth factors and medium growth supplements in our quest to reveal pathways that may be contributing to growth of MCF-7X cells. In this respect, the most obvious mitogenic factor that we have discovered for MCF-7X to date, other than estrogen, is the iron transporter transferrin (Li & Qian 2002). Of note, MCF-7X cells express transferrin and show markedly increased levels of its receptor. Provocatively, PI3K signaling has been shown to interact with transferrin/ transferrin receptor by regulating its trafficking (Jess et al. 1996). Hence our studies in MCF-7X may have revealed a previously unknown role for the PI3K enzyme in its promotion of estrogen-deprived states. Excitingly, both PI3K signaling and transferrin receptor have been linked with proliferative activity and disease progression in breast cancer samples, suggesting clinical relevance (Elliott et al. 1993, Perez-Tenorio et al. 2002). Detailed study of the networking between PI3K, transferrin and ERα in MCF-7X cells and its evolution during estrogen deprivation is obviously still required from our group. However, our data clearly demonstrate that ERα-dependent adaptive resistance to estrogen withdrawal can occur by mechanisms that do not always enhance sensitivity to residual estrogens or recruit classical growth factor receptors (notably EGFR/HER2/IGF-1R), but again involves PI3K as an essential signaling element.

Increased growth factor signaling elements can contribute to resistance to estrogen deprivation and antiestrogens in the presence of reduced or absent ERα expression/signaling in breast cancer cells

Several studies have shown that increased growth factor signaling is capable of promoting proliferation of breast cancer cells under conditions of markedly reduced ERα expression and/or signaling. Thus, we have demonstrated that endocrine responsive MCF-7 cells that are significantly growth inhibited by the ERα down-regulator, fulvestrant, may be stimulated to proliferate in the presence of this agent by exogenous heregulin β1 or IGF-1 (Fig. 2A) despite their ERα being barely detectable. Under such conditions, such growth factors appear able considerably to up-regulate MAP kinase and Akt phosphorylation in a manner that is proportional to their ability to induce cell proliferation, and the cells are refractory to any endocrine manipulation (Tang et al. 1996, Atlas et al. 2003) (Fig. 2C).
Similarly, following inhibition of the EGFR/ERα autocrine growth signaling loop in our acquired tamoxifen resistant sub-line using fulvestrant, re-activation of MAP kinase and Akt by exogenous heregulin β1 (Fig. 2D) appears sufficient to maintain high levels of cell growth in the presence of fulvestrant (Fig. 2B) where again the ERα protein is virtually absent and ERα/ERE activity is abrogated (Hutcheson et al. 2003, Nicholson et al. 2003, 2004). Since similar actions are produced by EGF and TGFα (Fig. 2B), extreme levels of growth factor signaling appear able to promote a ‘complete endocrine insensitive’ state.

Moreover, there is evidence that increased growth factor signaling can also act to reduce ERα expression and/or function. Growth factor dose–response and time-course studies suggest that while ERα activity and transcription can be activated as described in the section ‘Increased growth factor signaling elements contribute to adaptive hypersensitivity in breast cancer cells by interplay with ERα’ above, under certain extreme (as yet poorly defined) conditions blockade of these events is also a possible outcome. Since chronic ERα activation by estrogen can be associated with ERα downregulation (Pink & Jordan 1996), it is certainly feasible that constitutive/chronic activation of ERα by growth factor signaling could similarly result in a decline in ERα. Stoica and colleagues (Martin et al. 1995, Stoica et al. 1997, 2000a),b) systematically describe the capability of exogenous EGF, IGF-I, TGFβ and 12-o-tetradecanoyl pherbol-13-acetate (TPA) to down-regulate ERα mRNA and protein in MCF-7 cells via mechanisms involving increased growth factor signaling through EGFR, PI3K/Akt, phosphorylated (p) PKA and PKCs. In the case of prolonged treatment with the PKC activator TPA, ERα protein levels were reduced by 80% and there was a parallel decrease in ERα mRNA, ERα ligand binding,
ERα binding to DNA and ERα/ERE activity (Martin et al. 1995). TPA depletion of ERα appeared to be blocked by a PKC inhibitor (Saceda et al. 1991) and involved downstream activation of the AP-1 nuclear transcription factor complex (Doucas & Yaniv 1991). Further agents shown to depress ERα or its transcriptional activity include heregulin β1 (Mueller et al. 1995, Tang et al. 1996) and retinoids (Demirpence et al. 1992).

At its extremes, sustained growth factor suppression of ERα expression/function may even promote ERα negativity. This condition appears achievable in vitro during extended challenge with endocrine strategies. Such cells are subject to the influences of prolonged, elevated growth factor signaling that evolves during treatment. For example, we have recently noted that our fulvestrant-resistant cells, whose growth is promoted primarily via increased EGFR/MAP kinase signaling in the presence of minimal ERα levels (McClelland et al. 2001), have now developed a fully ERα-phenotype following extended culture in this pure antiestrogen (Fig. 3). Interestingly, while early passages of the fulvestrant-resistant sub-line were able to regain full ERα signaling on removal of fulvestrant (McClelland et al. 2001), our recent studies have revealed that the long-term resistant cells are unresponsive to estrogens on antiestrogen removal and significant levels of ERα are not recovered even after several months withdrawal. Prolonged estrogen deprivation has similarly been associated with evolution of an ERα− phenotype in T47D cells (Murphy et al. 1989, 1990, Pink et al. 1996), as exemplified by the T47D:C4 sub-line that expresses TGFrα and EGFR, as well as markedly elevated PKCα and AP-1 activity (Murphy et al. 1990, Tonetti et al. 2000, Chisamore et al. 2001).

Further supportive evidence for this transition can be drawn from several transfection studies where growth factor signaling elements introduced into ERα+ breast cancer cells can act to deplete ERα and impair ERα function. In our own study, we have shown that constitutive up-regulation of MEK1 in MCF-7 cells leads to a substantial increase in MAP kinase activation, decreased ERα levels and marked loss of progesterone receptor (PR) expression (R A McClelland, J M W Gee & R I Nicholson, unpublished observations). Similarly, El-Ashry and colleagues have noted precipitous falls in ERα mRNA and protein following transfection of constitutively active HER2, MEK (Δmek), Raf1 (Δraf) or ligand-stimulated EGFR into MCF-7 cells (Liu et al. 1995, Pietras et al. 1995, El-Ashry et al. 1997, Oh et al. 2001), with a parallel loss of estrogen-mediated induction of pS2 and PR and a marked suppression of activity of ERE-reporter gene constructs in transient transfection experiments that could not be overcome by estradiol treatment. Not surprisingly, the severe impact of elevated growth factor signaling on ERα expression and function also resulted in estrogen independence and antiestrogen resistance. Overexpression of Akt, PKCα and AP-1 components has similarly been linked with decreased ERα function, loss of ERα and endocrine-resistant states (Doucas & Yaniv 1991, Tzukerman et al. 1991, Smith et al. 1999).

In total, these data provide substantial evidence that while increased growth factor signaling can act to facilitate ERα function, at its extremes it may promote tumor cell growth in an apparently ERα-independent manner and may even encourage dislocation from ERα signaling and promote evolution of an ERα− phenotype. Interestingly, tumors with exaggerated EGFR signaling, whether ERα+ or ERα−, can be endocrine insensitive on presentation (Nicholson et al. 1994, 2004, Gee et al. 2001, 2002), and while not examined in any depth, such a phenotype may also contribute to the significant cohort of patients who fail to respond to second-line endocrine challenge (Gee et al. 2002). The conditions under which this transition to ERα+ complete endocrine insensitivity or ERα negativity occurs remains poorly defined; however, it is perhaps significant that our fulvestrant-resistant sub-line that shows markedly reduced ERα mRNA and ERα protein appears more susceptible to the generation...
Increased growth factor signalling

Endocrine responsive

ER+ ▸ ER+
Estrogen hypersensitive/supersensitive/responsive

ER+ ▫ ER+
Reduced estrogen sensitive

ER+ ▫ ER-
Estrogen unresponsive

Growth factor inhibitors

Figure 4 Model of growth factor-driven mechanisms of resistance to estrogen withdrawal. The black lines represent the experimental influences associated with increased growth factor signaling in breast cancer cells, while the red lines represent the potential reversal of such events by growth factor inhibitors.

of an ER− phenotype than either our tamoxifen or estrogen-withdrawn variants.

Such data raise the intriguing possibility that sustained growth factor signaling may underlie clinical ER− negativity. About 30% of tumors are ER− at diagnosis, while a proportion of ER+ tumors lack the receptor at the time of tamoxifen relapse in the adjuvant or metastatic setting. It is already established that the ER− gene is silenced via CpG island methylation or histone deacetylation in a proportion of clinical breast cancers that are ER− on presentation (Parl 2003). Indeed, Yang et al. (2001) have shown that ER− negativity in the MDA-MB-231 and -435 cell lines can be partially reversed by the DNA methyl transferase 1 (DNMT1) inhibitor, aza-2-deoxycytidine (5-aza-dC) and by the histone deacetylase (HDAC) inhibitor, trichostatin (TSA), an event that restores some estrogen responsive-ness from ERE reporter constructs and has obvious therapeutic implications for the future treatment of some ER− patients. However, if the above described association between chronic growth factor signaling and ER− loss occurs clinically, it may also be feasible to manipulate growth factor signaling to reinitiate the ER+ phenotype in a proportion of ER− patients. In support of this association, growth factor signaling elements do appear most extreme in ER− breast cancer, as evidenced by the established inverse association between ER− and EGFR in clinical disease (Nicholson et al. 1994) and across multiple ER− cell lines together with prominent ERK1/2 MAP kinase signaling (Biswas et al. 2000).

Therapeutic implications for the future treatment of resistant breast cancer

Many of the mechanisms that are emerging from laboratory studies, although complex, suggest that altered growth signaling cross-talking with ER+, as noted with antiestrogens, plays a central role in the development of acquired resistance to estrogen withdrawal. While detailed evaluation of these mechanisms is now clearly essential within clinical breast cancers from appropriate AI adjuvant trials and from examination of relapse material, model system data are already providing considerable rationale for designing anti-growth factor strategies, as well as improving anti-hormonal strategies, to treat or even prevent this disease state.

Given the concept of adaptive hypersensitivity that has arisen from the above models, one might expect that
tamoxifen has only limited value in acquired resistance to estrogen deprivation since this drug could be perceived as an agonist (although surprisingly, there is model system data to suggest some efficacy may still be apparent; Long et al. 2002). However, it is feasible that continued optimization and improvement of estrogen deprivation strategies to further deplete estrogen levels may prove a fruitful avenue (Lonning 2001, Santen et al. 2004). Similarly, use of alternative AIs may be relevant for the development of resistance to initial therapy if they more potently reduce estrogen levels, since a lack of complete AI cross-resistance between the competitive inhibitors and inactivators has been reported (Lonning 2001). A unifying feature of all the acquired resistant models appears to be the value of near-maximally depleting ERα protein. Hence, the use of the pure antiestrogen, fulvestrant, to abrogate ERα/growth factor cross-talk may also be rational in acquired resistance. This agent is certainly effective following patient relapse on tamoxifen (Howell et al. 1996, Pritchard 2003, Santen et al. 2004), and preliminary data from acquired letrozole-resistant xenografts generated from aromatase-transfected MCF-7 cells (MCF-7-Ca) also indicate fulvestrant sensitivity (Lon et al. 2002). Finally, since model system data reveal toxicity of estrogens at higher concentrations, patients becoming resistant to estrogen deprivation may benefit from estrogens administered at pharmacological doses (Lonning 2001, Jordan et al. 2003). Clearly, further research is needed to define the optimal type and sequence of endocrine agents in this setting (Pritchard 2003).

Excitingly, the above described model system and emerging clinical data also provide considerable impetus to consider anti-growth-factor therapies on the development of resistance to estrogen deprivation or indeed other endocrine strategies. Although it is currently unclear whether the transitions shown in Fig. 4 are linked or distinct, and what the determinants are for their establishment, it is certainly encouraging that our armoury of inhibitors for growth factor signaling elements is exponentially increasing and that many do appear to be tolerated in cancer patients (Johnston et al. 2003, Jones et al. 2004). In the future, therefore, it is highly probable that such inhibitors will be clinically examined in breast cancer as a novel means of (i) treating endocrine resistant and insensitive states, (ii) preventing/delaying emergence of endocrine resistance and (iii) regenerating endocrine responsiveness in de novo-resistant or acquired-resistant disease.

The successful application of several signal transduction inhibitors in the Santen and Dowsett adaptive hypersensitivity models and parallel acquired antiestrogen-resistant sub-lines are highly encouraging with regards to possible therapeutic strategies. Targeting growth factor receptor expression/activation (e.g. EGFR, IGF-1R, HER2), elements interactive with ERα (e.g. c-src kinase), or key downstream signaling elements (e.g. PI3K/AKT; Ras/Raf/MAP kinase) can clearly be growth inhibitory in these acquired-resistant models. Our own group has shown that blockade of EGFR signaling in tamoxifen- or fulvestrant-resistant cells with gefitinib inhibits cell growth, motility and invasiveness, with responses lasting up to 6 months before gefitinib resistance emerges (McClelland et al. 2001, Knowlden et al. 2003b, Nicholson et al. 2003, 2004). This compares favorably with the time taken to develop antiestrogen resistance in vitro (~3 months; Knowlden et al. 2003b). Within our new ERα+ dependent model of resistance to estrogen deprivation, sensitivity to PI3K blockade is still apparent, despite the cells being largely refractory to EGFR, HER2 or IGF-1R inhibition. Furthermore, given that multiple signaling elements are clearly synergistic in determining adaptive resistance to estrogen deprivation in several of the models, a particularly exciting approach may be to logically combine anti-growth factor strategies (e.g. PI3K/Akt and MAP kinase inhibitor combination). Alternatively, ‘dirty’ agents that hit multiple signaling pathways, such as the Hsp90 inhibitor, 17-allylamino-17-demethoxygeldanamycin (17AAG), known to promote degradation of multiple client proteins including tyrosine kinase receptors, signaling intermediates and steroid receptors and already demonstrated to be inhibitory in tamoxifen resistance, may prove valuable (Beliaeff et al. 2003).

There are also now several reports that logical combination of anti-hormonal and various anti-growth-factor strategies (e.g. anti-HER2, EGFR or Ras signaling) may be particularly powerful in preventing emergence of resistance, as well as restoring anti-hormone responsiveness in resistant cells (Wakeling et al. 2001, Johnston et al. 2003, Jones et al. 2004). Our own studies have demonstrated that when gefitinib is administered to anti-hormone-sensitive cells in combination with tamoxifen or fulvestrant (in anticipation of the development of EGFR signaling as a resistance mechanism), combination treatment promotes a much improved anti-proliferative and apoptotic effect than achieved with either drug alone (Gee et al. 2003, Nicholson et al. 2004). Indeed, apoptosis is so high using combination therapy that total cell kill is achieved and development of anti-hormone resistance is prevented (Gee et al. 2003). We hypothesize that since anti-hormones induce EGFR expression, early signaling from this receptor stimulates a survival pathway which reduces anti-hormone-mediated cell kill. Thus, the anti-hormone-treated cells, although initially growth inhibited, survive and eventually establish a resistant state driven by the EGFR pathway, an event abrogated by co-treatment with gefitinib (Gee et al. 2003). In a similar manner, we
have recently described that co-targeting of EGFR and IGF-1R in our acquired tamoxifen-resistant cells is superior to inhibition of either pathway alone, where IGFR-1R signaling clearly forms the survival and resistance mechanism recruited during treatment with gefitinib alone (Nicholson et al. 2004). Moreover, combination studies from Massarweh et al. (2002) have demonstrated that gefitinib delays resistance to estrogen deprivation and improves tamoxifen’s anti-tumor activity in their de novo tamoxifen-resistant HER-2 transfected MCF-7 cells.

This article has also presented the available clinical and experimental data implicating growth factor signaling in ERα+ de novo resistance. Increased HER2/EGFR signaling is a feature of ERα+ de novo tamoxifen-resistant clinical material, phenotypic data implying that anti-growth factor strategies would be worthy of consideration in such tumors. Interestingly, emerging clinical data indicate that such ERα+ patients still respond to estrogen deprivation. The underlying growth mechanism for such tumors may therefore involve elevated EGFR/HER2 signaling cross-talking with ERα, as described for acquired tamoxifen resistance. Addition of anti-HER2 or anti-EGFR strategies together with AIs could thus have potential to improve response and inhibit a proportion of such ERα+ de novo tamoxifen-resistant disease. Disappointingly, however, the recently published clinical trial of fulvestrant versus tamoxifen (Howell et al. 2004) shows that in the first-line setting tamoxifen is equivalent to fulvestrant, the data shedding doubt on the proposed ERα cross-talk with HER2/EGFR signaling in de novo resistance. There remains some concern as to whether an appropriate dose of fulvestrant was achieved in this trial, and certainly earlier studies with fulvestrant did not achieve complete ERα down-regulation, an event believed to discriminate its actions from SERMs. Nevertheless, continued study of appropriate de novo resistant models and further clinical trial material, notably AI-resistant samples, clearly remains essential if we are fully to understand de novo resistance. Indeed, diverse cellular mechanisms outside the remit of this article, including aberrations in cell cycle components and tumor suppressor genes, have all been linked previously with de novo resistance, data suggesting that growth regulation of this state is not simply an extrapolation of acquired resistance mechanisms.

Finally, experimental growth factor challenge and transfection studies suggest that growth factor signaling elements aberrantly expressed may be able to promote de novo resistance under conditions of reduced ERα, and even in an ERα-independent manner. Extreme or chronic, sustained growth factor signaling, arising during therapy or apparently de novo, can diminish ERα expression/function, dislocate cells from ERα signaling promoting endocrine insensitivity, and generate ER negativity in models. Treatment of these insensitive states with appropriate anti-growth factors may again be possible, while such agents may also reverse this transition and re-instate the anti-hormone responsive phenotype. Excitingly, in vitro blockade of constitutive EGFR, HER2, or MAP kinase with MEK inhibitors or dominant negative constructs can re-instate ERα expression in de novo-resistant cells (Oh et al. 2001), and preliminary studies indicate that reversion of the ERα− phenotype and re-instatement of endocrine responsiveness is initiated in a proportion of advanced HER2+ breast cancer patients by use of hereceptin to inhibit such growth factor signaling (Munzone et al. 2003).

This article, in total, establishes a proof of principle that blocking growth factor signaling cascades could be therapeutically effective (particularly in combination with anti-hormonal strategies) and it is certainly encouraging that preliminary data associated with the use of gefitinib in breast cancer patients resistant to tamoxifen are demonstrating clinical benefit, especially in ERα+ acquired resistant disease (Robertson et al. 2003). We eagerly anticipate the results of various ongoing and proposed trials examining the efficacy of a spectrum of such agents in de novo and acquired endocrine resistance, in particular those aiming to treat or prevent resistance to estrogen deprivation.

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