THERAPEUTIC RESISTANCE

Biological determinants of endocrine resistance in breast cancer

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Abstract | Endocrine therapies targeting oestrogen action (anti-oestrogens, such as tamoxifen, and aromatase inhibitors) decrease mortality from breast cancer, but their efficacy is limited by intrinsic and acquired therapeutic resistance. Candidate molecular biomarkers and gene expression signatures of tamoxifen response emphasize the importance of deregulation of proliferation and survival signalling in endocrine resistance. However, definition of the specific genetic lesions and molecular processes that determine clinical endocrine resistance is incomplete. The development of large-scale computational and genetic approaches offers the promise of identifying the mediators of endocrine resistance that may be exploited as potential therapeutic targets and biomarkers of response in the clinic.

Aromatase inhibitors
Drugs that function by blocking aromatase, the enzyme that converts androgens to oestrogens in tissues including the breast and adipose tissue. Examples include anastrazole, letrozole and exemestane.

ER-positive breast cancers
In current clinical practice, ER-positive breast cancers are those with immunohistochemically detectable ERα levels.

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The steroid hormone oestrogen is central to normal female physiology, reproduction and behaviour, through its effects on cellular processes including cell proliferation and cell survival. These effects are mediated by nuclear oestrogen receptors (ERα and ERβ; BOX 1). ERα is responsible for many of the effects of oestrogen on normal and cancerous breast tissue, through ligand-activated transcriptional regulation (genomic actions) and by acting as a component of membrane and cytoplasmic signalling cascades (non-genomic actions)[1] (FIG. 1).

Sustained exposure to endogenous or exogenous oestrogen is a well-established cause of breast cancer[2,3], underpinning the use of anti-oestrogens and aromatase inhibitors in breast cancer prevention[4–6]. At least 70% of breast cancers are classified as ER-positive breast cancers[7], and interfering with oestrogen action has been a mainstay of breast cancer treatment for more than a century. Early therapies included surgical removal of the ovaries, but the synthesis of competitive inhibitors of oestrogen–ER binding during the 1970s led to the first, and to date most successful, targeted cancer therapy: the selective oestrogen receptor modulator (SERM) tamoxifen[8]. Adjuvant therapy with tamoxifen almost halves the rate of disease recurrence and reduces the annual breast cancer death rate by one-third, making a significant contribution to the 25–30% decrease in breast cancer mortality in the past two decades[9]. Subsequently, other new, effective endocrine therapies have been developed that target oestrogen synthesis (such as aromatase inhibitors[10]) or ER signalling (such as other SERMs and 'pure' anti-oestrogens[11]).

One-third of women treated with tamoxifen for 5 years will have recurrent disease within 15 years[9], and so endocrine-resistant disease may represent up to one-quarter of all breast cancers. Therefore, two major challenges for the successful treatment of breast cancer are the development of more specific biomarkers that predict therapeutic response to endocrine therapy and the identification of new therapeutic targets for endocrine-resistant disease. This Review summarizes and evaluates the recent insights into the mechanisms of endocrine resistance that have been made through candidate gene approaches, as well as more global gene expression profiling and functional genetic screens. We necessarily focus on tamoxifen resistance, as the experience with this drug is more extensive and the clinical data more mature than for other drugs. Many of the broad concepts discussed will probably also apply to resistance to aromatase inhibitors and other anti-oestrogens, although the lack of clinical cross-resistance[10–12] indicates that some resistance mechanisms are independent.

Molecular mechanisms of resistance

The primary mechanism of *de novo* or intrinsic resistance to tamoxifen is lack of expression of ERα. Recently, a second intrinsic mechanism has been documented in which patients carrying inactive alleles of cytochrome P450 2D6 (*CYP2D6*) (approximately 8% of Caucasian women) fail to convert tamoxifen to its active metabolite, endoxifen, and are consequently less responsive to tamoxifen[13]. By contrast, a plethora of mechanisms

At a glance

- Endocrine therapies that target oestrogen action (anti-oestrogens and aromatase inhibitors) are widely used and successful breast cancer therapies, but many women treated with these therapies will relapse with endocrine-resistant disease.
- Mechanisms of endocrine resistance in oestrogen receptor (ER)-positive breast cancers include loss of ERα expression and expression of truncated isoforms of ERα and ERβ, post-translational modifications of ERα, increased AP1 activity and deregulation of ER co-activators, increased receptor tyrosine kinase signalling leading to the activation of the Erk and PI3K pathways, and deregulation of the cell cycle and apoptotic machinery.
- Gene expression signatures that are predictive of poor outcome in women treated with tamoxifen commonly contain ER target genes, as well as genes involved in proliferation, apoptosis, and invasion and metastasis. Many of these signatures are also predictive of outcome in women who have not been treated with tamoxifen and so are markers of intrinsic biology rather than specific to tamoxifen responsiveness.
- Gene expression signatures representing particular biological processes (for example, cell cycle progression, cell death and invasion) or pathways (for example, RB deregulation, MYC overexpression and E2f activation) can also predict outcome in women treated with tamoxifen and point towards possible mechanisms for endocrine resistance.
- Functional genetic screens have successfully identified several genes, the loss or overexpression of which can reduce anti-oestrogen sensitivity in cell lines and is associated with clinical endocrine resistance.
- Insights into the mechanisms of resistance have suggested possible therapeutic approaches for endocrine-resistant ER-positive breast cancer, for example tyrosine kinase inhibitors. Further potential therapeutic targets may emerge from combining large-scale genomic and transcriptomic data with large-scale functional analyses.

have been postulated to account for acquired resistance following prolonged exposure to tamoxifen, some of which may also account for intrinsic resistance in the clinic. Much of the published information on these potential molecular mechanisms has been derived from ERα-positive breast cancer cell lines and from variants of these cell lines selected for adaptation to sustained exposure to anti-oestrogens or withdrawal of oestrogen. Such models identify mechanisms that can induce tamoxifen resistance *in vitro* rather than those that actually mediate resistance in patients with breast cancer, and they have several other potential limitations. These include the degree to which the few ERα-positive breast cancer cell lines studied reflect the range of ER-positive phenotypes *in situ* and the absence of epithelial–stromal and tumour–host interactions that probably modulate sensitivity *in vivo*. Furthermore, the mechanisms that are responsible for the clinical observation that tamoxifen-resistant cancers often respond to second-line endocrine therapies[10–12] remain unclear.

Notwithstanding these potential limitations, studies of candidate genes involved in oestrogen signalling (FIG. 1) or anti-oestrogen regulation of cell proliferation and survival (FIG. 2) and more global unbiased approaches using cell line models have yielded important concepts and hypotheses that correlate with tamoxifen resistance in the clinic, and they have provided a basis for new therapeutic approaches. Deregulation of various aspects of oestrogen signalling is a common mechanism for resistance, but unrelated mechanisms that provide tumour cells with alternative proliferative and survival stimuli also confer resistance.

As detailed information on the biology of the many molecules implicated in tamoxifen resistance *in vitro* and the means by which they cause resistance is summarized in a series of excellent recent reviews[11,12,14–21], we focus here on recent developments that shed light on potential mechanisms of prognostic or predictive importance. As shown by the examples in TABLE 1, most of the molecules that modulate tamoxifen sensitivity in experimental models are correlated with disease outcome not only in women treated with tamoxifen but also in a wider population of patients with breast cancer. Furthermore, because adjuvant tamoxifen has been the 'therapy of choice' in ER-positive breast cancer for more than 25 years, most available patient cohorts do not allow the comparison of therapeutic responsiveness and outcome in well-matched control populations that differ only with respect to tamoxifen therapy. Therefore, it is difficult to distinguish specific differences in tumour reponse to tamoxifen from the broader effects of the underlying biology of the disease.

ER and co-regulators. Response to tamoxifen is rare in ER-negative breast cancer, and so ERα expression is currently the principal biomarker of response to endocrine therapy. Early studies implicated the loss of ERα expression or ERα mutations as potential mechanisms of acquired resistance. However, loss of ERα expression occurs in only a minority (15–20%) of resistant breast cancers[22] and <1% of ER-positive tumours have ERα mutations[14,20,23]. More recently, expression of a new truncated variant of ERα, ERα36, in the presence of full-length ERα has been associated with reduced responsiveness[24]. The development of antibodies that can distinguish between ERα, ERβ and naturally occurring ERβ variants (BOX 1) has led to the identification of responses in ERβ-positive but ERα-negative cancers and a potential role for the carboxy-terminally truncated variants of ERβ (ERβ2/cx and ERβ5) in tamoxifen responsiveness[25,26]. In addition, the oestrogen-related receptor ERRγ is overexpressed and mediates tamoxifen resistance in lobular invasive breast cancer models[27].

One mechanism by which ERα regulates gene expression is through protein–protein interactions with other transcription factors — for example, activator protein 1 (AP1), specificity protein 1 (SP1) and nuclear factor-κB (NF-κB) (FIG. 1). Increased AP1 and NF-κB transcriptional activity are also associated with endocrine resistance[28–30]. ERα function is regulated by post-translational modifications (phosphorylation, methylation and sumoylation) that influence interactions with other proteins, including transcriptional co-regulators[19] and cytoplasmic signalling molecules (FIG. 1). There is significant evidence to show that effects on these end points contribute to endocrine resistance[18,20,31]. Overexpression and increased phosphorylation of ERα co-activators, particularly nuclear receptor co-activator 3 (NCOA3; also known as AIB1 or SRC3), leads to constitutive ERα-mediated transcription, which confers resistance *in vitro* and in xenograft models[12,16] and is associated with reduced responsiveness to tamoxifen in patients[32]. Transient methylation of ERα at R260 by protein arginine *N*-methyltransferase 1 (PRMT1) results

Adjuvant therapy
A drug treatment (for example, chemotherapy or endocrine therapy) that is given after the primary therapy (for example, surgery and/or radiotherapy), with the aim of increasing the overall effectiveness of treatment.

SERMs
Drugs such as tamoxifen that bind the oestrogen receptor and thereby block the effects of oestrogen on tissues such as the breast but that function similarly to oestrogen in other tissues such as bone. Unlike oestrogen, these drugs are not steroidal in structure.

'Pure' anti-oestrogens
Drugs that bind the oestrogen receptor, thereby blocking the effect of oestrogen, but have no detectable oestrogen-like effects. Most have a steroidal structure.

Intrinsic resistance
The failure to respond to initial drug therapy.

Cytochrome P450 2D6 (*CYP2D6*)
A member of the large and diverse superfamily of cytochrome P450 enzymes. CYP2D6 catalyses the conversion of tamoxifen into its active metabolites, endoxifen and 4-hydroxytamoxifen. It is highly polymorphic, so its activity is variable between individuals.

Acquired resistance
In contrast to intrinsic resistance, an initial response to drug therapy followed by subsequent disease progression.

in the formation of cytoplasmic complexes that contain ERα, PI3K, the tyrosine kinase SRC and focal adhesion kinase (FAK; also known as PTK2) and that activate Akt (FIG. 1). However, it is not known whether this methylation event, which is frequent in breast cancer[33], is associated with the endocrine response. Another example is the ER co-activator PELP1, which in many breast cancers localizes to the cytoplasm, where it can confer tamoxifen resistance[31]. PELP1 functions as a scaffold that modulates ER interaction with SRC, leading to activation of SRC and the Erk family kinases and also promotes oestrogen activation of PI3K[31] (FIG. 1).

Receptor tyrosine kinase signalling. The bidirectional crosstalk between ER and receptor tyrosine kinase signalling is evidenced by the early observations of reciprocal expression of ER and members of the epidermal growth factor receptor (Egfr) family such as EGFR and ERBB2 (also known as HER2)[34]. Growth factors of the Egf and insulin-like growth factor (Igf) families can modulate tamoxifen sensitivity *in vitro*[35]; although breast cancer cells are quiescent and insensitive to growth factor stimulation following treatment with the pure anti-oestrogen ICI 182780 (fulvestrant), tamoxifen treatment does not lead to growth factor insensitivity[36]. This has focused attention on receptor tyrosine kinase expression and function as potential mediators of endocrine resistance. Increased expression of EGFR, ERBB2 and IGF1 receptor (IGF1R) can elicit tamoxifen resistance[37–40], as can activation of the components of their downstream signalling pathways, particularly the Erk and PI3K pathways[41–43]. In some cases, deregulation of these signalling pathways occurs as a result of genetic or epigenetic modifications, such as amplification of *ERBB2*, activating mutations in *PIK3CA*, which encodes a catalytic subunit of type I PI3Ks, and loss of heterozygosity or methylation of *PTEN*, a tumour suppressor that inhibits the PI3K pathway[20,21]. In other cases, however, deregulation of these pathways reflects aberrations in upstream regulators, such as the activation of Akt in association with the loss of *PTEN* expression or overexpression of *ERBB2* (REFS 20,21) and activation of IGF1R and ERBB3 following the loss of *PTEN*[40]. How these events mediate tamoxifen resistance has not been fully elucidated, but several potential contributing factors have been suggested (FIGS 1,3): decreased ERα expression mediated by ERK activation; loss of ER-mediated repression of EGFR and ERBB2 and consequent activation of mitogenic signalling cascades; ligand-independent activation of ER or its co-activators through phosphorylation; upregulation of key cell cycle regulators, for example MYC and the D-type and E-type cyclins, through constitutive activation of mitogenic signalling pathways; and the inhibition of apoptosis through constitutive activation of survival signalling.

Overexpression of ERBB2 is one of the best-characterized mechanisms of endocrine resistance[21]. Recent evidence implicates the loss of transcriptional repressors and amplification of *ERBB2* as mechanisms that are responsible for increased expression of this receptor. The X-linked tumour suppressor forkhead box P3 (FOXP3) and the zinc finger transcription factor GATA4 can repress *ERBB2* expression, even in a cell line with an approximately tenfold amplification of *ERBB2*, and their expression is negatively correlated with ERBB2 expression in breast cancer[44,45]. In addition, a recent pivotal study showed that ERα-mediated repression of *ERBB2* is dependent on competition between the paired-domain transcription factor PAX2 and the ERα co-activator NCOA3 for binding and regulation of *ERBB2* transcription and, in turn, tamoxifen responsiveness[46]. A direct relationship between FOXP3 or GATA4 expression and tamoxifen responsiveness has not been established. However, increased PAX2 expression and consequent repression of *ERBB2* was associated with increased survival following tamoxifen treatment, and loss of PAX2 expression in the presence of increased NCOA3 expression predicted a poor outcome[46], indicating that this mechanism is of direct clinical relevance.

Members of the Src family of tyrosine kinases, particularly SRC itself, and their downstream targets are also commonly overexpressed in breast cancer and have been implicated in resistance. The Src substrate BCAR1 (also known as CAS) is a focal adhesion adaptor protein that activates proliferative, survival and invasion pathways. It can induce tamoxifen resistance when overexpressed *in vitro*[47], and BCAR1-overexpressing breast cancers are less responsive to tamoxifen[48]. BCAR1 binds and activates SRC with consequent phosphorylation of the Src substrates EGFR and signal transducer and activator of transcription 5B (STAT5B) and effects on downstream signalling pathways[20]. However, recent data suggest that the ability of BCAR1 to confer anti-oestrogen resistance may not require interaction with SRC[49]. The putative guanine nucleotide-exchange factor BCAR3, which synergizes with BCAR1 to activate SRC[50], also causes tamoxifen resistance *in vitro*[51]. In addition, BCAR3 activates Rac and p21-activated kinase 1 (PAK1)[52]; the latter is itself implicated in tamoxifen resistance through ERα phosphorylation[53].

Box 1 | **Oestrogen receptors**

The oestrogen receptors ERα and ERβ are encoded by separate genes located on different chromosomes. They have a similar overall domain structure, consisting of a central DNA-binding domain flanked by two autonomous transcriptional activation domains, one of which, AF-2, is positioned in the ligand-binding domain and is ligand dependent (reviewed in REF. 11). The AF-2 domain also mediates interactions with co-activators that increase ER transcriptional activity[19,127]. Isoforms of both ERα and ERβ have been described, but the most relevant in the context of endocrine resistance are a truncated variant of ERα, ERα36 (REF. 24), and two isoforms of ERβ, ERβ2/cx and ERβ5, in which amino-terminal truncations remove some of the ligand-binding domain[26].

In the breast, ERα-positive epithelial cells located in the ductal lumen facilitate the development of a branched ductal tree, which functions as a scaffold for milk-producing alveoli. Not all luminal cells express ERα, but ERα-null mice develop only a rudimentary mammary ductal tree and are unable to lactate[128], indicating that the ERα-positive cells make an essential contribution to mammary development. By contrast, the mammary glands of ERβ-null mice develop normally[128]. When the two receptors are co-expressed in breast cancer cell lines, ERβ functions as an antagonist of ERα, impairing the ability of oestrogen to stimulate proliferation[129].

Neoadjuvant
A drug treatment that is given weeks to months before surgery, often to reduce the size of tumours before surgery.

Cyclin E1
Cyclin E1 and cyclin E2 are regulatory subunits of kinase complexes that contain CDK2 as their catalytic subunit and regulate the G1 to S phase cell cycle transition.

Cell cycle regulators. Data from experimental model systems, supported by clinical correlations, indicate that anti-oestrogens are both cytostatic and cytotoxic. Neoadjuvant endocrine therapy leads to decreased proliferation[54], and in cell culture anti-oestrogen treatment leads to a G1 phase-specific cell cycle arrest and a consequent reduction in growth rate[55]. Not unexpectedly, the molecules pivotal to the anti-oestrogen effects on cell cycle progression (FIG. 2a) have central roles in the control of G1 phase progression downstream of polypeptide growth factor mitogens, as well as oestrogen. Aberrant expression of several such oestrogen and anti-oestrogen targets confers resistance *in vitro* and is associated with reduced tamoxifen responsiveness in patients. Overexpression of MYC, cyclin E1, cyclin D1 or the cyclin D1 splice variant cyclin D1b, or the inactivation of the RB tumour suppressor — an important substrate for cyclin-dependent kinases (CDKs) that are active in G1 phase — and the decreased expression of the CDK inhibitors p21 or p27, results in decreased anti-oestrogen sensitivity *in vitro*[56–64]. MYC overexpression and consequent tamoxifen resistance is accompanied by transcriptional repression of *CDKN1A* (which encodes p21)[65], relieving the inhibitory effect of p21 on cyclin E1–CDK2 complexes. Cyclin D1 overexpression leads to an increased abundance of cyclin D1–CDK4 complexes (which indirectly activate cyclin E1–CDK2 (REF. 58) by sequestering p21 and p27) and to the activation of cyclin E2–CDK2 by increased transcription of *CCNE2* (which encodes cyclin E2)[66]. In addition to its cell cycle regulatory role, cyclin D1 interacts with several transcription factors, including ERα and STAT3 (REF. 67). Tamoxifen induces cyclin D1 binding to ERα at the expense of cyclin D1–STAT3 binding, activating both STAT3 and ERα — this is an additional mechanism by which cyclin D1 overexpression can affect the tumour's response to tamoxifen[68]. Therefore, MYC and cyclin D1 overexpression can potentially affect anti-oestrogen sensitivity at several levels.

Supporting the clinical relevance of the aberrant expression of these molecules, there is accumulating evidence that overexpression of MYC or cyclin D1 is associated with tamoxifen resistance in patients[69,70]. There is also more limited evidence for a relationship between the overexpression of cyclin E1, RB inactivation and reduced expression of p27 and clinical response[62,69–71]. In breast cancer, overexpression of MYC, cyclin D1 and cyclin E1 is at least two to three times more common than amplification of the corresponding genes[69], and RB inactivation is also more common than *RB1* deletion or mutation[62]. The reasons for this probably include the activation of upstream mitogenic signalling pathways and the deregulation of transcriptional regulators, including the E2f family. The gene encoding p27, *CDKN1B*, is rarely mutated or deleted in breast cancer, but p27 expression is frequently reduced by oncogenic activation of mitogenic signalling (for example, by ERBB2 overexpression or Src activation) and increased p27 degradation[71]. The microRNAs (miRNAs) miR-221 and miR-222 reduce p27 expression and confer resistance to tamoxifen, although the precise mechanism of resistance is unclear, as these miRNAs also reduce ERα expression and are overexpressed in ERBB2-overexpressing breast cancers[72,73]. There are conflicting data on the relationship between p21 expression and outcome in breast cancer[69], and the role of p21 in tamoxifen response in breast cancer has not been studied extensively, although the ERBB2 repressor FOXP3 is essential for p21 expression[74] and there is some evidence for p21 deregulation in cancers with ERBB2 overexpression or Akt activation[70]. Akt activation results in the mislocalization of p21 to the cytoplasm; predominantly cytoplasmic localization of p21 has been associated with poor response to tamoxifen[75].

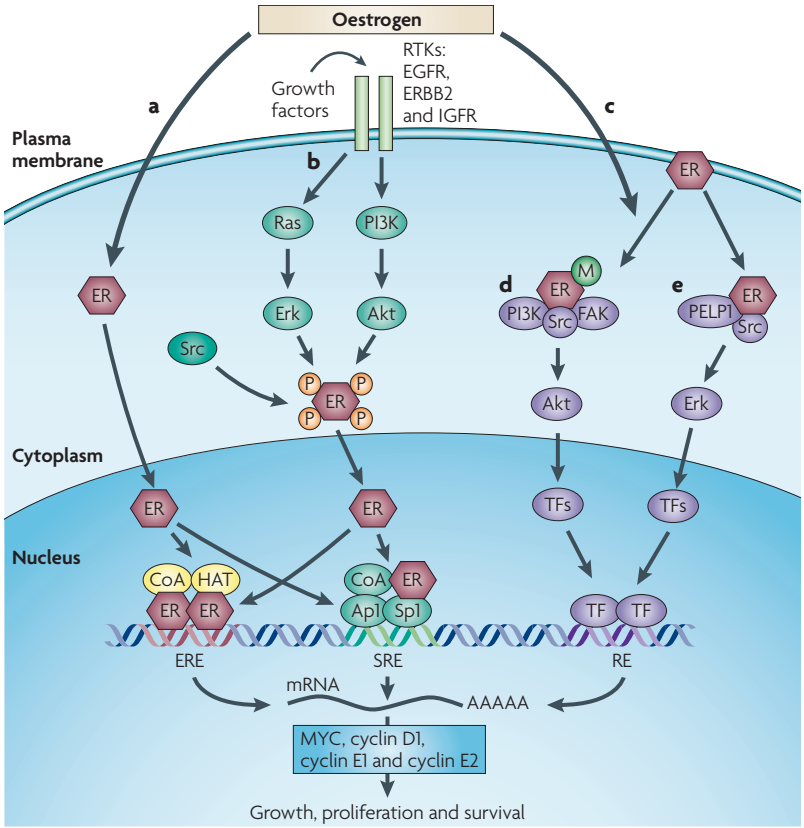

Figure 1 | **Oestrogen action at the molecular level.** Three distinct pathways of oestrogen regulation of gene expression (reviewed in REFS 1,11,31) are shown. First, in classic oestrogen signalling, ligand-bound oestrogen receptor (ER) activates gene expression — either through direct binding of dimeric ER to specific DNA response elements, EREs, in complexes including co-activators (CoAs) and histone acetyl transferases (HATs), or through protein–protein interactions with other transcription factors, particularly members of the activation protein 1 (Ap1) and specificity protein 1 (Sp1) families — to facilitate binding to serum response elements (SREs) and activation of transcription (**a**). Second, ER can also be activated as a consequence of signalling events downstream of receptor tyrosine kinases (RTKs) such as the epidermal growth factor receptor (EGFR), ERBB2 (also known as HER2) and the insulin-like growth factor receptor (IGFR) (**b**). Phosphorylation (P) by the Erk or Akt serine/threonine kinases leads to ligand-independent activation of the ER. Third, signalling can be mediated through non-genomic mechanisms by ER that is localized at the cell membrane or in the cytoplasm (**c**). Ligand binding induces the assembly of functional protein complexes that involve other signalling molecules and that activate intracellular signalling cascades, resulting in transcription factor (TF) activation. Two recently characterized mechanisms that ultimately activate transcription independently of ER binding to DNA are illustrated: ligand-induced methylation (M) of ER and formation of an ER–PI3K–Src–focal adhesion kinase (FAK) complex that activates Akt (**d**), and activation of Erk by ER–Src–PELP1 complexes (**e**).

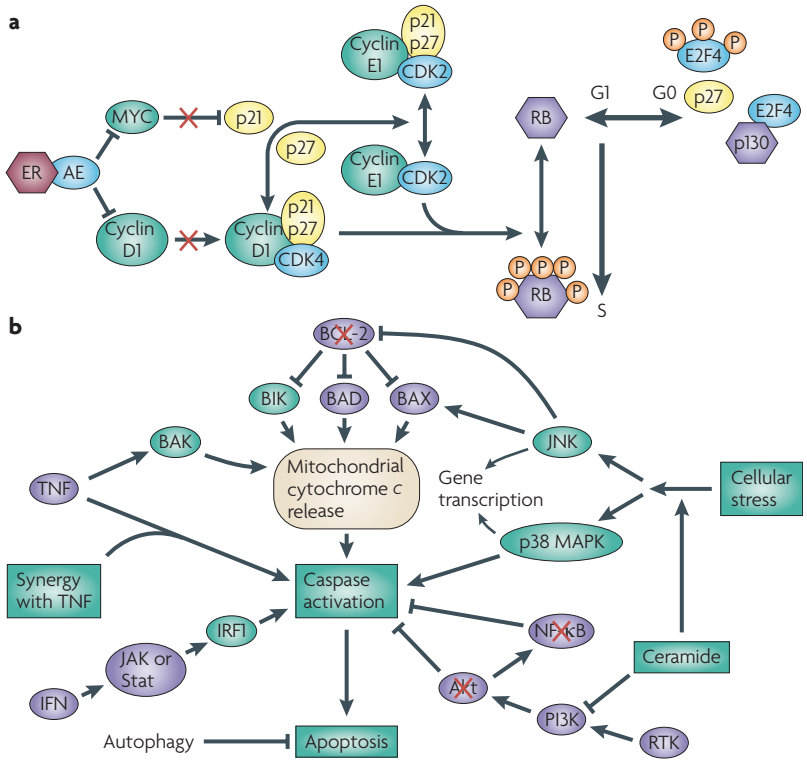

Figure 2 | **Anti-oestrogen action on the cell cycle and apoptotic pathways.** **a** | Anti-oestrogen (AE) treatment of cultured breast cancer cells leads to oestrogen receptor (ER) binding and subsequent rapid decreases in the expression of MYC, followed by decreased expression of cyclin D1. Downregulation of MYC leads to de-repression of *CDKN1A* (which encodes p21) transcription. In addition, because cyclin D1–cyclin-dependent kinase 4 (CDK4) complexes function as a cellular 'sink' for the CDK inhibitors p21 and p27, the reduction in cyclin D1–CDK4 abundance makes p21 and p27 available for cyclin E1–CDK2 binding, and so indirectly contributes to the inhibition of cyclin E1–CDK2 activity. The decrease in activity of both CDK2 and CDK4 prevents RB phosphorylation (P) and therefore impedes transition into S phase. Treatment with the pure anti-oestrogen ICI 182780, but not tamoxifen, leads to an increase in the expression of p27 and molecular markers that are characteristic of quiescence (G0), that is, the formation of p130–E2F4 complexes and the accumulation of hyperphosphorylated E2F4. These effects are reviewed in REF. 55. **b** | Proteins and processes that are upregulated during anti-oestrogen-induced apoptosis are indicated in green (reviewed in REF. 76); red crosses indicate proteins that are downregulated. Apoptotic concentrations of tamoxifen elicit caspase activation downstream of responses such as activation of the stress kinases Jun N-terminal kinase (JNK) and p38 MAPK, activation of the intracellular second messenger ceramide, transcriptional downregulation of anti-apoptotic molecules including BCL-2, and upregulation of pro-apoptotic molecules such as IRF1, BIK and possibly BAK. In addition, anti-oestrogens have effects on the interferon (IFN) and nuclear factor-κB (NF-κB) pathways, and on survival signalling through Akt downstream of receptor tyrosine kinases (RTKs), as well as synergistic effects on tumour necrosis factor (TNF)-mediated apoptosis. These pro-apoptotic effects of tamoxifen are opposed by autophagy[77]. JAK, janus kinase; Stat, signal transducer and transcription activator.

Cyclin D1
The regulatory subunit of a kinase complex that functions as a growth factor sensor to regulate G1 phase cell cycle progression. The catalytic subunits of cyclin D1-dependent kinases are CDK4 and CDK6.

Cell survival signalling and apoptosis. Treatment with high (micromolar) concentrations of anti-oestrogen, oestrogen withdrawal (mimicking the effects of aromatase inhibitors) or aromatase inhibitor treatment of cells transfected with aromatase leads to the activation of the cellular stress response and apoptosis in breast cancer cells[17,76]. The molecular mechanisms are not well defined, but several molecular consequences that promote apoptosis have been documented, including the regulation of Bcl-2 family members and increases in the apoptotic second messenger ceramide[17,76] (FIG. 2b). Crosstalk between the apoptotic effects of anti-oestrogens and the tumour necrosis factor (TNF) pathway, as well as anti-oestrogen effects on survival signalling through the PI3K–Akt, NF-κB and interferon pathways, is also likely to contribute to anti-oestrogen-mediated apoptosis[17] (FIG. 2b). Finally, intriguing recent observations indicate that autophagy is a mechanism of cell survival in breast cancer cells that are resistant to apoptotic concentrations of tamoxifen[77].

It has been difficult to establish the role of apoptosis in the clinical setting. Neoadjuvant studies have yielded conflicting data and have been limited by small patient numbers and the methodological challenges of measuring apoptosis *in vivo*[78]. Nevertheless, many signatures of response to endocrine therapy include genes with roles in apoptosis, as discussed below. As tumour growth reflects the balance between cell proliferation and cell death, disruption of this balance by effects on survival signalling and apoptosis are expected to affect clinical response. There is accumulating evidence for the increased expression of anti-apoptotic molecules, for example BCL-2 and BCL-X_L, and decreased expression of pro-apoptotic molecules, for example BAK, BIK and caspase 9, in attenuated responses to tamoxifen[17]. Although many of these responses are probably consequences of the activation of survival signalling through the PI3K–Akt pathway, as a consequence of overexpression of receptor tyrosine kinases and increased 'non-genomic' signalling from cytoplasmic ER, other pathways have been documented. For example, increased DNA-binding and transcriptional activity of NF-κB are features of tamoxifen-resistant cells[30], and tamoxifen sensitivity can be restored by parthenolide, a specific NF-κB inhibitor[30,79]. Tamoxifen insensitivity *in vitro* is also associated with the downregulation of IRF1, an interferon-responsive putative tumour suppressor that binds NF-κB and is essential for apoptosis. Furthermore, overexpression of a splice variant of human X-box-binding protein 1 (XBP1), a transcription factor that controls the unfolded protein response, is also associated with tamoxifen resistance *in vitro* and poor survival in patients with breast cancer treated with tamoxifen[80–82]. NF-κB, XBP1 and IRF1 expression are correlated in patients with breast cancer[83], which may indicate that these molecules function in a common pathway[14].

Signatures of tamoxifen responsiveness

The advent of genome-wide gene expression analysis allowed clinical material from patients of known responsiveness to tamoxifen to be used as a means of gaining broad insights into the potential mechanisms of endocrine resistance. It also helped in the development of clinically relevant markers of response and potential mechanisms of resistance. This is not without its own limitations, for example the difficulty of obtaining tumour tissue at the time when resistance has developed, rather than before therapy.

Selection on the basis of disease outcome. The identification of women who are unlikely to respond to endocrine therapy, but who may benefit from chemotherapy, is a

Table 1 | **Pathways associated with tamoxifen resistance *in vitro***

Pathway	Molecular aberration	Clinical correlates	
		Poor outcome*	Tamoxifen resistance‡
ER signalling			
ERs and ERRs	ERα loss (methylation)	Yes[14]	Yes[14]
	ERα36 (truncated variant)	No[24]	Yes[24]
	ERα phosphorylation	Yes[130]	Yes[130]
	ERα methylation (by PRMT1)	ND	ND
	ERβ, ERβ2/cx and ERβ5	Yes[23,25]	Yes[23,25]
	ERRγ	ND	ND
ER-associated transcription factors and co-activators	AP1 overexpression	Yes[28–30]	Yes[28–30]
	NF-κB activation	Yes[131]	Yes[131]
	NCOA1 overexpression	Yes[132]	Yes[132]
	NCOA3 amplification	Yes[32,133]	Yes[32,132]
	PELP1	ND	ND
	CBP and p300 overexpression	Yes[134]	Yes[134]
Growth factor receptors and cytoplasmic signalling			
Receptors	EGFR overexpression and *ERBB2* amplification	Yes[21,135]	Yes[21,135]
	PAX2 loss leading to ERBB2 de-repression	Yes[46]	Yes[46]
	IGF1R overexpression	Yes[136]	ND
	FGFR overexpression	ND	Yes (only FGFR4)[137]
MAPK signalling	Mek and Erk activation	Yes[138]	Yes[138]
	CDK10 methylation	ND	Yes[114]
PI3K signalling	Akt activation and overexpression	Yes[139,140]	Yes[139,140]
	PTEN loss	Yes[141]	Yes[141]
SRC and SRC-interacting proteins	SRC activation	ND	ND
	BCAR1	No[111]	Yes[48]
	BCAR3 overexpression	No[111]	No — associated with favourable response[111]

pressing clinical need that has driven much of the work aimed at identifying clinically useful markers of tamoxifen response. Gene selection on the basis of correlations between expression and patient outcome, with the goal of identifying a minimum gene set that retains robust predictive ability in women treated with tamoxifen, has led to the development of several gene signatures (TABLE 2), some of which are the subject of testing in prospective clinical trials (reviewed in REF. 84). Other signatures derived using a broader group of patients with breast cancer can also distinguish prognostic groups within ER-positive cancers treated with tamoxifen (TABLE 2).

Consistent with the idea that deregulation of ER signalling and upregulation of alternative mitogenic pathways is a common mechanism of resistance, essentially all these signatures contain a substantial proportion of genes that are ER targets, are involved in ER action or have roles in cell proliferation and survival (TABLE 2). Genes involved in apoptosis, invasion and cell motility are also consistently included[85] (TABLE 2). Perhaps surprisingly, *MYC*, *CCND1* (which encodes cyclin D1) and *RB1* are rarely included in the signatures, despite the predominance of proliferation markers and the importance of these molecules in anti-oestrogen effects on proliferation. However, signatures predominantly composed of MYC-responsive or RB- and E2f-responsive genes are associated with poor outcome in women treated with tamoxifen[62,86], suggesting that the absence of these genes reflects the limitations of assessing their activity on the basis of their expression alone.

Because of their shared biological features, many of the signatures selected on the basis of correlations with outcome are largely concordant, both with each other and with more generally derived prognostic signatures that have not been tested for association with response to tamoxifen, for example the 'Mammaprint' signature[87–89]. In a recent meta-analysis that compared nine prognostic signatures, the subset of genes related to proliferation within each signature was at least as good a predictor as the whole signature[90]. However, the remaining genes were also often significantly correlated with outcome[90], indicating that biological processes other than proliferation may also be important. These probably include apoptosis and invasion or metastasis, as signatures predominantly composed of genes with roles in these cellular processes are predictive of poor outcome after tamoxifen treatment[86,91,92].

Bcl-2 family
A protein family of up to 25 members that are classified according to their structure and function as anti-apoptotic (BCL2-like) or pro-apoptotic (multidomain BAX-like and 'BH3-only') proteins.

Autophagy
A cellular response in which the cell metabolizes its own contents and organelles to maintain energy production, often in response to stressful stimuli. Although such a process can eventually result in cell death, it can also be used to maintain cell survival.

Table 1 (cont.) | **Pathways associated with tamoxifen resistance *in vitro***

Pathway	Molecular aberration	Clinical correlates	
		Poor outcome*	Tamoxifen resistance‡
Cell cycle			
Cyclins	Cyclin D1 amplification or overexpression	Yes[69]	Yes[69]
	Cyclin D1b overexpression	Yes[142]	ND
	Cyclin E1 overexpression	Yes[69]	Yes[143]
MYC	MYC amplification and overexpression	Yes[69,144]	Yes[69]
CDK inhibitors	Cytoplasmic p21 expression	ND	Yes[75]
	Low p27 expression	Yes[71,145]	Yes[71,145]
RB	RB inactivation	ND	Yes[62]
Apoptosis and cell survival signalling			
Bcl-2 family members	Low BCL-2 expression	Yes[146]	Yes[147]
	BIK	ND	ND
	Low BAD expression	Yes[148]	Yes[148]
Survival signalling	(IRF1)	ND	ND
	XBP1 (spliced variant)	ND	Yes[82]

*Yes indicates poor outcome in women with breast cancer, whether or not treated with tamoxifen.‡Yes indicates poor outcome in women treated with tamoxifen. AP1, activator protein 1; CBP, CREB-binding protein; CDK, cyclin-dependent kinase; EGFR, epidermal growth factor receptor; ER, oestrogen receptor; ERRγ, oestrogen-related receptor-γ; FGFR, fibroblast growth factor receptor; IGF1R, insulin-like growth factor receptor; IRF1, interferon regulatory factor 1; ND, not done; NF-κB, nuclear factor-κB; NCOA, nuclear receptor co-activator; PRMT1, protein arginine *N*-methyltransferase 1; XBP1, X-box-binding protein 1.

Biology as a selection criterion. Biomarkers of therapeutic response are expected to include genes that are mechanistically involved in conferring drug resistance, but signatures of tamoxifen response derived solely from clinical outcome data have provided only limited mechanistic insights. There is little overlap between their component genes and, consequently, there are few obvious starting points for functional studies. Potential contributing factors include dependence of the signature composition on the patient set used to derive it, and minimization of the signature size to increase clinical practicality, with the result that multiple non-overlapping signatures of equal predictive power could potentially be derived from the same analysis[93]. Therefore, any single prognostic or predictive signature contains a somewhat arbitrary set of genes, and this selectivity can confound attempts to understand the underlying biology.

A potentially more mechanistically revealing approach is the inclusion of facets of the underlying biology in the selection criteria for genes that constitute a signature. Because of the importance of oestrogen action in breast cancer biology and the evidence for deregulation of oestrogen signalling as a major mechanism of resistance, oestrogen-regulated genes are common starting points for the derivation of signatures that are predictive of outcome in women treated with tamoxifen[86,94–96] (TABLE 2). The progesterone receptor (PR) is a well-established marker of response to endocrine therapy[97]; PR expression occurs rarely in the absence of functional ER, implying that PR-positive cancers, and cancers expressing high levels of other oestrogen-induced genes, are likely to be more dependent on ER signalling. However, although a signature of oestrogen-induced genes selected on the basis of co-expression with PR is correlated with longer survival in tamoxifen-treated women[96], many oestrogen-induced genes are correlated with poor outcome[86,94,95]. It is clear that oestrogen-regulated genes are not homogeneous but are instead regulated by different mechanisms (for example, direct ERα–DNA binding at oestrogen response elements compared with indirect ERα tethering to DNA through interactions with other transcription factors (FIG. 1)) and can be divided into biologically distinct subsets[86,91] that may have independent relationships with response to therapy.

Intrinsic biology or altered response? An important clinical question concerns the degree to which the correlation between individual signatures and outcome in women treated with tamoxifen is a consequence of response to therapy rather than inherently aggressive or indolent biology. As with individual candidate genes (TABLE 1), many outcome signatures, even those derived specifically from women treated with tamoxifen, are correlated with outcome irrespective of treatment (TABLE 2) and may therefore reflect the underlying biology as well as, or instead of, response to treatment. Differential sensitivity to endocrine therapy is observed in the different subtypes of ER-positive breast cancer that are defined by common patterns of gene expression[98,99]. Of these, the best characterized are the luminal A and B subtypes. The luminal A subtype has higher ER expression and lower proliferation indices than the remaining luminal cancers have, and a better outcome independent of tamoxifen therapy[90,98–100]. The subtypes are characterized by different patterns and degrees of copy number abnormalities[101], indicating that at least some differences in clinical

Unfolded protein response
A cellular response to stress that senses the misfolding of proteins in the endoplasmic reticulum. It activates a series of pathways that help the cells survive proteotoxicity that is caused by unfolded proteins or activate mechanisms of cell death.

Concordant
Clinical biomarkers and signatures are concordant if they classify the same patients as 'high risk'.

Multivariate analysis
A statistical analysis of the relationship between multiple parameters (variables) to identify those that have a dominant effect on outcome (termed independent predictors of outcome) and those that are dependant or redundant.

Biological concepts analysis
A bioinformatic approach in which related information is grouped together into a 'biological concept', and associations between different 'concepts' are sought.

response to tamoxifen may arise through differences in intrinsic tumour biology rather than through differences in tamoxifen response *per se*.

Differences in response also arise from differences in the rate of cell proliferation. Although proliferation is reduced in almost all ER-positive breast cancers following neoadjuvant endocrine therapy, the level of proliferation following short-term therapy is a better predictor of outcome than its pretreatment level or the magnitude of the decrease in proliferation following treatment[102,103]. This suggests that poor treatment response does not result from intrinsically high proliferation but rather from an ability to maintain high levels of proliferation in the presence of tamoxifen. Increased proliferation that occurs as a consequence of deregulated mitogenic signalling pathways may be more readily inhibited by tamoxifen than is increased proliferation that results from genetic or epigenetic events targeting individual genes directly involved in cell cycle progression (for example, *MYC* or *CCND1* amplification). In addition, the effects of the deregulation of cell cycle regulatory genes are likely to be gene specific. Consequently, to better understand the mechanisms of tamoxifen resistance it might be informative to distinguish different mechanisms of deregulation of proliferation or different types of proliferative defects.

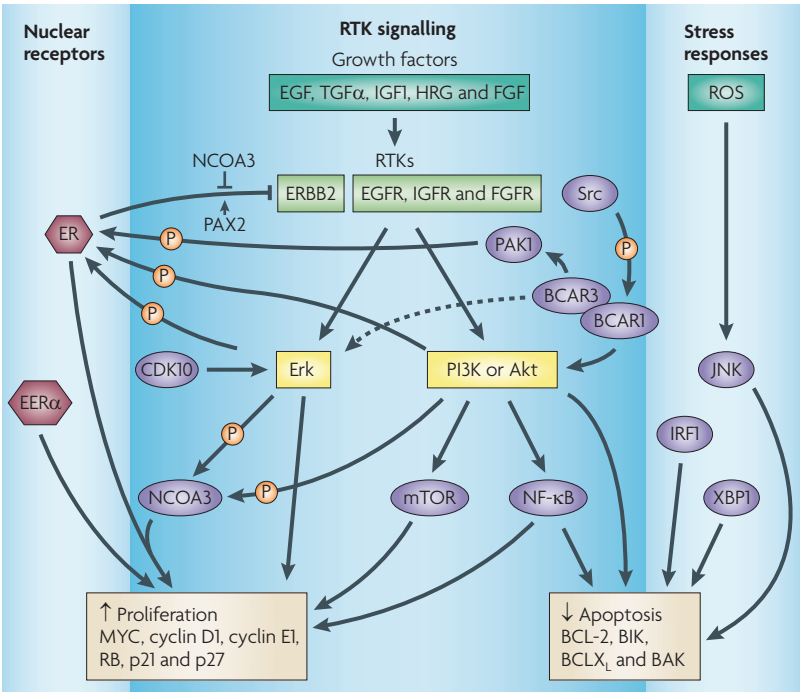

Figure 3 | **Molecular mechanisms of endocrine resistance.** Acquired tamoxifen resistance in breast cancer cells is mediated by either modulation of the oestrogen receptor (ER) pathway or aberrant or compensatory cellular signalling pathways controlled by growth factor receptors that negate the anti-proliferative and pro-apoptotic effects of tamoxifen[11,12,14–21]. In tamoxifen-resistant cells, these end points are regulated by nuclear receptors (ER and EERα) and their co-activators, for example nuclear receptor co-activator 3 (NCOA3; also known as AIB1 or SRC3); receptor tyrosine kinases (RTKs), including the epidermal growth factor receptor (EGFR), ERBB2, insulin-like growth factor receptor (IGFR) and fibroblast growth factor receptor (FGFR); RTK ligands, including transforming growth factor-α (TGFα) and heregulin (HRG), and downstream signalling pathways; and cellular stress responses, including those downstream of reactive oxygen species (ROS). CDK10, cyclin-dependent kinase 10; JNK, Jun N-terminal kinase; P, phosphorylation; PAK1, p21-activated kinase 1; PAX2, paired box 2 transcription factor; NF-κB, nuclear factor-κB; XBP1, X-box-binding protein 1.

Insights from computational approaches

Dissection of biological processes. A limitation of many clustering algorithms used to derive gene signatures is their dependence on global behaviour across the entire test set of profiles, at the expense of identifying more specific features that may be strongly related to only a small group of patients or conditions. In addition, an individual gene is often allocated to a particular cluster, although genes commonly contribute to different biological processes or are regulated by multiple stimuli. However, some algorithms do allow the identification of partially overlapping gene sets that show coordinate expression in only particular contexts, and so these tend to be functionally coherent. One such algorithm yielded eight biologically consistent gene set 'modules' in breast cancer expression profiles, including one module that was largely composed of genes with roles in apoptosis[91]. These genes were downregulated when MCF-7 breast cancer cells were treated with tamoxifen, suggesting that their expression may reflect active ER signalling[91]. Consistent with this idea, high expression of this module was associated with longer survival in several patient populations, including in women treated with tamoxifen.

A complementary approach is to identify biologically coherent signatures on the basis of a candidate gene approach or a functional annotation. For example, the high expression of a gene signature consisting of genes that are differentially expressed following RB loss, or the expression of constitutively active (growth-inhibitory) RB mutants in rodent fibroblasts, was associated with shorter disease-free survival in women treated with tamoxifen[62]. In another study, a combination of gene ontology and pathway analysis that used a curated database of functional annotations led to the subdivision of oestrogen-regulated genes into networks that represented specific biological processes, for example two distinct aspects of cell proliferation (cell cycle progression and increased cell size (cell growth)), apoptosis and survival signalling, and transcriptional regulation[86]. The networks representing cell proliferation and apoptosis were predictive of poor outcome in women treated with tamoxifen[86]. Previous analyses had pointed to both proliferation and apoptosis genes as potential markers of outcome in women treated with tamoxifen. This study provides evidence that these may identify distinct subpopulations with different mechanisms of resistance, as the cell proliferation-related signatures and the apoptosis signature were independent predictors of outcome in multivariate analysis[86].

Molecular mechanisms. Although data from large-scale genomic studies of breast cancer are increasingly available, few studies have adopted global approaches to identify specific molecular aberrations that might be associated with response to endocrine therapy. However, a global biological concept analysis identified associations

Table 2 | **Signatures predicting response to endocrine therapies**

Signature	Selection criteria	Clinical correlates		Biological processes
		Poor outcome*	Tamoxifen resistance‡	
Breast cancer subtypes[98,100]	Hierarchical clustering of breast cancers	Yes	Yes (luminal A compared with luminal B)	Proliferation (luminal A compared with luminal B)
HOXB13/IL17RB expression ratio[149]	Differential expression in recurrent compared with non-recurrent breast cancers treated with adjuvant tamoxifen; association with recurrence	Yes	Yes	ER targets and invasion (HOXB13)
21-gene signature:16 cancer-related and 5 controls (Oncotype Dx)[150]	250 manually selected genes tested for association with outcome following adjuvant tamoxifen	Yes	Yes	ER targets, proliferation[85], apoptosis[85], invasion and motility[85], signalling[85] and *ERBB2* amplification
81-gene discriminatory signature and 44-gene predictive signature[151]	Differential expression in progressive disease compared with objective response in recurrent disease treated with tamoxifen	No[89]	Yes	ER action and ER targets, apoptosis, extracellular matrix formation, immune response and 17q21–22 amplification
76-gene signature[152]	Correlation with outcome in breast cancer	Yes	Possibly	Proliferation and cell cycle[85], and apoptosis[85]
REF. 94	Oestrogen-induced genes used to cluster ER-positive breast cancer: groups have differing outcomes	Yes	Yes	ER targets, proliferation and cell cycle, and apoptosis
Genomic grade signature[153]	Distinguishes grade 1 and grade 3 breast cancer	Yes	Yes[88]	Proliferation and cell cycle[85], apoptosis[85], motility[85] and immune response[85]
'TuM1' 33 genes[91]	Modular analysis linked to biological function	No	Yes	Apoptosis
59-gene RB-deficiency signature[62]	Genes deregulated by *RB1* loss or repressed by RB activation	ND	Yes	Proliferation and E2f targets
36-gene signature[154]	Segregation of relapse and relapse-free breast cancers treated with adjuvant tamoxifen	ND	Yes	ER action and ER targets, proliferation, apoptosis, cell survival, adhesion and motility, signalling, metabolism and immune response
REFS 155,156	Segregation of responsive and non-responsive breast cancers treated with neoadjuvant aromatase inhibitor with or without tamoxifen: oestrogen-responsive gene subset identified	ND	Yes	ER targets, cell cycle, cell movement, apoptosis, and TGFβ and ERBB2 signalling
36 genes[96]	Oestrogen-induced, underexpressed in ER-negative breast cancer and correlated with PR	No	Yes	ER targets
181 genes in 13 biological clusters[157]	Clusters of co-expressed genes in breast cancers treated with adjuvant tamoxifen; 13 were combined to predict outcome after adjuvant tamoxifen	ND	Yes — adjuvant tamoxifen; no — tamoxifen at relapse except for one cluster alone	Cell cycle and proliferation, apoptosis, motility, and signalling of TGFβ, EGF, IGF and PDGF
256 genes in 4 signatures[86]	Functional annotation of oestrogen-regulated genes	ND	Yes	ER targets, Myc targets, cell cycle, proliferation, cell growth, apoptosis, survival signalling, and invasion and motility
47 genes[158]	Differential expression at the time of progression on tamoxifen therapy compared with non-recurrent cancers	ND	Yes — some individual genes	ER targets, immune response, transcriptional regulation, proliferation, invasion and adhesion, and apoptosis

*Yes indicates poor outcome in women with breast cancer, whether or not treated with tamoxifen. ‡Yes indicates poor outcome in women treated with tamoxifen. EGF, epidermal growth factor; ER, oestrogen receptor; HOXB13, homeobox B13; IGF, insulin-like growth factor; IL17RB, interleukin 17 receptor B; ND, not done; PDGF, platelet-derived growth factor; PR, progesterone receptor; TGFβ, transforming growth factor-β.

between a signature of early relapse in ER-positive breast cancer and the activation of MYC- and E2f-responsive pathways, together with chromosomal aberrations at loci including 8q24 (the location of the *MYC* gene)[104]. The E2f and MYC biological concepts seemed to make independent contributions to the likelihood of disease progression[104], despite the considerable overlap in the known functions of these genes. This may be because

Synthetic lethal
In genetics, a phenomenon in which the combination of two otherwise non-lethal mutations results in an inviable cell. Used in the context of functional screens to indicate a screen in which the end point is apparent in only some conditions, for example in the presence of a specific genetic lesion.

aberrations in these pathways occur independently and characterize biologically distinct phenotypes, as a subset of ER-positive breast cancers is characterized by a high probability of MYC pathway deregulation but a low probability of E2f pathway deregulation[105,106].

Functional genetic screens

In parallel with the emergence of increasingly sophisticated bioinformatic and genomic tools, there has been an increase in the feasibility of global functional screens. Gain-of-function screens in breast cancer cells have identified >40 candidate tamoxifen resistance genes[47,51,107–110]. These include genes encoding cell surface receptors (*EGFR*, *ERBB2*, platelet-derived growth factor receptor-β (*PDGFRB*) and colony-stimulating factor 1 receptor (*CSF1R*)) and their ligands (neuregulin, which is a ligand for some Erbb receptors, and *FGF17*), intracellular signalling molecules (*BCAR1*, *BCAR3*, *AKT1*, *AKT2*, *SRC* and *GRB7*) and transcriptional regulators (*BCAR2* (also known as *TRERF1*) and the ER co-repressor *NCOR2* (also known as *SMRT*)), as well as genes of poorly understood function (for example, *BCAR4* and *TLE3*). Many have roles in signalling pathways that are implicated in endocrine resistance, such as the Erk and PI3K–Akt pathways (as summarized above and in TABLE 1), illustrating the potential for identifying functionally relevant pathways using this approach. Genes derived from unbiased functional screens will not necessarily be deregulated in cancer, and even if they do show differential expression there is no guarantee that this will correlate with a therapeutic response. However, some of the genes that confer tamoxifen resistance in functional genetic screens are overexpressed in breast cancer and are independent predictors of response in women treated with tamoxifen at relapse[48,111–113].

An RNA interference screen to identify kinases for which decreased expression can reduce tamoxifen sensitivity yielded 20 candidates, including multiple components of the Erk signalling pathway, other intracellular signalling molecules and several kinases related to CDC2, the prototypical CDK[114]. One of these, CDK10, was examined in more detail. The *CDK10* gene was silenced by methylation in 7 of the 34 breast cancers examined, and low CDK10 expression was associated with poor outcome in women treated with adjuvant tamoxifen[114]. Downregulation of CDK10 in cultured breast cancer cells led to an ETS2-dependent increase in *RAF1* transcription and activation of the Erk pathway. Therefore, downregulation of CDK10 seems to modulate tamoxifen sensitivity through effects on intracellular signalling, rather than through more direct effects on cell cycle progression. This screen also identified sensitizers of tamoxifen response, many of which were in the PI3K pathway, including *PIK3C2B*, *PDK1* (which is activated by PI3K) and the PDK1 substrates *PRKCZ* and *AKT1*, as well as several other activators of Akt[115]. The sensitizers identified in a parallel small-molecule screen also included many inhibitors of Akt[115], again emphasizing the importance of the PI3K pathway as a determinant of the response to tamoxifen.

Implications for therapy

Endocrine-resistant ER-positive disease accounts for approximately one in four breast cancers but, similarly to the 'triple-negative' phenotype that does not express ER, PR or ERBB2, it is not well served by current targeted therapies. The possibility of identifying new targets for therapy in resistant disease, or patients who may benefit from additional treatment with existing therapies, provides a strong impetus to identify markers and mediators of therapeutic resistance. Some candidate endocrine resistance genes can also affect response to other therapies; for example, *BCAR1* confers resistance to adriamycin[116], and cyclin D1 confers resistance to the EGFR-targeted therapy gefitinib[117]. This adds to the challenge of identifying alternative therapies in endocrine-resistant disease. Recent data suggest that integrative bioinformatics and/or targeted large-scale screening may assist in meeting this challenge. One bioinformatic study identified highly significant links between genes repressed by inhibition of PI3K and genes activated by MYC, suggesting that PI3K inhibition may be an effective therapy for MYC-dependent cancers[104]. Together with evidence from the combined small-molecule and RNA interference screen discussed above that highlighted inhibition of Akt as a means of potentiating tamoxifen responses *in vitro*[115], this observation argues that therapies targeting the PI3K–Akt pathway may be useful in endocrine-resistant breast cancer. Similar integrative analyses including data relating specifically to endocrine resistance may yield new targets for further analysis and testing. Synthetic lethal genetic and/or small-molecule screens using cell lines that are tamoxifen resistant, either because of adaption in culture or because of the introduction of specific genetic lesions, may also identify pathways specifically required in tamoxifen-resistant cells and the means of targeting them.

Current models of endocrine resistance (FIG. 3) identify many individual molecules that can affect antioestrogen sensitivity *in vitro* and are therefore potential targets for therapy in endocrine-resistant disease. The convergence of many potential resistance genes on the Erk and PI3K pathways suggests that inhibitors of these pathways that are currently in development may be useful in this setting. Data from cell line models indicate that inhibiting Src[118], BCAR1 (REF. 119), Mek–Erk[120], Akt[38,115,121,122], mTOR[39,123] or NF-κB[30,79] can restore or potentiate tamoxifen sensitivity. However, early clinical trials of EGFR- and ERBB2-targeted agents that reduce Erk signalling (gefitinib, erlotinib, trastuzamab and lapatinib) or mTOR inhibitors (everolimus and temsirolimus) in combination with endocrine therapies have yielded mixed results, which may be partly due to difficulties in identifying tumours that are dependent on these pathways and so are most likely to benefit from the additional therapy[124]. In addition, crosstalk and negative feedback loops between different signal transduction pathways may lead to cellular resistance to individual inhibitors. One approach to this problem is the use of inhibitors that target more than one kinase or the use of combinations of therapies to simultaneously target multiple pathways. Alternatively, end points shared

by multiple pathways — for example MYC and cyclin D1–CDK4 — could be targeted, which would have the benefit of more directly targeting proliferation, a central component in essentially all gene signatures that are predictive of endocrine resistance. Recent evidence indicates that targeting MYC may be an effective cancer therapy[125], and some small-molecule CDK inhibitors have entered clinical trials[126].

Concluding remarks

To date, much of the information on the mechanisms of endocrine resistance has come from studies that have considered relatively few genes. The application of integrative approaches that examine gene expression in the context of global genomic, proteomic or functional data to understand mechanisms of endocrine resistance is in its infancy and has relied on small-scale genomic and transcriptional data sets. Nevertheless, as summarized above, this approach has provided some pointers towards areas for further study. The next generation of high-throughput technologies allows genome-scale genetic and synthetic lethal screens, large-scale proteomic and phospho-proteomic profiling, and parallel transcriptome, epigenome and genome sequencing of both experimental models and well-annotated clinical samples. The further use of integrative bioinformatic approaches combined with these new technologies offers the potential for a 'systems biology' approach, in which cellular responses and pathways are considered as a whole. This may identify previously unconsidered mechanisms for the modulation of therapeutic response, as suggested by recent data implicating miRNAs in tamoxifen resistance[72], and thereby greatly increase the depth of our understanding of the mechanisms of endocrine-resistant disease. This, in turn, should aid in the identification of the specific genetic events that cause resistance and the means by which these events can be targeted therapeutically.

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