REVIEW

Understanding the mechanisms of aromatase inhibitor resistance

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Abstract

Aromatase inhibitors (AIs) have a central role in the treatment of breast cancer; however, resistance is a major obstacle to optimal management. Evidence from endocrine, molecular and pathological measurements in clinical material taken before and after therapy with AIs and data from clinical trials in which AIs have been given as treatment either alone or in combination with other targeted agents suggest diverse causes for resistance. These include inherent tumour insensitivity to oestrogen, ineffective inhibition of aromatase, sources of oestrogenic hormones independent of aromatase, activation of signalling by non-endocrine pathways, enhanced cell survival and selection of hormone-insensitive cellular clones during treatment.

Introduction

Some breast cancers require oestrogens for growth and, if deprived of these hormones, will regress. Consequently, oestrogen deprivation therapy is a major treatment strategy for hormone-dependent breast cancer. There are various forms of endocrine therapy but recently agents inhibiting the aromatase enzyme, which catalyzes the conversion of androgens to oestrogen, have been increasingly used [1]. These have evolved from rational drug development, which has generated inhibitors with exceptional potency and specificity [2]. In postmenopausal women, drugs such as letrozole, anastrozole and exemestane can inhibit aromatization of androgen *in vivo* by >99% [3], often decrease circulating oestrogens to undetectable levels [3,4] and, in hormone-dependent breast cancers, reduce tumour proliferation [5,6] and growth [7,8]. Third-generation aromatase inhibitors (AIs) are now front-line treatments for breast cancer [1]. However,

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response rates range between 35 and 70% in neoadjuvant studies [9,10], and benefits may be lower in advanced disease [11]. Acquired resistance after initial successful treatment also occurs [12]. Consequently, resistance is a major obstacle and optimal clinical management would benefit from early identification of resistance and the mechanisms by which resistance occurs. Patients with clinically resistant cancers could then be spared unnecessary side effects and ineffective treatment. Knowledge of the underlying reason for resistance would also facilitate the development and implementation of new therapies by which to bypass or reverse resistance. The present review will address these issues by i) distinguishing between different types of resistance and identifying potential complications and confounders, ii) summarizing key clinical observations and iii) integrating these with biological/molecular studies performed on tumours clinically resistant to AIs.

Types of resistance

Before considering specific issues relating to resistance, some brief definitions of different forms of resistance are presented.

Clinical versus other forms of resistance

Clinical 'resistance' to AIs is usually perceived as a lack of growth inhibition by AI treatment in that the therapy is ineffective in causing a decrease in tumour size. However, AI treatment often results in molecular (and pathological) changes in clinically resistant tumours [13,14]. Clinical resistance therefore needs to be distinguished from other forms of resistance, including that in which AI therapy fails to elicit any form of response (in the same way as dependence should be separated from sensitivity).

Primary versus acquired resistance

Resistance may be subdivided into primary (or *de novo*) and secondary to an initial treatment response (or acquired). Although having clinical implications, primary and acquired resistance may not be separate entities and underlying mechanisms of resistance may be shared. However, the inference is that 'acquired' resistance is the result of inductive changes or clonal selection caused by

treatment. Molecular changes that could impact on effectiveness of therapy have been observed following AI treatment [15,16].

Cross-resistance and non-cross-resistance

Some tumours resistant to AIs also appear non-responsive to other forms of endocrine therapy (that is, they are cross-resistant [17]); other AI resistant tumours are sensitive to other endocrine therapies (that is, there is no cross-resistance [18,19]). Non-cross-resistance can be subtle where, for example, tumours may be resistant to one AI (or class of AIs) but respond to another [20,21].

Observations from clinical trials

Knowledge contributing to the understanding of resistance to AI may be derived from i) randomised clinical trials comparing AIs with other forms of endocrine therapies, ii) randomised studies in which AIs have been compared with a combination of AIs plus a targeted agent and iii) studies in which patients with AI-resistant tumours have been given further treatment.

Comparison of AIs with other forms of endocrine therapies

Novel, third generation AIs (anastrozole, letrozole and exemestane) have greater efficacy and improved safety profiles compared with their predecessors when employed as treatment for hormone-responsive postmeno-pausal breast cancers [2,3,8]. Randomized clinical trials also show that third generation AIs are equivalent or superior in efficacy to tamoxifen [9-11,22,23] and may be effective in tamoxifen-resistant advanced breast cancer [24,25]. Despite the latter observation, prior resistance to other forms of endocrine therapy is associated with a decreased probability of response to an AI [26].

It is worth commenting on the time taken to elicit clinical response. Several neoadjuvant protocols show that longer treatment with an AI results in additional clinical benefit [27,28]. It is thus possible that a minority of apparently resistant tumours may be sensitive to the action of AIs but extended treatment is required before clinical response becomes manifest. This contrasts with the speed of response generally observed following chemotherapy.

Comparison of AIs with a combination of AIs plus a targeted agent

Clues to potential resistance mechanisms may be gleaned from studying agents that significantly change response rates when given in combination with AIs. The most informative studies are those involving selected targeted agents for which there is a rationale related to AI resistance. Targets include type I growth factor receptors, epidermal growth factor receptor, human epidermal growth factor receptor (HER)2 and phosphoinositide 3-kinase/mammalian target of rapamycin (mTOR) inhibitors. Results

from several of these clinical trials have recently been reported. For example, a preoperative study of letrozole with or without the mTOR inhibitor everolimus reported greater tumour shrinkage for the combination [29]. Furthermore, marked antiproliferative responses occurred in 57% of patients in the combination everolimus arm compared with 30% in the letrozole alone arm. This suggests that, in some tumours, AKT signalling is associated with letrozole resistance, an influence that may be abrogated by phosphoinositide 3-kinase/mTOR inhibitors.

Other combinations involve therapies that target the HER family of growth factor receptors using either antigrowth factor-receptor antibodies (for example, trastuzumab) or small molecule tyrosine-kinase inhibitors (such as gefitinib and lapatinib). A randomized trial of first-line gefitinib plus anastrozole versus anastrozole alone in women with oestrogen receptor (ER)-positive advanced breast cancer reported that patients who received the combination therapy experienced significantly longer progression-free survival (PFS) and an improvement in clinical benefit rate, but a lower response rate [30,31]. In neither of the mentioned trials using the combination of gefitinib plus anastrozole were patients selected on the basis of overexpression of growth factor receptors. However, two studies have included HER-2 status in selection criteria. Thus, in patients with known ER-positive/HER2-positive tumours, the addition of lapatinib to letrozole significantly reduced the risk of progression and improved median PFS; clinical benefit rate was also significantly greater for the combination [32]; a preplanned analysis was also able to show an impact of combination therapy on PFS in the HER2-negative population. Finally, a randomized phase III trial in patients with known hormone receptor-positive/HER2-positive metastatic breast cancer recently reported a doubling of PFS with the addition of trastuzumab to anastrozole compared with anastrozole alone [33]. In these combination studies, it is possible that the additional benefit of targeted therapy is separate from the endocrine effects of AIs. However, preclinical studies and measurements of biological markers suggest synergy or cross-talk between signalling systems. The hypothesis is therefore that acquired resistance to AIs in patients with ER-positive/HER2-negative tumours may be caused by adaptive epidermal growth factor receptor or HER2 upregulation and this might be prevented or delayed by agents directed against these targets.

Further treatment in patients with AI-resistant tumours

Important information about the nature of AI resistance may be derived from clinical studies in which patients with tumours resistant to an AI are given further treatment. It is especially interesting to review investigations in which therapy has involved another AI (Table 1). For

Table1. Sequential treatment using different classes of aromatase inhibitors

Initial treatment	Second treatment	n	Objective response (%)	Clinical benefit (%)	Time to progression (months)	Reference
Anastrozole or letrozole	Exemestane	23	8.7	43.5	5.1	[37]
Exemestane	Anastrozole or letrozole	18	22.2	55.6	9.3	[37]
Anastrozole	Exemestane	12			4.4	[73]
Exemestane	Anastrozole	11			1.9	[73]
Anastrozole	Exemestane	50	8.0	44.0	5.0	[74]
Anastrozole or letrozole	Exemestane	114	5.0	46.0	4.5	[75]
Anastrozole or letrozole	Exemestane	31	19.4	54.8	3.2	[76]
Anastrozole or letrozole	Exemestane	30	0.0	46.6	4.0	[77]
Anastrozole or letrozole	Exemestane	60	20.0	38.3	3.2	[78]
Anastrozole or letrozole	Exemestane	105	4.8	20	3.2	[36]

example, responses to formestane have been reported in patients failing aminoglutethimide [34,35], and clinical response to exemestane may follow the development of resistance to non-steroidal AIs [36] and, conversely, patients progressing after exemestane therapy have been shown to derive further benefits from treatment with letrozole or anastrozole [37]. These clinical studies indicate at least a partial non-cross-resistance between steroidal AIs and non-steroidal AIs [20,31,38]. In general, objective response rates with the second-line agent are not high, but clinical benefit is observed in 20 to 55% of patients regardless of the treatment sequence (for example, non-steroidal AI followed by steroidal AI or steroidal AI followed by non-steroidal AI). More recently, results have become available from the Evaluation of Fulvestrant versus Exemestane Clinical Trial (EFECT) in which patients with advanced hormone receptor-positive breast cancer refractory to a non-steroidal AI have been randomized to receive either fulvestrant or exemestane [39]. In the exemestane arm, objective response rate was observed in 6.7% and clinical benefit in 31.5% (the corresponding figures for the fulvestrant arm were 7.4 and 32%). Although many of the studies contain small numbers of patients, evidence is consistent and indicates that patients whose disease becomes resistant to one AI may still respond to a different class of AI. The molecular mechanisms underpinning AI non-cross-resistance are not immediately apparent (AIs have a common mechanism of action). However, the phenomenon and sequential responses to anti-oestrogens such as fulvestrant [39] suggest that growth in a proportion of AI-resistant tumours may be maintained by signalling through a functioning ER pathway.

Endocrine and molecular markers

The understanding of mechanisms of AI resistance has also been furthered by identification of i) molecular and endocrine markers that might distinguish between resistant and responsive cancers, ii) changes induced by AI therapy that might be associated with an AI-resistant phenotype and iii) genetic signatures and patterns that illustrate diversity of resistance.

Endocrine and molecular markers
Oestrogen receptors
A major cause of resistance to AIs and other endocrine therapies is absence of functional ER in tumours. For example, in the P024 neoadjuvant trial of letrozole versus tamoxifen [23], a small number of ER-negative tumours (protocol violators) were entered into the study and none responded to either drug. Patients with ER-negative tumours should not be offered therapy. However, many ER-positive tumours also do not respond to AIs. The challenge is how to discriminate accurately and on an individual basis which ER-positive tumours respond to treatment and those that do not.

HER2
HER signalling can result in ER phosphorylation (a critical step in ER activation) even in the absence of oestrogen [40]. However, the situation with regard to tumour HER2 overexpression and resistance to AIs is complicated. In the neoadjuvant setting, clinical response rates to AIs are similar in HER2-positive and -negative tumours [41,42]. At the same time, AIs often fail to reduce proliferation in ER-positive/HER2-positive breast cancers even amongst those that display a clinical response [41,43]; this suggests that growth factors other than oestrogen are driving proliferation, limiting the benefits of AIs in HER2-overexpressing tumours (this observation would also account for the poorer long-term outcomes in HER2-overexpressing breast cancer as reported in adjuvant trials with AIs [44,45]). Furthermore, numbers of ER-positive/HER2-positive breast cancers are small [46] and the pathway is unlikely to account for AI resistance in most tumours.

Other potential markers
Genetic polymorphisms have been identified and characterised in the aromatase gene and may have functional influences on the interaction between the enzyme protein, its substrate and inhibitors [47]. Thus, it is of interest that Wang and colleagues [48], examining tumour from breast cancer patients, reported that two tightly linked SNPs (rs6493497 and rs7176005) were significantly associated with a greater change in aromatase activity after AI treatment and that, in a separate group of cases, these two same SNPs were associated with higher plasma oestradiol levels in patients pre-AI and post-AI treatment. The authors hypothesised that SNPs in the *CYP19* gene may alter the effectiveness of AI therapy in the neoadjuvant setting. Others have reported interesting findings with regard to a SNP (rs4646) located in the 3' untranslated region of the aromatase *CYP19* gene. Thus, Colomer and colleagues [49] found that in patients with hormone receptor-positive metastatic breast cancer treated with the aromatase inhibitor letrozole, time to progression was significantly improved in patients with the rs4646 variant compared with the wild-type gene (17.2 versus 6.4 months; *P* = 0.02). In contrast, Garcia-Casado and colleagues [50] analysed DNA from peripheral blood of patients offered neoadjuvant letrozole; they showed that those carrying genetic variants of rs4646 had a lower PFS than patients homozygous for the reference variant. Ribosomal proteins have also been associated with resistance to an AI. Thus, mRNA expression of several ribosomal proteins has been reported to be significantly lower in letrozole-resistant tumours compared with responsive cases [51]. A study using letrozole alone or in combination with chemotherapy [52] examined a group of tumour proteins involved in apoptosis, cell survival, hypoxia, angiogenesis, and growth factor and hormone signalling; increased hypoxia-inducible factor-1 alpha and P44/42 mitogen-activated protein kinase (MAPK) were associated with resistance. Lastly, overexpression of low-molecular-weight cyclin E has been claimed to bypass letrozole-induced G1 arrest and thereby produce resistance [53]. Whilst all these studies are potentially important, being derived from appropriate clinical material and involving markers that could functionally impact on resistance mechanism for AIs, it should be noted that results have usually been based on a single series of breast cancers; there is an immediate need for independent confirmation using different cohorts of tumours.

Changes induced by aromatase inhibitor therapy
Recently, several studies have exploited preoperative or neoadjuvant protocols employing AIs to determine molecular responses to treatment [15,16,54-56]. Results are generally consistent. Thus, AIs suppressed expression of classical oestrogen-dependent and proliferation-related genes, such as *TFF1*, KIAA0101, *PDZK1*, *AGR2*, *ZWINT*, *IRS1*, *CDC2*, *CCND1*, *CCNB1*, *NUSAP1* and *CKS2*. The most consistently upregulated genes were enriched by 'stromal' signatures, including specific types of collagens (*COL3A1*, *COL14A1*, *COL1A2*), members of a small leucine-rich proteoglycan family (*DCN*, *LUM* and *ASPN*), genes associated with cell adhesion and intercellular matrix turnover (*MMP2*, *CD36*, *CDH11*, *ITGB2*, *SRPX*, *SPON1*, *DPT*) and immune-response-associated genes (*COLEC12*, *IL1R1*, *C1R*, *TNFSF10*). In the neoadjuvant studies, molecular changes could be related to clinical response [14,51,55,57]. Although classical markers of oestrogen sensitivity and proliferation were generally reduced with treatment in responsive tumours, their expression was also frequently decreased in resistant tumours [13,14]; consequently they differentiated poorly between response and resistance to AIs. In terms of genes that changed with therapy and also distinguished between responsive and resistant tumours, Miller and colleagues [51] drew attention to structural constituents of ribosomes (Figure 1). Thus, responsive tumours showed higher expression of ribosomal proteins before treatment and decreased expression after 2 weeks of letrozole therapy but, by contrast, baseline expression of ribosomal proteins was low in resistant tumours and was increased by treatment.

Mello-Grande and colleagues [55] examined gene expression profiling and response to neoadjuvant treatment with anastrozole and observed an enrichment of induction of T-cell anergy, positive regulation of androgen signalling, synaptic transmission and vehicle trafficking in non-responding tumours. In a further study, upregulation of ER coactivator mRNA and HER2 was observed during neoadjuvant treatment with either letrozole or anastrozole [56]. This is of interest since these are factors that influence oestrogen signalling and could potentially mediate acquired resistance to AIs.

It is self-evident that to differentiate between responsive and resistant tumours on the basis of changes on treatment, it will be necessary to sample tumours on multiple occasions. A further corollary is that if adaptive changes during treatment result in resistance, it is likely that there will be a necessity for a re-biopsy at time of recurrence/resistance to elucidate the nature/mechanism of resistance.

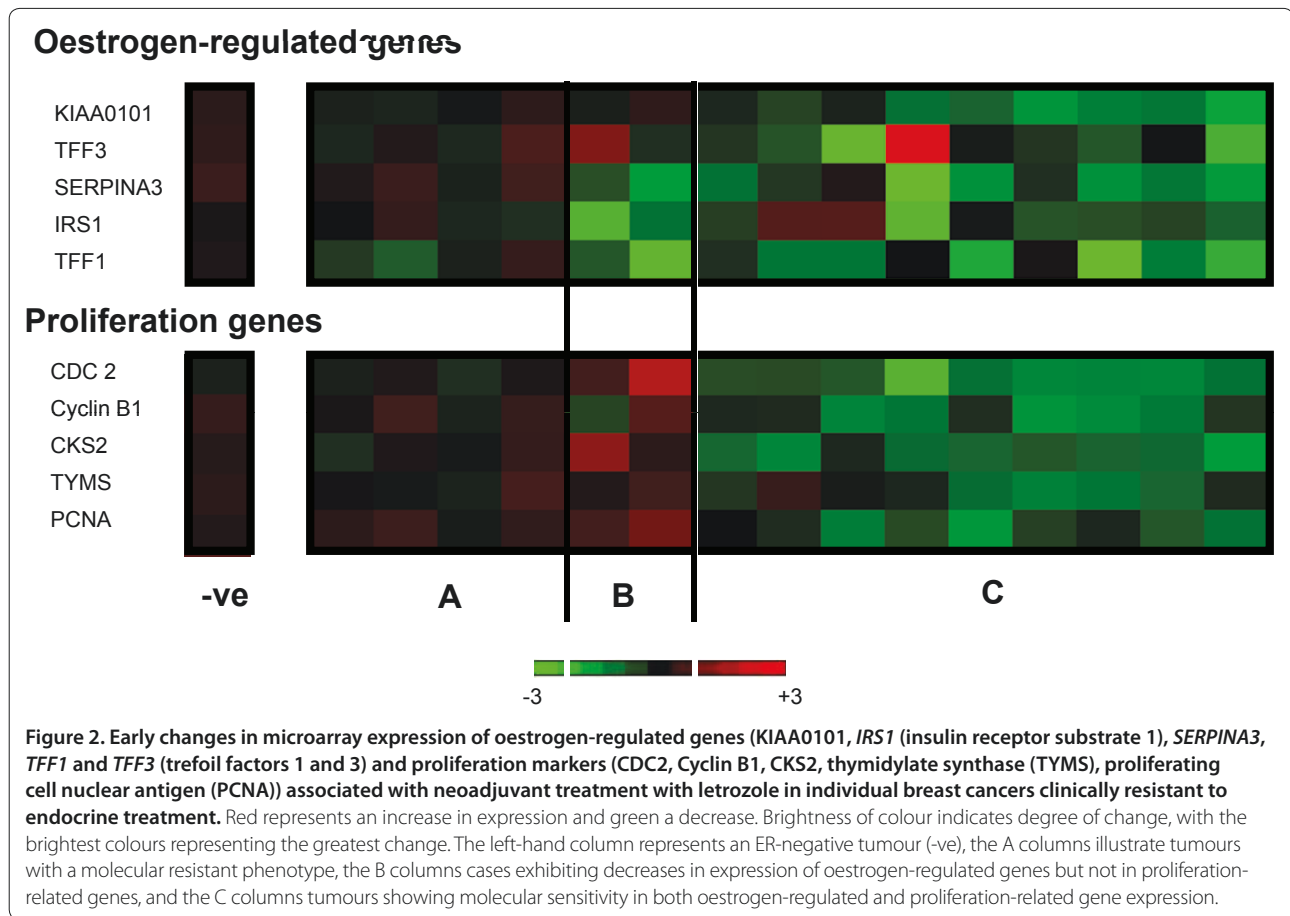### Molecular diversity of aromatase inhibitor resistance
Gene profiling data suggest that AI-resistant tumours are more diverse than responsive cases [57]. Resistant tumours can also be divided into subgroups using treatment-induced expression changes in genes associated with oestrogen regulation or proliferation [13]. Thus, letrozole-resistant tumours could be grouped into cases that
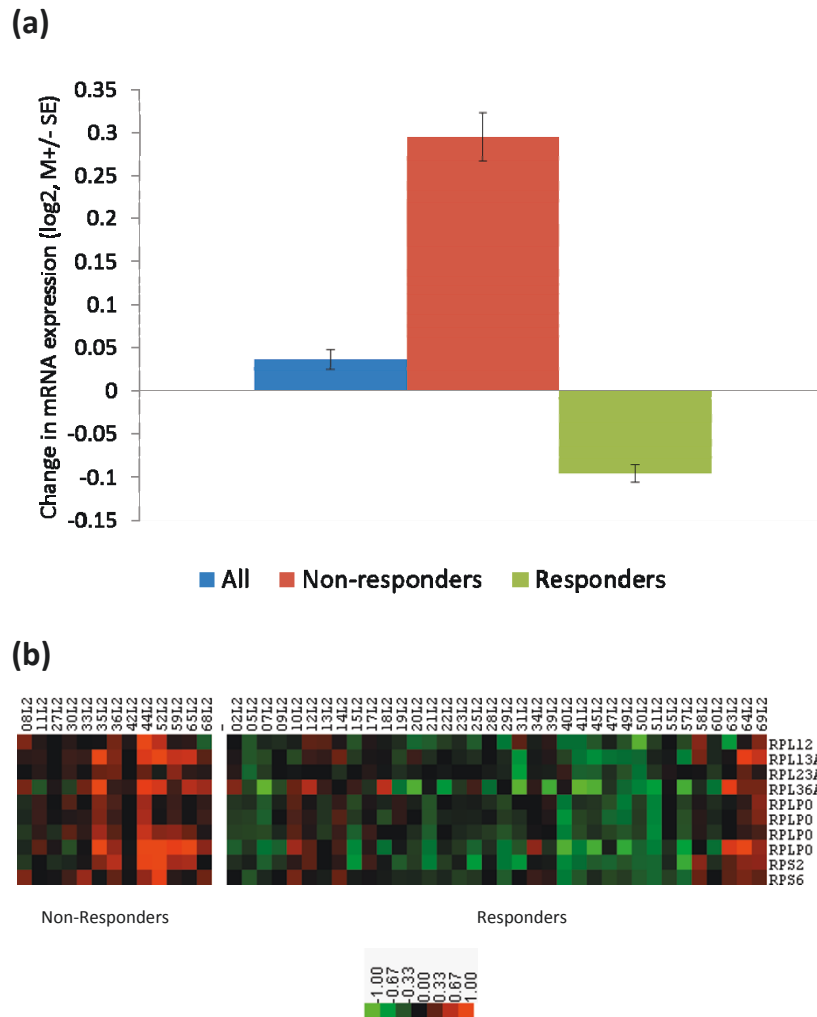
Figure 1. Effects of neoadjuvant treatment with letrozole on changes (within 10 to 14 days) in microarray expression of ribosomal proteins. (a) Average changes for the total group, the responding group and the non-responding group. Error bars represent standard errors of the mean. **(b)** Changes in individual tumours. Red represents an increase in expression and green a decrease. Brightness of colour indicates degree of change, with the brightest colours representing the greatest change. The RPLP0 gene is represented by four probe sets.

showed generally no molecular changes, decreases in oestrogen-regulated genes but not those related to proliferation, or general decreases in both oestrogen-regulated and proliferation genes (Figure 2). Some speculation on these findings is merited. Thus, cases with no general change in gene expression in response to letrozole appear to have the classical phenotype of oestrogen insensitivity. There are two major reasons for this. First, it is possible that although the tumours possess ER, the receptors are non-functional and not functionally connected to downstream signalling. However, it is also possible that the lack of molecular changes may be because the drug has failed to have endocrinological effects and tumour is not being exposed to oestrogen deprivation. Measurements of circulating and intra-tumoural oestrogens would distinguish between these

possibilities. The differential phenotype in which expression of oestrogen-regulated genes was mostly reduced but that for proliferation genes was generally increased illustrates a disconnection between expression of oestrogen signalling and proliferation genes. It is clear that these tumours are seeing oestrogen deprivation as evidenced by the decreases in oestrogen-regulated genes but it appears that proliferation (and therefore tumour growth) is being controlled by non-oestrogenic pathways. Reduced expression of both markers of oestrogen regulation and proliferation is a paradoxical phenotype in cases of clinical resistance. Whilst these tumours are categorized as clinical non-responders, they do react to oestrogen deprivation at molecular and proliferative levels. The major issue to clarify is why molecular and proliferative responses associated with oestrogen

Figure 2. Early changes in microarray expression of oestrogen-regulated genes (KIAA0101, *IRS1* (insulin receptor substrate 1), *SERPINA3*, *TFF1* and *TFF3* (trefoil factors 1 and 3) and proliferation markers (CDC2, Cyclin B1, CKS2, thymidylate synthase (TYMS), proliferating cell nuclear antigen (PCNA)) associated with neoadjuvant treatment with letrozole in individual breast cancers clinically resistant to endocrine treatment. Red represents an increase in expression and green a decrease. Brightness of colour indicates degree of change, with the brightest colours representing the greatest change. The left-hand column represents an ER-negative tumour (-ve), the A columns illustrate tumours with a molecular resistant phenotype, the B columns cases exhibiting decreases in expression of oestrogen-regulated genes but not in proliferation-related genes, and the C columns tumours showing molecular sensitivity in both oestrogen-regulated and proliferation-related gene expression.

deprivation by letrozole do not translate into clinical responses. There are several potential reasons. First, it may reflect limitations and inaccuracy of clinical measurements. Current clinical criteria for response assessment are often based on arbitrary empirical thresholds that may not accurately reflect biology of tumour response. For example, tumours shrinking with treatment are routinely categorized as 'clinically resistant' if the decrease fails to reach 50% reduction in tumour volume during therapy. These tumours might have become clinical responders with extended treatment. It should also be noted that treatment did not decrease expression of these genes to zero and, after therapy, expression is still measurable. Hence, it could be that the reductions in proliferation are not sufficient to produce a clinical response in the absence of other changes, such as an increase in cell death.
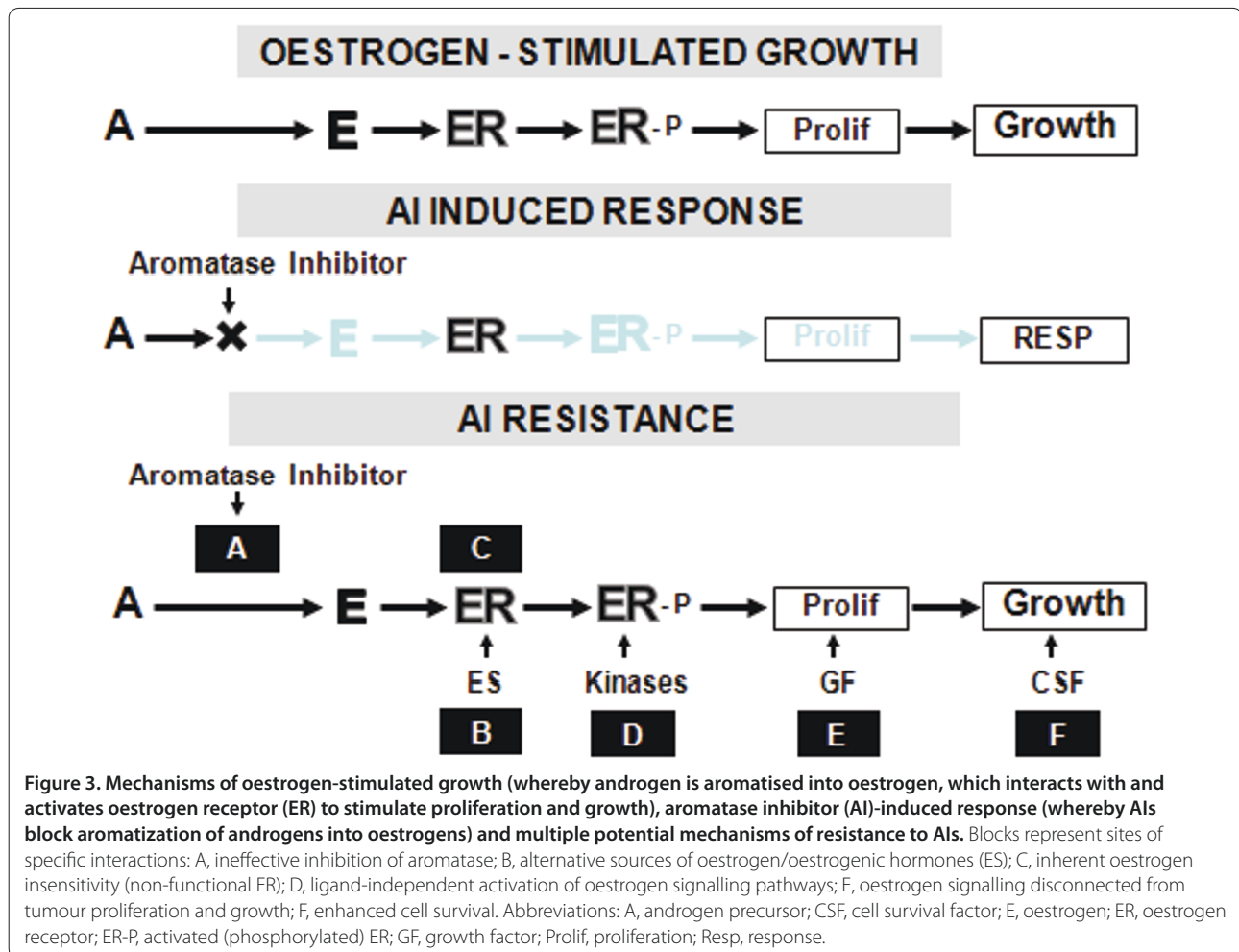
The authors suggest that a systematic molecular characterisation of changes in expression of classical oestrogen-regulated and proliferation-associated genes with short-term exposure to AIs will provide fundamental information relating to underlying mechanism of resistance and allow a more rational clinical management in individual patients. A prospective study is recommended for the future.

Mechanisms of resistance

It is clear that there are a multitude of mechanisms that could account for breast cancers appearing/being resistant to therapy with AIs [2,58]. This could also be deduced theoretically by considering the classical multi-step pathway of oestrogen stimulation of breast cancer growth and mechanism of action of AIs as illustrated in Figure 3. Because the influence of AIs may be compromised or bypassed at each step in the pathway, there are multiple opportunities for resistance. These will be considered under the headings illustrated in Figure 3: (A) ineffective inhibition of aromatase; (B) alternative sources of oestrogen/oestrogenic hormones; (C) inherent oestrogen insensitivity (non-functional ER); (D) ligand-independent activation of oestrogen signalling pathways; (E) oestrogen signalling disconnected from tumour proliferation and growth; (F) enhanced cell survival; and outgrowth of hormone-insensitive cellular clones (not illustrated).

Ineffective inhibition of aromatase

There are several reasons by which AI may fail to inhibit aromatase effectively and residual oestrogen may maintain tumour growth. These include poor drug potency, adverse pharmocokinetics/pharmacogenetics,

Figure 3. Mechanisms of oestrogen-stimulated growth (whereby androgen is aromatised into oestrogen, which interacts with and activates oestrogen receptor (ER) to stimulate proliferation and growth), aromatase inhibitor (AI)-induced response (whereby AIs block aromatization of androgens into oestrogens) and multiple potential mechanisms of resistance to AIs. Blocks represent sites of specific interactions: A, ineffective inhibition of aromatase; B, alternative sources of oestrogen/oestrogenic hormones (ES); C, inherent oestrogen insensitivity (non-functional ER); D, ligand-independent activation of oestrogen signalling pathways; E, oestrogen signalling disconnected from tumour proliferation and growth; F, enhanced cell survival. Abbreviations: A, androgen precursor; CSF, cell survival factor; E, oestrogen; ER, oestrogen receptor; ER-P, activated (phosphorylated) ER; GF, growth factor; Prolif, proliferation; Resp, response.

compensatory endocrine loops and altered aromatase phenotype. Early-generation AIs did not completely block oestrogen biosynthesis [3,4] whereas later generation AIs were potent and able to produce clinical responses in tumours resistant to inferior inhibitors (see [38] for details). Although measurable differences in potency are apparent between the current generation of inhibitors, there is no direct evidence to suggest that this is associated with cross-resistance. Furthermore, in the absence of confounding factors, effective inhibition of aromatase by third generation inhibitors appears to occur in most postmenopausal patients [59,60], suggesting that ineffective suppression of oestrogen is only likely to be the cause of resistance in occasional cases.

Pharmacokinetics/pharmacogenomics may have adverse influences on AIs [61]. There are drug interactions between tamoxifen and some AIs; concomitant administration of tamoxifen with either anastrozole or letrozole decreases plasma levels of the AIs (letrozole by 30 to 40% and anastrozole by 20 to 30%). However, oestrogen suppression does not seem to be compromised [62,63]

and clinical relevance is likely to be limited. High/raised levels of aromatase may prevent effective blockade by inhibitors. For example, high levels of aromatase in the premenopausal ovary and compensatory feedback loops, which increase levels of gonadotrophins, are associated with ineffective inhibition of ovarian aromatase by AIs. Consequently, in premenopausal women, AIs are generally used with a luteinizing hormone releasing hormone (LHRH) agonist to block the rise in gonadotrophins [64]. SNPs in the aromatase gene have been associated with resistance to AIs, suggesting an aromatase phenotype that is resistant to AIs. Differential sensitivity to AIs has been observed in some breast cancers, but it is comparatively rare and has not been associated with mutations in aromatase [65]. Finally, ineffective aromatase inhibition may be related to treatment compliance issues.

Alternative sources of oestrogen/oestrogenic hormones

AIs block endogenous synthesis of oestrogen but have no effects on the synthesis of other steroid classes, which

can interact with ER (such as adrenal androgens) [2], and on exogenous oestrogens/oestrogenic compounds, including synthetic estrogens, industrial pollutants, and phytoestrogens. However, if these alternative sources of oestrogenic factors were a common cause of resistance to AIs, it might be expected that anti-oestrogens (which block the action of oestrogenic factors irrespective of source) would have superior clinical benefits to AIs whereas generally they do not [9-11,66]. Moreover, there is evidence that tamoxifen can act as an oestrogen to compromise the action of AIs. Thus, the experience of combining tamoxifen with anastrozole in the Arimidex, Tamoxifen Alone or in Combination (ATAC) trial was that disease-free survival in patients taking the combination of anastrozole plus tamoxifen was significantly less than in those taking anastrozole alone (and similar to tamoxifen alone) [66]. The basis for this probably resides in the accentuation of the oestrogen agonist properties of tamoxifen [67-69], which become apparent in the low oestrogen environment produced by AIs.

Inherent oestrogen insensitivity (non-functional ER)

Stimulatory effects of oestrogen on the growth of hormone-dependent breast cancers are mostly mediated through ERs. It is confirmed by the fact that AIs are unlikely to produce responses in ER-negative tumours [70]. However, many tumours resistant to AIs have ER-positive phenotypes [71], and the major challenge is to comprehend why, if AIs produce effective oestrogen deprivation, they do not result in tumour regression. One possibility is that ER is non-functional. RNAs encoding variant and mutant ERs have been reported in breast cancer [72]; abnormal receptors may bind oestrogens but not transmit a signal. Tumours with non-functional ERs would be inherently insensitive to hormone stimulation (and refractory to AI therapy) despite being ER-positive. Other critical components of ER signalling are co-regulators [71]. Coregulator abnormalities or imbalance may dislocate signalling so that growth is independent of oestrogen and not susceptible to AIs.

Ligand-independent activation/stimulation of oestrogen signalling pathways

ER signalling may be activated independently of oestrogen [71]. For example, HER2 signalling can result in ligand-independent ER phosphorylation [40]. Although numbers of ER-positive HER2-positive tumours are small [46], other kinases such as MAPKs and insulin-like growth factor 1 receptor/AKT are capable of activating and supersensitizing ER signalling [12]. It is thus relevant that overexpression of MAPK has been found in breast cancers resistance to letrozole [52]. These considerations underpin the proposed use of appropriate signal transduction inhibitors in combination or sequence with AIs

[12,52]. Involvement of ligand-independent ER signalling may also explain cases with lack of cross-resistance between AIs and anti-oestrogens.

Oestrogen signalling disconnected from tumour proliferation and growth

Certain breast cancers appear clinically resistant despite fully functional ER and effective oestrogen deprivation. However, it may be that proliferation and growth are stimulated by oestrogen-independent pathways. In this setting, AI treatment would reduce expression of classically oestrogen-regulated genes but not those associated with cellular proliferation. The phenotype has been described in some breast cancers clinically resistant to neoadjuvant treatment with letrozole [13,58].

Cell survival

Most tumours that appear clinically resistant to AI are nevertheless molecularly sensitive to the drugs insofar as the expression of both oestrogen-regulated and proliferation-associated genes and proteins decreases with treatment [13,14,58]. To explain this form of resistance, it is necessary to suggest that the therapeutic reduction in proliferation leaves residual cell cycling which, together with efficient cell survival mechanisms, maintains tumour growth.

Adaption with treatment/outgrowth of hormone-insensitive cellular clones

This scenario suggests that at the outset of treatment, tumours may have a responsive phenotype or be composed of a mixture of AI-responsive and -resistant cells. Under the pressure of treatment either adaptive intracellular changes occur (transforming a responsive phenotype into one with resistant characteristics) or there is an outgrowth of resistant cellular clones (present at the outset of treatment) with a survival advantage over other cells that are susceptible to therapy. This type of mechanism would be particularly applicable to resistance secondary to an initial response or 'acquired' resistance. Adaptive changes with AI treatment, such as increased/ changed expression of HER2 and ER co-regulators and loss of ER, have been described (although most breast cancers with acquired resistance to AIs remain ER-positive after treatment [12,71]).

Conclusion

In order to understand the nature of resistance to AIs, this review has drawn upon endocrine, molecular and pathological measurements made in clinical material taken before and after therapy with AIs and upon observations from clinical trials in which AIs have been given as treatment either alone or in combination with other targeted agents. The major message from these studies is

that no single reason can account for resistance in all cases and that there are multiple and diverse mechanisms by which breast cancers may avoid the restraints of AI therapy. The consequences of this are that a battery of tests and predictive markers may be needed in order to elucidate the nature of resistance in individual tumours and that if rational treatments to avoid or reverse resistance are based on an underlying mechanism, they also will be both varied and individually targeted.

In terms of general identification of resistance, assessment of ER is essential. However, in ER-positive tumours, additional markers are needed both to identify resistance and pinpoint its nature. Status of ER signalling may be directly assessed by measuring the degree/type of ER phosphorylation and levels of ER coactivators/corepressors, and indirectly by analyzing profiles of oestrogen-regulated genes. Measurement of proliferation markers, relevant growth factors, their receptors and kinase activity may complement the ER signalling assessment. Treatment adherence and efficiency of aromatase inhibition may be monitored by measuring blood levels of drugs, oestrogen and other hormones, aromatase activity, pharmacokinetics of AIs and pharmacogenetics of aromatase. As well as multiple assessments, dynamic measurements may be necessary - neoadjuvant studies indicate that most clinically resistant tumours show a variety of molecular responses and these may help identify more precisely the exact nature of resistance in individual tumours. This may entail sequential biopsies of tumour during treatment and, in the case of acquired resistance, at the time of recurrence.

If resistance to AIs occurs through a diverse set of mechanisms, it follows that therapy aimed at preventing or reversing resistance is to be designed rationally by targeting, and understanding of the specific cause of resistance in individual cases will be necessary. In this respect, the use of neoadjuvant and short-term preoperative protocols may be particularly informative - AIs and other signal-transduction modifying agents can be administered to patients and the primary tumour monitored for molecular and pathological effects. These approaches are particularly promising because they may be coupled with new pathological methodologies and molecular techniques. A future can be envisaged in which patients may be selected for specific treatment regimes after molecular profiling and phenotyping at genomic, transcriptomic and proteomic levels in tumour taken before treatment and after a short period of therapy. These results will be used to detect early evidence of resistance and to select rational treatments to avoid resistance (by using appropriate targeting drugs either in combination or in sequence with AIs). These measures can be expected to increase and prolong clinical benefits of AIs whilst circumventing resistance.

Abbreviations

AI, aromatase inhibitor; ER, oestrogen receptor; HER, human epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PFS, progression-free survival; SNP, single-nucleotide polymorphism.

Competing interests

The authors declare that they have no competing interests.

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