Downstream targets of growth factor and oestrogen signalling and endocrine resistance: the potential roles of c-Myc, cyclin D1 and cyclin E

Alison J Butt1,2, Catriona M McNeil1,2, Elizabeth A Musgrove1,2 and Robert L Sutherland1,2

1Cancer Research Program, Garvan Institute of Medical Research, St Vincent’s Hospital, Darlinghurst, New South Wales 2010, Australia
2St. Vincent’s Clinical School, Faculty of Medicine, University of New South Wales, Randwick, New South Wales 2052, Australia

(Requests for offprints should be addressed to R L Sutherland; Email: r.sutherland@garvan.org.au)

Abstract

Antioestrogen therapy is a highly effective treatment for patients with oestrogen-receptor (ER)-positive breast cancer, emphasising the central role of oestrogen action in the development and progression of this disease. However, effective antioestrogen treatment is often compromised by acquired endocrine resistance, prompting the need for a greater understanding of the down-stream mediators of oestrogen action that may contribute to this effect. Recent studies have demonstrated a critical link between oestrogen’s mitogenic effects and cell cycle progression, particularly at the G1 to S transition where key effectors of oestrogen action are c-Myc and cyclin D1, which converge on the activation of cyclin E-cdk2. These components are rapidly upregulated in response to oestrogen, and can mimic its actions on cell cycle progression, including re-initiating cell proliferation in antioestrogen-arrested cells. Here we review the roles of c-Myc, cyclin D1 and cyclin E in oestrogen action and endocrine resistance, and identify their potential as markers of disease progression and endocrine responsiveness, and as novel therapeutic targets in endocrine-resistant breast cancer.

Endocrine-Related Cancer (2005) 12 S47–S59

Introduction

Since its first clinical use in the early 1970s, the antioestrogen tamoxifen (TAM) has been the most widely prescribed endocrine treatment for breast cancer in both the advanced disease and adjuvant settings, with a significant impact on survival for patients with endocrine-responsive disease. However, despite its widespread clinical efficacy, response is often short-lived, and resistance to endocrine therapy remains a major obstacle in the successful treatment of this disease. A major issue for the development of novel endocrine therapies is that, although oestrogen has been implicated as a major aetiological factor in the tumorigenic process in the breast, the downstream effects of its actions remain to be fully characterised. To this end, several recent studies have centred upon the identification and characterisation of oestrogen targets that may give clues to the factors influencing therapeutic response, and could, potentially, lead to new approaches to the treatment of breast cancer, particularly endocrine-resistant disease.
Oestrogen and antiestrogen signalling in breast cancer

Although the genetic and environmental factors that lead to the initiation of breast cancer remain largely undefined, it is known that cumulative exposure to endogenous and exogenous oestrogens plays a crucial role in the development and progression of this disease (Colditz 1998). Epidemiological evidence has shown clear associations between an increased risk of developing breast cancer and early age of menarche, late menopausal age, pregnancy, serum oestrogen levels and use of hormone replacement therapies. These effects are thought to be mediated predominantly via the mitogenic properties of oestrogens, although effects of oestrogen on cell death, cell motility and invasion may also contribute.

The effects of oestrogen are mediated through nuclear ligand-activated transcription factors, the oestrogen receptors (ERs). Two ERs have been characterised, ERα and ERβ, although studies using ER knockout models have shown that ERα is the predominant mediator of the mitogenic effects of oestrogen in the mammary gland (Hewitt et al. 2005). ERα contains two C4-type zinc fingers and binds as a dimer to palindromic regulatory promoter elements termed oestrogen response elements (EREs) within specific target genes. The binding of oestrogen to the ERs induces an increase in receptor phosphorylation resulting in a conformational change that exposes the DNA-binding region and transcriptional activation domains. The stable ER dimer binds to an ERE in the target gene, allowing formation of a transcription complex (Beekman et al. 1993). ERs can also regulate gene expression without directly interacting with DNA, via protein–protein interactions with heterologous transcription factors such as the Fos/Jun activating protein-1 (AP-1) complex and Sp1 (Kushner et al. 2000).

Two distinct regions within ERα contribute to its transcriptional activity. The amino-terminus contains a ligand-independent activation function (AF-1), and a ligand-regulated AF-2 domain is present within the carboxyl-terminal ligand binding domain. Depending on the promoter and cell type examined, AF-1 and AF-2 can regulate transcription independently or synergistically (Tzukerman et al. 1994). The effects of antioestrogens are thought to be mediated via modulation of the following activation functions: the pure, steroidal antioestrogen, ICI 164,384 prevents activation of AF-1 and AF-2, and the antagonistic activity of TAM results from inhibition of AF-2 while its agonistic activity is mediated by activation of AF-1 (Berry et al. 1990). The response of the two ERs to antioestrogens is also distinct. Several studies have demonstrated that TAM and ICI 164,384 have an ERα-specific partial agonist/antagonist function, but an antagonist effect through ERβ (McDonnell et al. 1995, Barkhem et al. 1998).

In addition to hormone-mediated activation, there is now compelling evidence of ligand- and DNA-independent activation of ER through cross-talk with cell-surface tyrosine kinase receptors and their intracellular signalling cascades (see Fig. 1). Polypeptide growth factors such as epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I), transforming growth factor (TGF)-α and heregulin can activate ER and increase expression of ER target genes in breast cancer cells (Pietras et al. 1995, Bunone et al. 1996, Lee et al. 1997), with some suggestion that the mitogenic effects of these factors may, in part, be mediated via ER signalling. The clearest demonstration of this is in the uteri of ERα knockout mice, where wild-type, functional levels of EGF and EGF receptor (EGFR) are maintained, but the mitogenic actions of EGF are ablated (Curtis et al. 1996). It is postulated that this cross-talk may act to amplify growth factor signalling pathways, thereby enhancing mitogenesis in ER-positive tissues.

The majority of evidence indicates that modification of the phosphorylation status of the ER by cellular kinases may be an important point of convergence between growth factor and ER signalling pathways. The amino-terminal AF-1 domain of ERα is required for growth factor signalling (Ignar-Trowbridge et al. 1996), and is phosphorylated on serine 118 by the mitogen-activated protein kinase (MAPK) pathway following treatment with EGF or IGF (Kato et al. 1995). Ligand-independent activation of ERα has also been demonstrated in MCF-7 cells via the activation of phosphatidylinositol (PI) 3-kinase/Akt (Martin et al. 2000, Campbell et al. 2001, Stoica et al. 2003). Studies utilizing specific inhibitors of MAPK and PI 3-kinase have demonstrated that these pathways have some influence on oestrogen-induced mitogenesis in breast cancer cells (Lobenhofer et al. 2000). Furthermore, Stoica et al. (2003) have shown that a constitutively active Akt mutant can mimic the effects of oestrogen in breast cancer cells.

Several in vivo studies have emphasised the physiological relevance of growth factor and ER cross-talk in breast cancer progression and endocrine responsiveness. Dysregulation of growth factor receptor signalling is a hallmark of malignant progression in the breast (Slamon et al. 1987), and is often associated with increased activation of MAPK and PI 3-kinase
pathways (Sivaraman et al. 1997, Coutts & Murphy 1998, Santen et al. 2002). Thus, enhanced interactions between growth factors and ERs during tumour progression could lead to ligand-independent activation of ER and a consequential loss of therapeutic responsiveness (Weigel & Zhang 1998, Campbell et al. 2001, Dowsett et al. 2001). A further complexity is demonstrated by studies showing that long-term oestrogen-deprivation is associated with increased EGF-mediated growth response and MAPK activation in breast cancer cells (Miller et al. 1994, Jeng et al. 2000, Yarden et al. 2001, Martin et al. 2003). Support for these proposed mechanisms of endocrine resistance has been demonstrated in experimental models, where endocrine insensitivity is associated with increased EGFR and PI3 kinase activity (McClelland et al. 2001, Knowlden et al. 2003, Jordan et al. 2004), and treatment with specific tyrosine kinase inhibitors improves endocrine-responsiveness and can reverse or delay TAM-resistant tumour growth (Kurokawa et al. 2000, Gee et al. 2003). This concept has also been translated to the clinical setting, where trials are currently underway combining endocrine therapy with EGFR tyrosine kinase- or HER2-specific inhibitors (Robertson et al. 2003). There is also the potential that such downstream signalling molecules may have clinical relevance as predictive markers of endocrine response.

Cyclins and Myc as mediators of oestrogen action in breast cancer

In terms of downstream transcriptional signalling, one of the earliest responses to oestrogen is increased c-Myc expression, which occurs within 15 min of oestrogen stimulation (Dubik et al. 1987, Dubik & Shiu 1988). The DNA binding region of ERα is

The c-Myc protein is a nuclear transcription factor that displays high-affinity, site-specific DNA-binding activity when complexed with its cellular partner, Max (Blackwood & Eisenman 1991). Myc has profound mitogenic effects on breast cancer cells through its ability to modulate regulators of cell cycle progression (Hanson et al. 1994, Nass & Dickson 1997). Inhibition of c-Myc expression abrogates oestrogen-stimulated breast cancer cell proliferation (Watson et al. 1991), and blocks cell cycle progression leading to a G1 arrest (Heikkila et al. 1987). Furthermore, induction of c-Myc can mimic the effects of oestrogen and induce antioestrogen-arrested cells to reinitiate cell cycle progression (Prall et al. 1998), implicating Myc as a prominent mediator of oestrogen action in breast cancer cells.

While regions in the C-terminal dimerisation domain are required to elicit the mitogenic effects of c-Myc, there is also evidence suggesting that N-terminal regions play an important role (Oster et al. 2003). The N-terminus contains a transcription regulation domain with two evolutionarily conserved motifs termed Myc box I and Myc box II (Oster et al. 2003). Our recent studies using specific c-Myc mutants have demonstrated a requirement for the N-terminal region including Myc box II in Myc-mediated cell cycle progression in breast cancer cells (CM Sergio, AJ Butt, EA Musgrove & RL Sutherland, unpublished data).

Numerous genetic targets for c-Myc activation and repression have been identified (Dang 1999), and its expression has been associated with activation of cell cycle regulators (Jansen-Durr et al. 1993, Rudolph et al. 1996, Perez-Roger et al. 1997). However, a major mechanism governing Myc’s effects on cell cycle progression in breast cancer cells, appears to be via the activation of cyclin E/Cdk2 through loss of the cyclin dependent kinase (CDK) inhibitor, p21^{WAF1,Cip1} (Prall et al. 1998, Lai et al. 2001, Mukherjee & Conrad 2005). In this respect, c-Myc’s actions closely mimic those of oestrogen (Foster & Wimalasena 1996, Planas-Silva & Weinberg 1997, Prall et al. 1997, 2001). The mechanism governing this action is not entirely clear, although it is thought to involve estrogen-mediated inhibition of the CDK inhibitor, p21^{WAF1,Cip1} (Planas-Silva & Weinberg 1997, Prall et al. 1997). Cyclin D1 expression also elicits similar effects to c-Myc on the activation of cyclin E-Cdk2 (Prall et al. 1998). However, in this model system overexpression of cyclin D1 did not induce c-Myc expression or vice versa, suggesting that oestrogen-stimulated cell cycle progression is mediated initially by distinct c-Myc and cyclin D1 pathways that converge on the activation of cyclin E-Cdk2 (Prall et al. 1998).

In addition to its effects on cyclin D1, oestrogen also elicits rapid activation of cyclin E-cdk2 in breast cancer cells (Foster & Wimalasena 1996, Planas-Silva & Weinberg 1997, Prall et al. 1997, 2001). The mechanism governing this action is not entirely clear, although it is thought to involve estrogen-mediated inhibition of the CDK inhibitor, p21^{WAF1,Cip1} (Planas-Silva & Weinberg 1997, Prall et al. 1997). Cyclin D1 expression also elicits similar effects to c-Myc on the activation of cyclin E-Cdk2 (Prall et al. 1998). However, in this model system overexpression of cyclin D1 did not induce c-Myc expression or vice versa, suggesting that oestrogen-stimulated cell cycle progression is mediated initially by distinct c-Myc and cyclin D1 pathways that converge on the activation of cyclin E-Cdk2 (Prall et al. 1998).

In addition to their role as oestrogen targets, cyclins and Myc also represent an important point of convergence between growth factor and ER signalling cascades (see Fig. 1). Both oestrogen and insulin/IGF-I can stimulate the expression of cyclins D1 and E, as well as c-Myc via MAPK and PI-3 kinase pathways (Dufourny et al. 1997, Prall et al. 1997, Lai et al. 2001, Mawson et al. 2005), with recent studies suggesting that both c-Myc and cyclin D1 may require growth radio-therapies (Teixeira et al. 1995, Huang et al. 1997), but may also be a significant component of oestrogen-stimulated breast tumour growth in vivo (Kyprianou et al. 1991). However, their relevance in clinical responses to antioestrogen therapies remains an open question.

The effects of oestrogen on cell cycle progression are also tightly linked to increased expression of cyclin D1. Cyclin D1 induction in breast cancer cells shortens G1 and can rescue growth factor deprived and antioestrogen-arrested cells enabling them to complete the cell cycle (Musgrove et al. 1994). Cyclin D1 expression in breast cancer cells has consistently been found to associate with ER positivity (Buckley et al. 1993, Barbarechi et al. 1997, Utsumi et al. 2000). While oestrogen rapidly induces cyclin D1 expression, antioestrogens have a converse acute, inhibitory effect (Musgrove et al. 1993, Watts et al. 1994, Altucci et al. 1996, Prall et al. 1997, Wilcken et al. 1997). Furthermore, abrogation of cyclin D1 activity by cyclin D1 antibodies or the cdk4 inhibitor p16\textsuperscript{INK4A}, blocks oestrogen-induced G1-S phase progression (Lukas et al. 1996), indicating that oestrogen acts, at least in part, through upregulation of cyclin D1 expression. Like c-Myc, cyclin D1 expression can mimic the effects of oestrogen allowing cell cycle re-entry in antioestrogen-arrested breast cancer cells (Prall et al. 1998, Wilcken et al. 1997).

In addition to its effects on cyclin D1, oestrogen also elicits rapid activation of cyclin E-cdk2 in breast cancer cells (Foster & Wimalasena 1996, Planas-Silva & Weinberg 1997, Prall et al. 1997, 2001). The mechanism governing this action is not entirely clear, although it is thought to involve estrogen-mediated inhibition of the CDK inhibitor, p21^{WAF1,Cip1} (Planas-Silva & Weinberg 1997, Prall et al. 1997). Cyclin D1 expression also elicits similar effects to c-Myc on the activation of cyclin E-Cdk2 (Prall et al. 1998). However, in this model system overexpression of cyclin D1 did not induce c-Myc expression or vice versa, suggesting that oestrogen-stimulated cell cycle progression is mediated initially by distinct c-Myc and cyclin D1 pathways that converge on the activation of cyclin E-Cdk2 (Prall et al. 1998).

In addition to their role as oestrogen targets, cyclins and Myc also represent an important point of convergence between growth factor and ER signalling cascades (see Fig. 1). Both oestrogen and insulin/IGF-I can stimulate the expression of cyclins D1 and E, as well as c-Myc via MAPK and PI-3 kinase pathways (Dufourny et al. 1997, Prall et al. 1997, Lai et al. 2001, Mawson et al. 2005), with recent studies suggesting that both c-Myc and cyclin D1 may require growth
factor signalling to mediate their mitogenic effects (Mawson et al. 2005). The PI3-kinase pathway can also regulate cyclin D1 stability and intracellular localisation via its inhibitory effects on glycogen synthase kinase 3β. The latter phosphorylates cyclin D1 leading to its translocation from the nucleus to the cytoplasm (Diehl et al. 1998, Hamelers et al. 2002) and increased degradation. MAPK activity has also been linked to increased cyclin D1 expression at both the mRNA and protein level (Balmanno & Cook 1999).

**Cyclins and Myc as potential players in endocrine resistance**

While there have been many studies in which cohorts of archival breast tumours have been analysed for expression of c-Myc and various cyclins, the relationship between these putative markers and response to endocrine therapy in such cohorts is often undefined. Thus, it is in cell culture systems that the evidence for the involvement of cyclins and c-Myc in the response to antioestrogens is most compellingly demonstrated.

The role of c-Myc in the proliferative response to oestrogens is discussed above. Importantly, there is also evidence to suggest that c-Myc plays a role in the development of antioestrogen resistance. Antioestrogen treatment in the form of oestrogen withdrawal, aromatase inhibition, TAM and faslodex, all down-regulate c-myc mRNA and induce cell cycle arrest (Carroll et al. 2002, Thiantanawat et al. 2003), while down-regulation of c-Myc with synthetic antisense oligonucleotides parallels the effects of antioestrogens on cell cycle progression (Carroll et al. 2002). Conversely, the acquisition of oestrogen-independence in MCF-7 cells maintained in oestrogen-deprived medium is associated with the upregulation of selected oestrogen-regulated genes including ER, and c-myc (Jeng et al. 1998). Furthermore, overexpression of c-Myc alone is able to partially reverse the growth suppressive effects of the antioestrogen ICI 182,780 in MCF-7 cells (Prall et al. 1998, Venditti et al. 2002).

The amplification of growth factor receptor signalling cascades can also converge on activation of c-Myc, thus potentially influencing endocrine responsiveness. High levels of erbB2/erbB3 signalling are frequently observed in breast cancer and lead to persistent Ras and Akt activity via amplification of MAPK and PI-3 kinase pathways. Ras has been shown to phosphorylate c-Myc at serine-62 leading to stabilization of the protein (Sears et al. 1999), and activation of the PI-3 kinase pathway stimulates translation of c-myc mRNA species (West et al. 1998, Sears et al. 1999) and stabilization of the protein (Sears et al. 2000). Furthermore, c-Myc protein levels are reduced by an ErbB2 inhibitor (PD153035) and this effect is reversed by ectopic expression of c-myc (Neve et al. 2000). This is consistent with the clinically observed antioestrogen resistance seen in breast cancers overexpressing erbB2. The synergistic interaction between deregulated c-Myc and EGFR signalling has also been seen in mammary tumours in transgenic mice (Nass & Dickson 1998). It is notable that co-amplification of erbB2 and c-myc is associated with poorer survival in several clinical cohorts (Roux-Dosseto & Martin 1989, Cuny et al. 2000, Al-Kuraya et al. 2004) although data are conflicting in this regard (Schlotter et al. 2003).

At a clinical level, the impact of MYC amplification and expression on prognosis and in particular, response to endocrine therapy is less clear than would be suggested from cell line studies. MYC is frequently amplified in human breast cancers (Table 1), and is significantly associated with tumour grade, lymph node status, and post-menopausal status (Deming et al. 2000). Furthermore, MYC amplification increased the risk of relapse and death (Naidu et al. 2002, Schlotter et al. 2003, Al-Kuraya et al. 2004). However, there are few data evaluating the effect of MYC amplification on response to endocrine therapy in patients with breast cancer, and those that exist are inconsistent with observations from in vitro studies. Schlotter et al. (2003) found in a cohort of 181 patients with node negative breast cancer that although MYC amplification predicted for recurrence, no differences were detected in the response to TAM treatment among patients with and without gene amplification. In another study, those patients with MYC amplification tended to have a slightly longer progression-free survival on endocrine therapy (Berns et al. 1995). However, it is difficult to draw conclusions regarding the role of c-Myc from these small studies. Future clinical investigations using retrospective tumour cohorts need to consider factors such as the rates of TAM use, the levels of ER and Her2/neu expression, and the endocrine effects of chemotherapeutic agents in premenopausal women.

| Table 1 Aberrations of cell cycle regulators in breast cancer |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Frequency range (%) |     Mean (%)     |                 |                 |                 |                 |                 |                 |
| MYC amplification| 4–52             | 19              |                 |                 |                 |                 |                 |                 |
| c-Myc overexpression| 11–70           | 38              |                 |                 |                 |                 |                 |                 |
| 11q13 amplification| 9–17             | 13              |                 |                 |                 |                 |                 |                 |
| Cyclin D1 overexpression| 28–81           | 45              |                 |                 |                 |                 |                 |                 |
| Cyclin E overexpression| 28–35           | 32              |                 |                 |                 |                 |                 |                 |
| Decreased p27| 50–63            | 57              |                 |                 |                 |                 |                 |                 |

**Table 1** Aberrations of cell cycle regulators in breast cancer.
Similarly, in clinical cohorts the role of p21WAF1/Cip1 in overall outcome and response to antioestrogens remains the subject of debate. Some investigators show that p21WAF1/Cip1 expression predicts responsiveness to antioestrogens (Pellikainen et al. 2003), while other studies have not shown prognostic benefit in multivariate analyses (Gohring et al. 2001, O’Hanlon et al. 2002). In contrast, other investigators have shown a negative association between cytoplasmic p21WAF1/Cip1 expression and outcome (Caffo et al. 1996, Winters et al. 2003, Xia et al. 2004). These conflicting data may reflect the fact that p21 WAF1/Cip1 function in these tumours can also be influenced by p53 status, titration by cyclin-CDK complexes and intracellular localisation.

Like Myc, evidence demonstrating the role of cyclins in mediating the proliferative effects of oestrogen, suggest they may also be involved in the development of antioestrogen resistance. Of these, it is the cyclins D1 and E that have been most comprehensively studied. In vitro studies have demonstrated that a reduction in cyclin D1 mRNA and protein expression is an early and critical event in antioestrogen action (Musgrove et al. 1993, Watts et al. 1995). Furthermore, constitutive and inducible expression of cyclin D1 rescues breast cancer cells from antioestrogen-induced growth arrest (Wilcken et al. 1997, Prall et al. 1998, Hodges et al. 2003). However, previous studies from this laboratory have shown that constitutive over-expression of cyclin D1 in T-47D breast cancer cells, can decrease sensitivity to the antioestrogen ICI 182,780 in the short-term (up to 48 h, see Fig. 2), suggesting that cyclin D1 may abrogate the early cell cycle effects of antioestrogen inhibition (Hui et al. 2002). Sustained expression of cyclin D1 is also seen in breast cancer cells during their acquisition of TAM resistance (Kilker et al. 2004). In these cells ER expression and function remain intact, and the pure antioestrogen, ICI 164,384 retains its anti-proliferative effects via suppression of cyclin D1. This is consistent with the clinically observed benefit seen in patients with TAM-resistant tumours who are able to derive benefit from second line therapy with ER down-regulators (Howell et al. 2005). Interestingly, over-expression of cyclin D1 confers complete resistance to the growth inhibitory effects of progestins (Musgrove et al. 2001). Cyclin D1 can also potentiates the transcriptional activity of the ER independently of estradiol, with some evidence that this effect is not inhibited by antioestrogens (Neuman et al. 1997, Zwijsen et al. 1997). This suggests a further mechanism by which the overexpression of cyclin D1 frequently observed in breast cancers, could lead to sustained ER signalling and endocrine resistance.

**Figure 2** Overexpression of either cyclin D1 or cyclin E modulates the response to ICI 182,780. (A) Western analysis of cyclin D1 and cyclin E expression in T-47D cells stably transfected with empty vector, human cyclin D1 or human cyclin E. (B) Acute effects of cyclin D1 and cyclin E overexpression on the response to ICI 182,780. After treatment of proliferating cells with ICI 182,780 at the concentrations shown, cells were harvested and stained with ethidium bromide. The S phase fraction was determined by flow cytometry and represented relative to vehicle treated controls. Data points indicate mean ± S.D. of duplicate experiments (Hui et al. 2002).
The situation is less clear when in vitro hypotheses are tested in a clinical setting. Cyclin D1 is overexpressed in ~45% of breast cancers (Table 1), although there are conflicting data regarding the overall clinical outcome seen with cyclin D1 amplification and overexpression. A large number of studies have examined the prognostic effect of changes in cyclin D1, and several show that a poor outcome is associated with amplification at the 11q13 locus (Dickson et al. 1995). This negative effect was seen in another cohort, but in ER+ve patients only (Seshadri et al. 1996). Subsequently, Bieche et al. (2002) demonstrated a shortening of relapse free survival in association with CCND1 amplification. Many other studies have reported conflicting relationships between cyclin D1 overexpression and clinical outcome. Variation in methodologies, adjuvant treatment, ER assessment, and the heterogeneity inherent in human populations may account for some of this variability. Certainly it is difficult to draw definitive conclusions about the relationship between cyclin D1 expression and prognosis from these studies.

When the more specific question of the role of cyclin D1 in endocrine responsiveness in clinical cohorts is addressed, again the data are often conflicting. Kenny and colleagues found that increased expression of cyclin D1 mRNA was associated with a reduced response to TAM treatment (Kenny et al. 1999). Stendahl et al. (2004) showed similar results using immunohistochemistry in their cohort of 167 patients. However, others have shown a trend towards superior response to TAM in metastatic ER+ve tumours that overexpress cyclin D1 (Han et al. 2003a). Thus, the true impact of cyclin D1 on the response and resistance to antioestrogens in a clinical setting remains the subject of debate, and is urgently in need of further study in large cohorts of known therapeutic responsiveness.

It is clear that cyclin E-cdk2 complexes are crucial in mediating oestrogen-induced progression through the G1-S phase of the cell cycle. However, there also exist in vitro data for the role of cyclin E in the development of antioestrogen resistance. Studies in MCF-7 cells have shown that a three-fold overexpression of cyclin E is able to abrogate TAM-mediated growth arrest (Dhillon & Mudryj 2002). Hui et al. (2002) have also demonstrated that cyclin E overexpression confers partial resistance to the acute, inhibitory effects of ICI 182,780, although to a lesser extent than that observed with cyclin D1. Nonetheless, in clonogenic survival assays overexpression of both cyclin D1 and cyclin E confer significant resistance to the growth inhibitory effects of ICI 182,780 (see Fig. 2). Cyclin E is overexpressed in ~30% of breast cancers (Table 1), and studies of protein expression in tumour specimens have shown that cyclin E levels correlate strongly with disease-specific and overall survival in stage I-III disease. In addition, the production of low-molecular weight isoforms of cyclin E, which is unique to tumour cells, appears to confer resistance to the effects of CDK inhibitors p21 and p27, and to the effects of antioestrogens in MCF-7 cells (Akli et al. 2004). It has also been noted that in experimental systems, overexpression of the low molecular weight isoforms of cyclin E is associated with a defect in progression through S phase with concomitant accumulation of chromosomal instability (Akli et al. 2004). Importantly, this work demonstrated that cyclin E outperformed other independent clinical and pathological risk factors, and is consistent with the data from several other clinical studies showing adverse outcome in association with cyclin E overexpression (Kim et al. 2001, Keyomarsi et al. 2002, Han et al. 2003b, Rudolph et al. 2003). However, on multivariate analysis a number of other clinical studies have failed to show any association between cyclin E expression and outcome (Bukholm et al. 2001, Rudolph et al. 2003). There is some evidence that cyclin E expression is associated with poor relapse-free survival specifically in patients treated with endocrine therapy (Span et al. 2003). Other studies have shown that antioestrogen treatment has no influence on disease-specific survival among ER+ve cyclin E overexpressors, suggestive that cyclin E confers resistance to antioestrogens (Keyomarsi et al. 2002). Again, more definitive conclusions on the role of cyclin E in endocrine resistance must await data from large, randomised treatment trials.

Do cyclins and Myc represent novel therapeutic targets in endocrine resistance?

Evidence from several studies suggest that the development of endocrine resistance is not due to alterations in ERα expression or function (Taylor et al. 1982, Clarke et al. 1993), although more recent data suggesting that sustained growth factor signalling is associated with loss of ERα expression through promoter methylation, indicates that this may not be universal (Nicholson et al. 2005). Furthermore, the fact that patients resistant to TAM respond to second-line therapies such as pure antioestrogens, aromatase inhibitors and progestins, imply that ER signalling pathways may remain, at least in part, functional. There is also clear evidence from both experimental
models and clinical studies, that the cross-talk between growth factor and ER signalling pathways is a potent influence on breast cancer progression and endocrine responsiveness.

Myc and cyclins, in particular cyclin D1, represent attractive therapeutic targets for breast cancer for a number of reasons. They are both rate-limiting for progression through G1 phase of the cell cycle (Musgrove et al. 1994, Prall et al. 1998), and have been implicated in the development of mammary hyperplasia and carcinogenesis (Wang et al. 1994, Nass & Dickson 1997). They are also frequently overexpressed in human breast cancers (Table 1), potentially influencing prognosis and response to therapy. Perhaps more importantly for treatment efficacy, they represent an important point of convergence downstream of both growth factor and ER signalling pathways (Fig. 1), and could, therefore, influence multiple signalling cascades, overcoming resistance. Results from recent studies combining growth factor inhibitors with TAM have provided support for this strategy (Robertson et al. 2003, Chu et al. 2005). There are also some encouraging results from therapies directly targeting activation of cyclin D1 and cyclin E (Zafonte et al. 2000, Mita et al. 2003). However, enthusiasm for these targets is tempered to some extent by the knowledge that c-Myc, cyclin D1 and cyclin E play pivotal roles in the control of proliferation and survival in normal cells. Nonetheless, the cell type-specific effects of these molecules, as noted in cyclin knockout models, and the tumour-specific expression of novel isoforms in the case of cyclin E, imply opportunities for tumour site-specific inhibition.

In conclusion, while antioestrogen therapies have had a significant impact on improving outcome for patients with breast cancer, our increased understanding of the signalling pathways involved in endocrine responsiveness has provided novel opportunities for new therapies. Evidence clearly implicates cyclins and Myc as the converging effectors of both oestrogen and growth factor actions in breast cancer, and thus, potential mediators of antioestrogen resistance. As such, they may represent promising targets, not only for therapeutic intervention, but also as markers of endocrine responsiveness.

Acknowledgements

Work in this laboratory is supported by the National Health and Medical Research Council (NHMRC) of Australia, The Cancer Council of NSW, the Association for International Cancer Research, the CureCancer Australia Foundation, the RT Hall Trust and the US Department of Defence Breast Cancer Research Program (DAMD17-99-1-9184). AJB and EAM are Research Fellows of the Cancer Institute NSW, and CMM is a National Health and Medical Research Council and Cancer Institute NSW Research Scholar.

References


Balmano K & Cook S 1999 Sustained MAPK kinase activation is required for the expression of cyclin D1, p21\textsuperscript{Cip1} and a subset of AP-1 proteins in CCL39 cells. Oncogene 18 3085–3097.


Beekman JM, Allan GF, Tsai SY, Tsai MJ & Omalley BW 1993 Transcriptional activation by the estrogen-receptor requires a conformational change in the ligand-binding domain. Molecular Endocrinology 7 1266–1274.


Berry M, Metzger D & Chambon P 1990 Role of the two activating domains of the oestrogen receptor in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen. EMBO Journal 9 2811–2818.


Kushner PJ, Agard DA, Greene GL, Scanlan TS, Shiu AU, Uht RM & Webb P 2000 Estrogen receptor pathways to...


McClelland RA, Barrow D, Madden TA, Dutkowski CM, Pamment J, Knowlden JM, Gee JM & Nicholoson RI 2001 Enhanced epidermal growth factor receptor signaling in MCF-7 breast cancer cells after long-term culture in the presence of the pure antiestrogen ICI 182,780 (Faslodex). Endocrinology 142 2776–2788.


Musgrove EA, Lee CS, Buckley MF & Sutherland RL 1994 Cyclin D1 induction in breast cancer cells shortens G1 and is sufficient for cells arrested in G1 to complete the cell cycle. PNAS 91 8022–8026.


Prall OWJ, Sarcevic B, Musgrove EA, Watts CK & Sutherland RL 1997 Estrogen-induced activation of cdk4 and cdk2 during G1-S phase progression is accompanied by increased cyclin D1 expression and decreased cyclin-dependent kinase inhibitor association with cyclin E-cdk2. Journal of Biological Chemistry 272 10882–10894.

Prall OWJ, Rogan EM, Musgrove EA, Watts CK & Sutherland RL 1998 c-Myc or cyclin D1 mimics estrogen effects on cyclin E-cdk2 activation and cell cycle reentry. Molecular and Cellular Biology 18 4499–4508.


Tzukerman MT, Esty A, Santiso-Mere D, Danielian P, Parker MG, Stein RB, Pike JW & McDonnell DP 1994 Human estrogen receptor transactivational capacity is determined by both cellular and promoter context and


