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c-Myc overexpression and endocrine resistance in breast cancer☆

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Abstract

The oncoprotein c-Myc is frequently overexpressed in breast cancer and ectopic expression in breast cancer cell lines attenuates responses to antiestrogen treatment. Here, we review preliminary data aimed at further elucidating a potential role for c-Myc in clinical endocrine resistance in breast cancer. Immunohistochemical and semi-quantitative PCR revealed that c-Myc protein and c-*myc* mRNA were frequently overexpressed in both ER-positive and ER-negative breast carcinoma. Furthermore, both constitutive and inducible c-Myc overexpression in MCF-7 breast cancer cell lines markedly reduced their sensitivity to the growth inhibitory effects of the pure antiestrogen ICI 182,780. In order to identify potential downstream targets of c-Myc that mediate this effect, Affymetrix microarrays were employed to examine the patterns of gene expression shared by MCF-7 cells stimulated by estrogen, or by induction of c-Myc. Approximately 50% of estrogen target genes identified 6 h after treatment were also regulated by c-Myc. One novel target, EMU4, was transcriptionally regulated by c-Myc. In addition, there was a strong correlation between c-*myc* and *EMU4* mRNA expression in a battery of breast cancer cell lines. These data confirm that c-Myc overexpression is a common event in breast cancer, and that this is associated with resistance to antiestrogens *in vitro*. Furthermore, the development of an experimental paradigm for the discovery of c-Myc and estrogen target genes associated with endocrine resistance provides a framework for the discovery and validation of genes involved in estrogen signalling, and c-Myc-mediated-antiestrogen resistance.

Keywords: Breast cancer; c-Myc; Endocrine resistance

1. Introduction

Breast cancer represents a major public health problem in industrialised and developing countries. While breast cancer-related survival has improved by ∼25% over the last 10–20 years as a result of improvements in screening and in local and systemic treatment, it remains a major cause of mortality among women, accounting for 15–20% of cancer related deaths [1].

The female sex-steroid estrogen plays a key role in the control of cellular proliferation in breast cancer [2]. For the ∼70% of women whose breast cancers express estrogen receptors (ER), modulation of estrogen synthesis (aromatase inhibitors) and ER signalling by blockade of ER (antiestrogens) remain the cornerstone of the systemic treatment of their disease. Treatments, such as tamoxifen reduce the

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relative risk of disease relapse and breast cancer-related death by 50% and 30%, respectively [3]. Nonetheless, many women treated with adjuvant endocrine therapy eventually relapse and die as a result of their breast cancer (in the order of 10–15% in the ATAC trial which enrolled a cohort of relatively good prognosis patients) [4].

The biological basis of *de novo* or acquired antiestrogen resistance as it is manifest in the clinic remains to be fully defined, although there is evidence to support a causal relationship with upregulation of growth-factor signalling pathways that cross-talk with ER signalling [5,6], adaptive hypersensitivity to estrogen deprivation, changes in the co-expression of ERβ, and loss or mutation of ERα itself (reviewed by Ring and Dowsett [7]). An area of research that has received somewhat less attention is the role played by aberrant expression and activity of cell cycle regulatory proteins in the development of antiestrogen resistance.

Estrogens and antiestrogens regulate a number of genes critical to cell cycle entry and progression at the G_1/S interface [8]. Of these, one of the key effectors of estrogen signalling is the transcription factor c-Myc. The expression of c-Myc is rapidly induced by estrogen treatment, and conversely, downregulated by antiestrogen treatment [9,10]. Furthermore, anti-sense oligonucleotides directed against c-Myc inhibit estrogen-induced cell proliferation in a manner similar to antiestrogens [10,11], while c-Myc overexpression recapitulates the effects of estrogen on cell cycle progression in cells arrested in G_0/G_1 by pre-treatment with either SERMS or pure antiestrogens [12].

In clinical cohorts, MYC gene amplification has been associated with the transition from *in situ* to invasive carcinoma [13], markers of aggressive phenotype [14,15] and poor prognosis in general [14,16–19]. It has been reported as an independent predictor of survival in patients treated with tamoxifen [20]. MYC gene amplification occurs in approximately 15–20% of patients with breast cancer [14]. However, overexpression of c-*myc* mRNA and c-Myc protein occurs more frequently, generally 30–50% [19,21,22], particularly in high-grade tumours [23]. While some studies link c-*myc* mRNA overexpression with adverse outcome [19], others studies do not [24]. Immunohistochemical studies have generally failed to demonstrate an association between c-Myc protein expression and outcome [25,26]. While some studies show an association between c-Myc overexpression and negative prognostic factors such as poor differentiation and high proliferation index [21], and others document co-operation with Bcl-2 overexpression in promoting lymph node metastasis [27], at present it is difficult to draw definite conclusions regarding the prognostic significance of c-Myc protein overexpression in breast cancer.

On the basis of these *in vitro* and clinical studies, we propose that altered expression of c-Myc and key downstream target genes may be involved in the development of antiestrogen resistance. Indeed, there is now an accumulating body of evidence from our laboratory and others in support of this concept, whereby inducible expression of c-Myc attenuates the anti-proliferative effects of antiestrogen treatment in MCF-7 cells [12,28–30]. Furthermore, sustained expression of c-Myc has been observed in the development of tamoxifen-resistant cell lines, suggesting that these molecular changes may contribute to clinical antiestrogen resistance [31].

In this brief review, we summarise the experimental approach, and present some preliminary data aimed at more clearly defining the role of c-Myc and selected target genes in the acquisition of antiestrogen resistance in breast cancer.

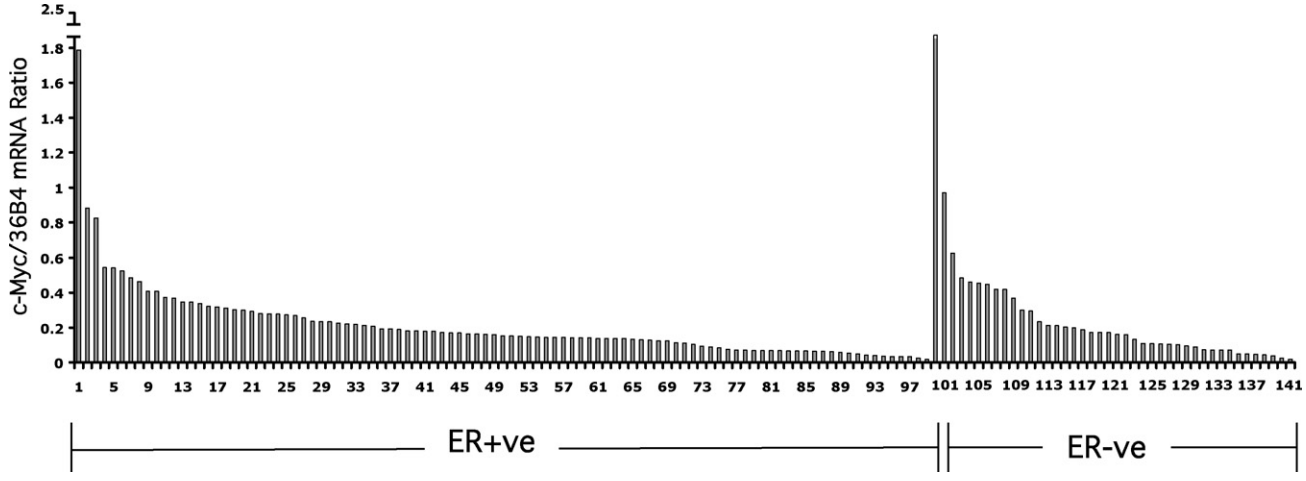

Fig. 1. c-Myc mRNA expression in clinical breast cancer. Expression of c-*myc* and the housekeeping gene 36B4 were analysed by RT-PCR in 141 primary breast tumour RNA samples using the Promega Reverse Transcription System (Promega, USA) and the Roche Lightcycler Fast Start DNA Master Sybr Green 1 kit (Roche, Germany), and a Rotor-gene RG-3000 (Corbett Research, Australia). A standard curve was generated for both c-*myc* and 36B4 from PCR product, and the relative expression calculated for each sample, normalised to 36B4 expression. The PCR reactions were performed in triplicate, from two independent reverse transcription reactions.

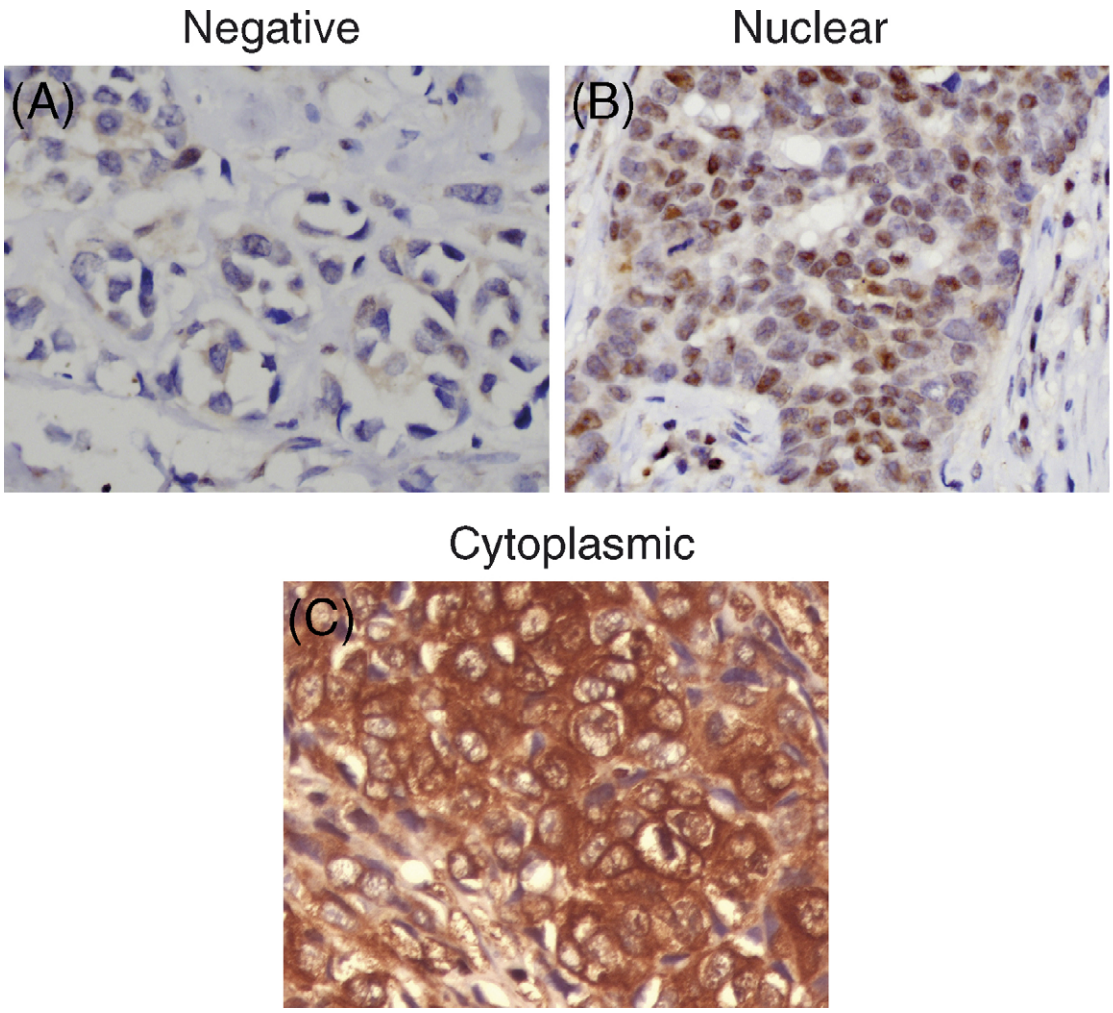

Fig. 2. Immunohistochemistry of c-Myc expression in breast cancer. c-Myc protein expression was measured using immunohistochemistry on tissue microarrays constructed from paraffin-embedded blocks of infiltrating ductal carcinomas from 289 patients [32] treated by a single surgeon (PC). Of these patients, cores from 217 were analysable for both c-Myc and ER. c-Myc staining was performed using mouse monoclonal anti-human c-Myc, 9E10 (Dako, USA), and ER staining was performed using mouse monoclonal anti-human ER, ID5 (Dako, USA). The cohort was scored for each antibody by two independent observers (CMMcN and JGK) one of whom was a pathologist, and both of whom were blinded to patient identity and outcome. For c-Myc, cores were assessed for both nuclear and cytoplasmic staining by percentage of cells staining positive, and the intensity of staining in the range 0–3. Examples of the staining patterns are illustrated: (A) negative, (B) nuclear, and (C) cytoplasmic. Data for the whole cohort are presented in Table 1.

2. Materials and methods

The human breast cancer tissue samples and *in vitro* cell culture models employed in this study have been described in detail in previous publications from this laboratory [12,32,33]. Specific details of individual experiments are presented in the legends to Figs. 1–6.

3. Results and discussion

3.1. c-myc mRNA and protein expression in clinical breast cancer

In order to determine the range of c-*myc* mRNA and c-Myc protein expression in samples of clinical breast cancer, we employed quantitative RT-PCR and immunohistochemistry on two independent cohorts of breast cancer patients where either RNA or tissue microarrays were available. Fig. 1 presents the mRNA expression data, relative to the housekeeping gene 36B4 in a cohort of 141 breast carcinomas. A wide range of relative c-*myc* mRNA expression was observed in both ER+ and ER− breast cancers with the relative range and frequency of expression similar between the two groups. Likewise, when protein expression was evaluated using immunohistochemistry in a separate cohort of 217 primary breast cancer samples, c-Myc overexpression was observed in both ER+ and ER− cancers. As detailed in Table 1, the prevalence of overexpression was slightly higher in the ER+ tumours when nuclear or cytoplasmic staining were evaluated individually or in combination, although the difference between the ER+ and ER− groups was most

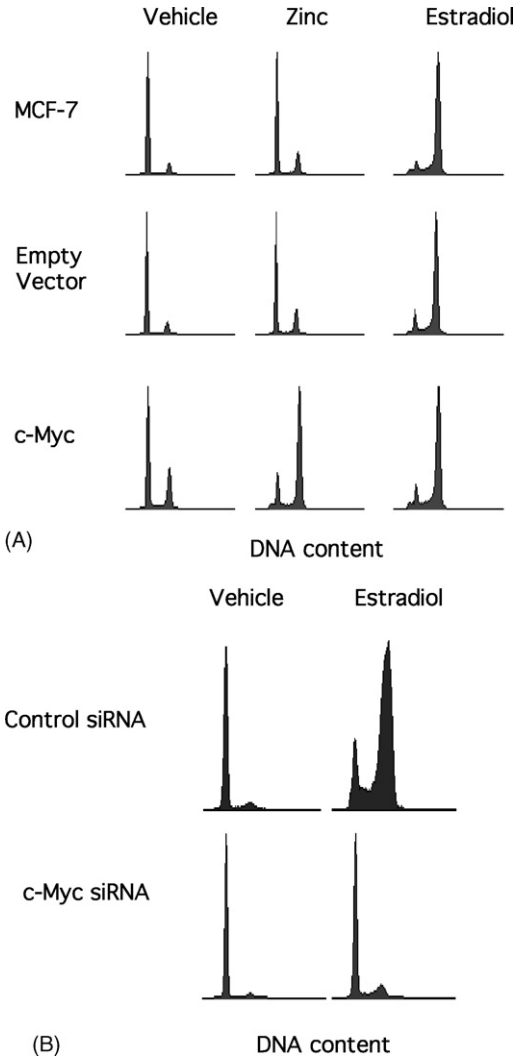

Fig. 3. c-Myc effects on cell cycle progression in antiestrogen-arrested cells. (A) MCF-7 cells inducibly expressing c-Myc under the influence of the zinc-inducible metallothionein promoter were arrested with 10 nM of the pure antiestrogen, ICI 182,780, for 48 h and compared to similarly-treated control cells bearing the empty vector only, and parental MCF-7 cells. Estrogen (100 nM 17β-estradiol) or zinc (65 μM) were added to arrested cells, and 48 h later changes in cell cycle phase distribution were measured by flow cytometry as previously described [12]. Nocodazole at a final concentration of 50 ng/mL was added to inhibit cytokinesis and allow all cells that progressed to accumulate in G_2/M. (B) After pre-treatment for 24 h with c-Myc siRNA or RISC-free control siRNA (Dharmacon, USA), MCF-7 cells were treated with 10 nM ICI 182,780 for 48 h to induce cell cycle arrest. Cells were then stimulated with estrogen (100 nM 17β-estradiol) for 40 h and changes in cell cycle phase distribution were measured by DNA flow cytometry as described above.

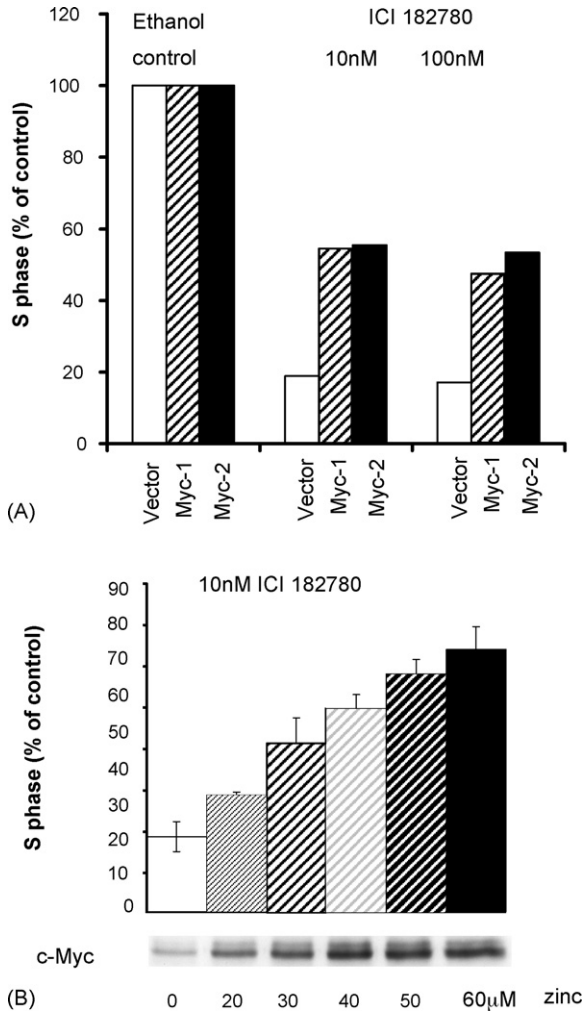

Fig. 4. Constitutive and inducible expression of c-Myc attenuates the growth inhibitory response to antiestrogens in vitro. (A) Two independent clones of MCF-7 cells constitutively overexpressing c-Myc (∼2-fold compared with wild type MCF-7 cells) were treated with ICI 182,780 at concentrations of 10 nM or 100 nM, or ethanol vehicle. After 48 h, changes in S phase were measured by DNA flow cytometry and expressed as a percentage of the S phase in control cells. (B) MCF-7 cells inducibly expressing c-Myc under the influence of a zinc-inducible promoter were treated with increasing concentrations of zinc while in exponential growth phase, and 3 h later were treated with 10 nM of the pure antiestrogen, ICI 182,780. After 24 h, changes in S phase were measured by DNA flow cytometry and expressed as a percentage of the S phase in vehicle (ethanol)-treated exponentially growing cells. In parallel, protein lysates were prepared from zinc-treated cells at 3 h and immunoblotted for c-Myc (as described previously [12]).

striking when only nuclear expression was evaluated (77% versus 47%). Fig. 2 depicts the predominant staining patterns observed in the breast cancer tissue microarray cores.

Together these data demonstrate that c-*myc* mRNA and c-Myc protein expression are detectable in the majority of breast cancers with elevated expression observed in both ER+ and ER− phenotypes. Such data challenge the view that c-Myc overexpression is predominantly a feature of ER− disease [14] and raise the question of the functional consequences of elevated c-Myc expression in ER+ tumours, particularly as it relates to response to endocrine therapies.

3.2. c-Myc is required for estrogen-induced cell cycle progression following antiestrogen arrest

To determine the relationship between c-Myc- and estrogen-induced cell cycle progression, clonal cell lines expressing c-Myc under the influence of the zinc-inducible,

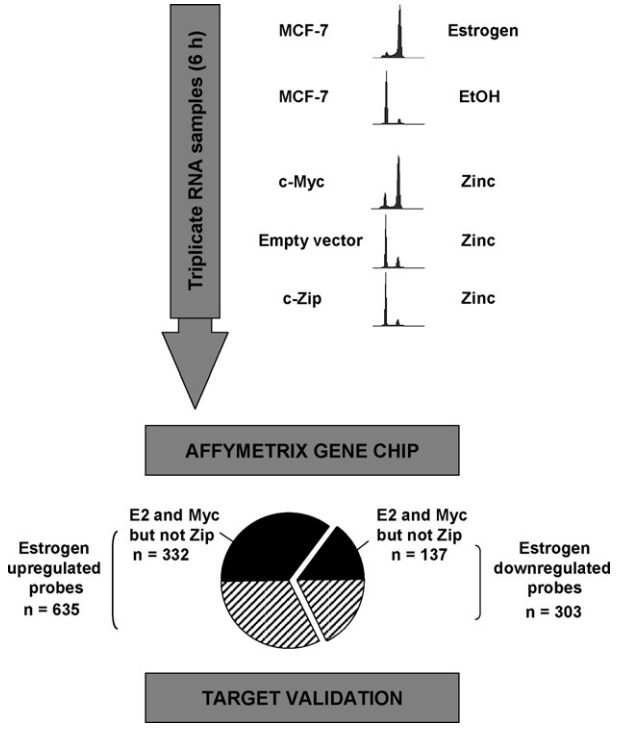

Fig. 5. An experimental paradigm for identifying estrogen-regulated Myc target genes in breast cancer cells. MCF-7 cells inducibly overexpressing c-Myc (wild type, and c-Zip, a deletion mutant of c-Myc in which the N-terminal transcriptional activation domain is absent), parental cells, and cells bearing the empty vector only were growth-arrested with 10 nM ICI 182,780 for 48 h. Parental cells were treated with estrogen (100 nM 17β-estradiol) or vehicle (ethanol), and cells transfected with c-Myc (wild type or c-Zip) or the empty vector were treated with zinc (65 μM). RNA was prepared from cells harvested at 6 h post-treatment, and analysed using the Affymetrix gene chip platform and Bayesian linear modelling methods in the *limma* package [36]. From the list of probes that were significantly regulated by estrogen (relative to vehicle-treated cells, adjusted $p < 0.01$), those also significantly regulated by c-Myc and not by c-Zip (relative to zinc-treated empty vector cells) were further selected to yield a number of downstream candidate genes for further *in vitro* validation studies.

metallothionein promoter were employed as described previously [10]. As depicted in Fig. 3A, induction of c-Myc expression with zinc was able to mimic the effects of estrogen action in initiating cell cycle progression in antiestrogen-arrested cells, such that the majority of the cell population exited from G_0/G_1 following treatment with estradiol or zinc. Cells were arrested in G_2/M due to concurrent treatment with nocodazole that inhibited further cell cycle progression and re-entry into G_1 phase. This effect was analogous to that seen in empty vector or parental MCF-7 cells upon the addition of estrogen (Fig. 3A).

Since, these data confirmed earlier experiments demonstrating that c-Myc induction was sufficient for cell cycle progression in antiestrogen-arrested cells we then determined if estrogen induction of c-Myc was necessary for estrogen-induced cell cycle progression. MCF-7 cells treated with both antiestrogen and siRNA directed against c-*myc* remained growth-arrested upon stimulation with estrogen (Fig. 3B). These data suggest not only that c-Myc induction is capable of replicating the proliferative effects of estrogen, but also that c-Myc expression is necessary for estrogen-induced cell cycle progression.

3.3. Constitutive or inducible expression of c-Myc attenuates the growth-inhibitory response to antiestrogens in vitro

The ability of the pure antiestrogen ICI 182,780 to decrease proliferation of MCF-7 cells expressing different levels of c-Myc was assessed in two experimental systems, using as an endpoint the percentage of the cell population in S phase 24–48 h after antiestrogen treatment. In the first study, MCF-7 cell lines that constitutively overexpressed c-Myc at a level ∼2-fold higher than that seen in empty vector control cells were employed, while in the second paradigm antiestrogen sensitivity was tested in cell lines inducibly expressing c-Myc. Fig. 4A demonstrates the attenuation of the anti-proliferative effect of ICI 182,780 in two clones of MCF-7 cells in which c-Myc was constitutively overexpressed. These data demonstrate that an ∼2-fold increase in c-Myc results in an ∼50% decrease in sensitivity to the anti-proliferative effect of ICI 182,780 in MCF-7 cells. In order to establish if higher levels of c-Myc overexpression can further attenuate the anti-proliferative effects of antiestrogens on exponentially growing cells *in vitro*, c-Myc overexpression was induced in 2 clonal cell lines expressing c-Myc under the influence of a zinc-inducible promoter as described above. c-Myc expression was rapidly induced following zinc treatment and after 3 h, the cells were treated with 10 nM ICI 182,780. After 24 h treatment with antiestrogen, cells with no c-Myc induction (i.e. 0 μM zinc added) demonstrated the expected decline in

Table 1
Immunohistochemical assessment of c-Myc expression in a cohort of 217 breast cancers

Positive staining	≥25% at ≥2 + intensity		≥50% at ≥2 + intensity		≥75% at ≥2 + intensity	
	ER +ve (n = 157 (72%))	ER −ve (n = 60 (28%))	ER +ve (n = 157 (72%))	ER −ve (n = 60 (28%))	ER +ve (n = 157 (72%))	ER −ve (n = 60 (28%))
Nuclear positive	121 (77%)	28 (47%)	97 (62%)	22 (37%)	45 (29%)	8 (13%)
Cytoplasmic positive	123 (78%)	41 (68%)	107 (68%)	22 (37%)	82 (52%)	33 (55%)
Nuclear and cytoplasmic positive	100 (46%)	23 (38%)	78 (47%)	18 (30%)	36 (23%)	8 (13%)

Positive staining was defined as ≥2 + intensity. The data were analysed at cut-offs of 25%, 50% and 75% of cancer cells positive. In our cohort, approximately 30% normal breast tissue cells stained for c-Myc with 2 + intensity or greater. The threshold for ER positivity was 10% for any degree of nuclear staining. Consistent with the representative nature of the cohort, 72% of the cancers were ER positive.

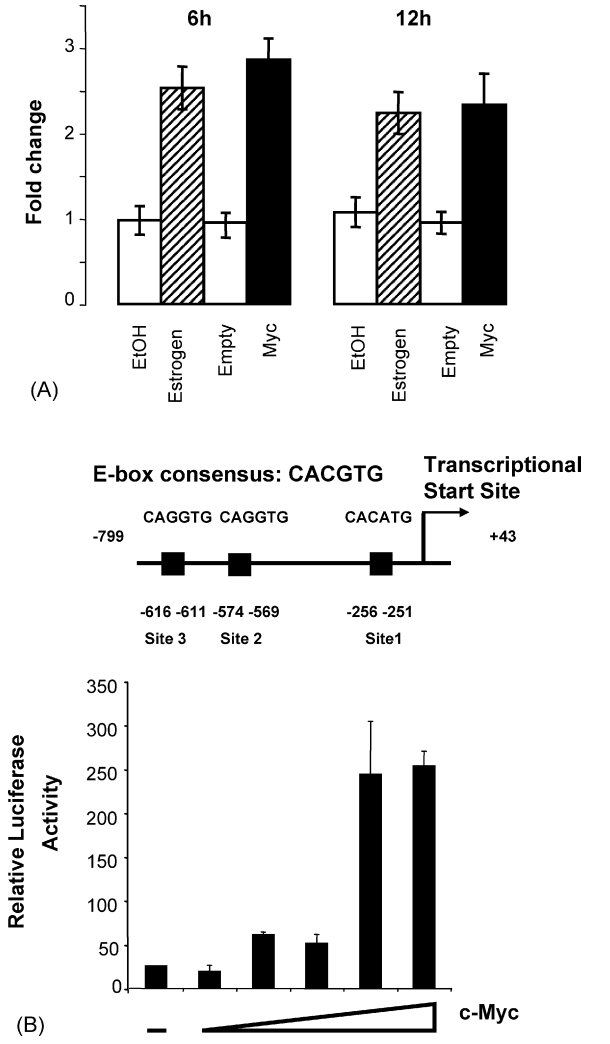

Fig. 6. EMU4 is an estrogen and c-Myc target gene. (A) Parental MCF-7 cells were antiestrogen-pretreated (ICI 182,780, 10 nM, 48 h) then treated with estrogen (100 nM 17β-estradiol) or vehicle (ethanol). In parallel, empty vector cells or cells expressing c-Myc under the control of the metallothionein promoter were pretreated with antiestrogen and then treated with zinc (65 μM). RNA was prepared, and real-time PCR was performed in triplicate using a RotorGene RG-3000 (Corbett Research, Australia). Data analyses were performed using the ΔC_t method relative to a 0 h control sample. The change in the expression of *EMU4* was measured at 6 and 12 h and is presented normalised to *GAPDH* level. (B) Schematic diagram illustrating the *EMU4* proximal promoter reporter construct. The sequence between −799 and +43 bp of the *EMU4* gene was ligated upstream of the luciferase cDNA in the pGL3-basic reporter construct. The locations of the potential c-Myc binding sites (E-boxes) are indicated. MCF-7 cells were transfected with the EMU4 luciferase reporter construct and increasing amounts of the c-Myc expression construct, pCDNA3.1-cMyc. All samples were cotransfected with the Renilla luciferase reporter construct, pRLSV40; which was used for normalisation of transfection efficiency. Cells were transfected using LipofectAMINE2000 (Invitrogen, Australia) and luciferase was assayed using the dual luciferase reporter assay system (Promega, USA) in accordance with manufacturer's recommendations. All transfections were performed in triplicate and are presented as mean ± S.D. All values are relative to the activity of the pGL3-basic reporter.

S phase to about 30% of that observed in untreated exponentially growing cells (Fig. 4B). However, as the concentration of zinc was incrementally increased, resulting in a concurrent increase in c-Myc expression (Fig. 4B, lower panel), the S phase following antiestrogen treatment was also incrementally increased. At the highest concentration of zinc (60 μM), the antiestrogen-induced decline in S phase was significantly attenuated to ∼80% of that seen in untreated cells (Fig. 4B, upper panel). Together these data clearly demonstrate that c-Myc overexpression can attenuate the growth inhibitory response to antiestrogen in a concentration-dependent manner and potentially, at even higher levels of expression, render cells completely insensitive to antiestrogens. These and other published data [28,29] further support a potential role for c-Myc overexpression in the development of endocrine resistance in breast cancer.

3.4. An experimental paradigm for identifying estrogen-regulated target genes in breast cancer

We next adapted the inducible c-Myc model to facilitate the identification of estrogen target genes that were regulated secondarily to estrogen induction of c-Myc expression. The downstream targets of both c-Myc and estrogen are numerous, reflecting their pleiomorphic cellular effects. Therefore, in order to identify genes that are downstream of both estrogen and c-Myc, gene expression profiling was undertaken following the scheme outlined in Fig. 5. MCF-7 cells expressing wild type c-Myc under the control of the metallothionein promoter were stimulated with zinc, while parental cells were treated with either estrogen or vehicle (ethanol). In addition, MCF-7 cells containing vector only, or a deletion mutant of c-Myc (c-Zip) in which the two N-terminal transcriptional activation domains, Myc box I and Myc box II, are absent [34], were stimulated with zinc. The gene expression profiles following 6 h of these treatments were then determined using Affymetrix GeneChips (U133 Plus V2.0), and probe sets significantly regulated by estrogen (relative to vehicle-treated control cells) were identified as those with $p < 0.01$ (adjusted for multiple hypothesis testing). Probe sets that were also significantly regulated by c-Myc (relative to zinc-treated empty vector cells), but not by c-Zip were also identified and compared with the estrogen-regulated probes. This analysis revealed that approximately two-thirds of the estrogen-regulated probes were upregulated, i.e. 635 of 968 significantly regulated probe sets, and of these approximately half, i.e. 322 of 635, were also regulated by c-Myc. A significant proportion of these genes had been previously identified as either estrogen- or c-Myc-regulated, indicating that the model can robustly identify genes of likely relevance to estrogen and c-Myc action.

A number of candidate genes were selected for further evaluation as downstream targets of estrogen and c-Myc action, on the basis of degree of upregulation, potential c-Myc binding sites in the promoter region, or other motifs suggestive of a functional role in cell proliferation. One such

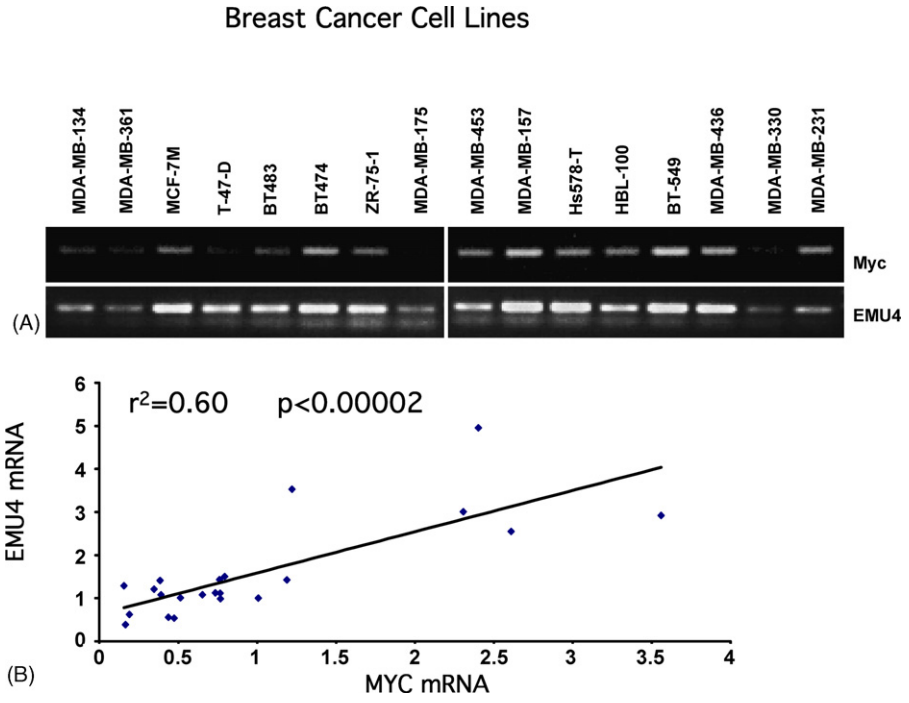

Fig. 7. EMU4 expression correlates with Myc expression in breast cancer cell lines. (A) Semi-quantitative RT-PCR was performed to assess the expression of c-*myc* and *EMU4* mRNA in total RNA isolated from a battery of breast cancer cell lines. PCR products were generated in 20–25 cycles, run on 1% agarose gels and stained with ethidium bromide, before UV visualisation and photography. (B) Total RNA from a larger series of breast cancer cell lines was used for real-time qRT-PCR in triplicate using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, USA) with Taq-Man probes for EMU4 and c-Myc, normalised using RPLP0. Data analyses were performed using the ΔC_t method, relative to expression in the 184 normal breast epithelial cell strain.

candidate gene was a novel gene that was upregulated by both estrogen and c-Myc, which we have named EMU4 (Estrogen- and Myc-upregulated) and is discussed further below.

3.5. *EMU4 is a novel estrogen and c-Myc target gene*

In order to confirm regulation of EMU4 by estrogen and c-Myc, parental MCF-7 cells and clonal cell lines expressing inducible c-Myc (as above), were pretreated for 48 h with ICI 182,780 then treated with ethanol, estrogen or zinc as appropriate, and RNA was then extracted for PCR analysis. Empty vector cells were also included as controls. Quantitative RT-PCR was performed for EMU4, 6 and 12 h after treatment. At 6 h, the EMU4 mRNA level in the inducible c-Myc cell line was increased relative to empty vector control cells by 2.5–3-fold. This induction continued to at least 12 h post-treatment, with a level of induction of 2–2.5-fold relative to control (Fig. 6A). A similar degree of induction of EMU4 mRNA was observed following 6 and 12 h treatment with estrogen validating the Affymetrix GeneChip data and confirming that EMU4 is indeed both an estrogen and c-Myc target gene in breast cancer cells.

To confirm a potential role for c-Myc in the transcriptional regulation of *EMU4*, a luciferase reporter construct containing ~800 bp of the *EMU4* proximal promoter was transfected into parental MCF-7 cells. This sequence included three putative c-Myc-responsive E-box motifs (Fig. 6B). When increasing amounts of a c-Myc expression vector were cotransfected with the EMU4 promoter luciferase reporter, an increase in luciferase expression was clearly apparent (Fig. 6B). These data demonstrate that c-Myc can act as a transcriptional activator of *EMU4* gene expression, identifying EMU4 as a novel c-Myc target gene.

3.6. *EMU4 and c-Myc mRNA expression are correlated in breast cancer cell lines*

To further investigate EMU4 as a potential c-Myc target gene in breast cancer, both *EMU4* and c-*myc* mRNA expression were analysed in a battery of human breast cancer cell lines using semiquantitative RT-PCR (Fig. 7A). These data demonstrated that *c-Myc* expression and *EMU4* expression are closely related. In a separate experiment using quantitative RT-PCR, a strong positive correlation between c-*myc* and *EMU4* mRNA expression in this series of 16 breast carcinoma cell lines was observed (Fig. 7B), suggesting that EMU4 expression may be a good surrogate marker of c-Myc activity in breast cancer.

4. Conclusions

Our *in vitro* studies demonstrate that both constitutive and inducible overexpression of c-Myc in the estrogen-responsive

breast cancer cell line MCF-7 confer partial resistance to the anti-proliferative effects of the antiestrogen ICI 182,780. Furthermore, this effect is dependent on the degree of c-Myc overexpression. These data are consistent with previous studies, in which inducible expression of c-Myc re-initiated cell cycle progression in antiestrogen-arrested cells [12,29]. Published data indicate that c-Myc likely exerts its proliferative effects, at least in part, through repression of cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$ expression [29]. It remains unclear whether this occurs through direct modulation of the $p21^{WAF1/CIP1}$ promoter by the binding of c-Myc to an E-box element [29], or indirectly *via* c-Myc titrating Sp1 away from the Spl binding sites in the $p21^{WAF1/CIP1}$ promoter [35]. However, the large number of c-Myc-regulated genes identified here in association with estrogen-induced mitogenesis raises the possibility that other downstream targets of c-Myc may contribute to the proliferative effect of estrogen.

To this end, we have used gene expression profiling to identify genes that are coordinately regulated with c-*myc* in association with the proliferative response to estrogen in breast cancer cells. One such target is the novel gene, EMU4. The level of *EMU4* mRNA was correlated with the level of c-*myc* expression following estrogen treatment in a battery of human breast cancer cell lines. Further work is required to evaluate the biological role of this novel c-Myc target and its relevance, if any, to c-Myc action in breast cancer.

Our data also confirm a broad range of c-Myc expression in human breast cancer at both the RNA and protein level. Furthermore, this spectrum of expression is displayed across both ER+ and ER− tumours. Thus, in the light of our *in vitro* data, aberrant expression of c-Myc in ER+ disease may be of clinical relevance in predicting sensitivity to antiestrogen therapy. The *in vitro* studies described here have led to the identification of a number of novel estrogen and c-Myc-target genes associated with the proliferative response to estrogen in breast cancer cells and are likely to be of relevance in addressing the important problem of antiestrogen resistance, and in contributing further to our broader understanding of the biology of the estrogen signalling pathways.

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