

## Review of: c-Myc suppresses p21<sup>WAF1/CIP1</sup> expression during oestrogen signalling and antioestrogen resistance in human breast cancer cells

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### Abstract of the original article:

Oestrogen rapidly induces expression of the proto-oncogene c-Myc. c-Myc is required for oestrogen-stimulated proliferation of breast cancer cells, and deregulated c-Myc expression has been implicated in antioestrogen resistance. In this report, we investigate the mechanism(s) by which c-Myc mediates oestrogen-stimulated proliferation and contributes to cell cycle progression in the presence of antioestrogen. The MCF-7 cell line is a model of oestrogen-dependent, antioestrogen-sensitive human breast cancer. Using stable MCF-7 derivatives with inducible c-Myc expression, we demonstrated that in antioestrogen-treated cells, the elevated mRNA and protein levels of p21<sup>WAF1/CIP1</sup>, a cell cycle inhibitor, decreased upon either c-Myc induction or oestrogen treatment. Expression of p21 blocked c-Myc-mediated cell cycle progression in the presence of antioestrogen, suggesting that the decrease in p21<sup>WAF1/CIP1</sup> is necessary for this process. Using RNA interference to suppress c-Myc expression, we further established that c-Myc is required for oestrogen-mediated decreases in p21<sup>WAF1/CIP1</sup>. Finally, we observed that neither c-Myc nor p21<sup>WAF1/CIP1</sup> is regulated by oestrogen or antioestrogen in an antioestrogen-resistant MCF-7 derivative. The p21 levels in the antioestrogen-resistant cells increased when c-Myc expression was suppressed, suggesting that loss of p21 regulation was a consequence of constitutive c-Myc expression. Together, these studies implicate p21<sup>WAF1/CIP1</sup> as an important target of c-Myc in breast cancer cells and provide a link between oestrogen, c-Myc, and the cell cycle machinery. They further suggest that aberrant c-Myc expression, which is frequently observed in human breast cancers, can contribute to antioestrogen resistance by altering p21<sup>WAF1/CIP1</sup> regulation.

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### Review

While the hormonal treatment of oestrogen receptor (ER)-positive tumours has been a major advance in

the treatment of breast cancer, a significant percentage of hormone receptor positive breast cancers are inherently antioestrogen resistant, or become antioestrogen resistant during treatment [1,2]. An understanding of how antioestrogens act and how resistance may develop is an important goal of breast cancer research. To date aberrations in a number of signalling pathways have provided potential mechanisms for antioestrogen resistance. These include cross-talk between the ER and cell-surface tyrosine

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kinase receptors, as well as alterations in the MAP kinase and PI3-kinase pathways [1,2]. An important focus of study has understandably been at the point of convergence of these mitogenic signalling pathways, that is control of cell proliferation, where the transcription factor c-Myc is an important player [3].

Studies in clinical cohorts have failed to demonstrate a relationship between c-Myc amplification or c-Myc expression and overall outcome, including response to therapy, in breast cancer [4,5]. This may relate to the wide variability of amplification and expression levels identified across different cohorts as a consequence of variation in experimental techniques, contamination by non-tumour cells, and heterogeneous study populations, as well as an absence of data pertaining to patient follow-up, tumour characteristics and treatment [4,5]. Nonetheless there are significant *in vitro* data implicating c-Myc in breast cancer cell proliferation, and evidence suggesting that it may also be involved in the development of anti-oestrogen resistance. Regulation of c-Myc is an early response to either oestrogen or anti-oestrogen treatment of breast cancer cells [6]. Overexpression of c-Myc reverses or attenuates anti-oestrogen inhibition of breast cancer cell proliferation [7,8], and antisense oligonucleotide-mediated suppression of c-Myc expression leads to an inhibition of proliferation that is accompanied by molecular changes that mimic the effects of anti-oestrogens [9].

In their recent article, Mukherjee and Conrad provide further mechanistic insight into the potential role of c-Myc in resistance to anti-oestrogens in breast cancer cells *in vitro* [10]. Consistent with a previous study using MCF-7 cells [7], inducible expression of c-Myc led to re-initiation of cell cycle progression in anti-oestrogen-arrested cells, although this was less effective than oestrogen treatment [10]. Thus, there appears to be a link between c-Myc overexpression and resistance to anti-oestrogens. Overall, the kinetics of cell cycle progression in response to oestrogen treatment or c-Myc induction were similar. However, some differences were apparent, particularly at later time points in the analysis, which may relate to differences in the magnitude and/or time course of c-Myc induction. However, it is also possible that they resulted from effects of oestrogen treatment that were independent of c-Myc, for example induction of cyclin D1 and activation of cyclin D1-CDK4 [7].

Consistent with previous data in which c-Myc decreased the transcription of the CDK inhibitor p21<sup>WAF1/CIP1</sup> (p21) in other cell types [11–13], Mukherjee and Conrad demonstrated that decreased p21 expression was a pronounced response to c-Myc induction in MCF-7 cells [10]. Other studies have shown that oestrogen also decreased p21 transcription during re-initiation of cell cycle progression

in anti-oestrogen-arrested cells and this was necessary for oestrogen-mediated CDK activation [14–17]. In addition, decreased p21 expression conferred resistance to the anti-proliferative effects of anti-oestrogens [18,19] and increased p21 expression inhibited the ability of oestrogen to overcome anti-oestrogen-mediated arrest [20]. Similarly, when Mukherjee and Conrad overexpressed p21 using a recombinant adenovirus, c-Myc could not promote proliferation in anti-oestrogen-arrested cells [10]. Furthermore, RNA interference experiments showed that when c-Myc expression was decreased, p21 expression remained high in the presence of oestrogen [10]. Collectively, these observations suggest that the ability of oestrogen to decrease p21 transcription might be mediated by c-Myc.

Mukherjee and Conrad used luciferase reporter assays to show that a fragment of the p21 promoter beginning at –194 bp, encompassing a region previously shown to bind c-Myc [13], was repressed by c-Myc in MCF-7 cells [10], with the implication that oestrogen/anti-oestrogen-mediated regulation of c-Myc would lead to regulation of p21 via the same promoter element. Others have localised the sequence required for increased p21 promoter activity in response to anti-oestrogen treatment to a similar region, between –143 and +8 [21]. Mutation of individual Sp1 sites within this region substantially attenuated the induction, and further assays including chromatin immunoprecipitation suggested a model in which an ER-Sp1 complex binds to this region in cycling cells, recruiting histone deacetylases that repress p21 transcription [21]. Treatment with the anti-oestrogen ICI 182,780 led to disruption of this association and enhanced recruitment of Sp1 to the p21 promoter [21]. Gartel *et al* [12] demonstrated that c-Myc bound Sp1 but did not directly interact with the p21 promoter, suggesting that c-Myc may repress p21 by titrating Sp1 away from the p21 promoter. In hormone-responsive cells, ER and c-Myc may collectively modulate Sp1 regulation of p21 transcription. Thus although the experiments of Mukherjee and Conrad suggest that oestrogen/anti-oestrogen effects on p21 transcription are primarily mediated by c-Myc, the precise mechanism by which this occurs requires further study.

An increase in p21 levels when c-Myc is decreased has not been observed in another study using MCF-7 cells [9], perhaps due to differences in experimental design, whereby p21 expression was evaluated after 8–16 h of antisense c-Myc-oligonucleotide treatment as compared to 48 h of c-Myc RNA interference. However, despite the apparent differences between these studies, the overall conclusion is that regulation of p21 expression is central to the ability of c-Myc to

modulate cell cycle progression in these cells, since overexpression of p21 using a recombinant adenovirus abrogated the ability of c-Myc to induce proliferation in antioestrogen-arrested cells [10], and decreased p21 expression allowed continued proliferation despite decreased c-Myc expression [9].

In a further set of experiments Mukherjee and Conrad investigated the potential role of c-Myc and p21 in resistance to antioestrogens in LCC9 breast cancer cells. These cells are an ER-positive, but oestrogen-independent and antioestrogen-resistant derivative of MCF-7 cells. They displayed no alteration in c-Myc or p21 expression after oestrogen or antioestrogen treatment [10]. When c-Myc was suppressed in LCC9 cells using RNA interference, p21 expression increased after antioestrogen treatment. It has been shown elsewhere that proliferation of antioestrogen-sensitive cells continues in the face of antioestrogen treatment if the increase in p21 is prevented using antisense oligonucleotides, or if c-Myc expression is maintained [8,18,19]. Collectively, these data suggest that the antioestrogen resistance observed in LCC9 cells may be due to altered p21 regulation by c-Myc.

The potential clinical importance of this article lies in its description of a mechanism through which c-Myc may mediate antioestrogen resistance. The questions that now arise include whether p21 modulation accounts for the majority of the antioestrogen resistance associated with c-Myc overexpression, and whether other upstream mediators are also involved. Although a previous study suggested that c-Myc induction resulted in cell cycle progression equivalent to the oestrogen response [7], Mukherjee and Conrad concluded that c-Myc induction alone was less effective than oestrogen treatment. Other investigators have demonstrated that c-Myc confers only partial antioestrogen resistance [8]. Thus the quantitative relationship between the level of c-Myc expression and oestrogen/antioestrogen sensitivity, and whether there is a level of c-Myc that can confer complete antioestrogen resistance, is unclear. An additional question relates to the degree to which c-Myc expression may influence antioestrogen resistance in the clinic. Further studies are required to dissect out other downstream targets of c-Myc with potential roles in steroid sensitivity, and upstream influences on p21 activity in order to direct research towards future therapeutic strategies for women with antioestrogen-resistant breast cancers.

Thus the important mechanistic study of Mukherjee and Conrad provides a framework for further testing these hypotheses in the laboratory and in tumour material from women with known therapeutic response to endocrine therapies.

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