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Journals Club

Review of: c-Myc suppresses $p21^{WAF1/CIP1}$ 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 $p21^{WAF1/CIP1}$, 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 $p21^{WAF1/CIP1}$ 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 $p21^{WAF1/CIP1}$. Finally, we observed that neither c-Myc nor $p21^{WAF1/CIP1}$ 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 $p21^{WAF1/CIP1}$ 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 $p21^{WAF1/CIP1}$ regulation.

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 antioestrogen resistance. Regulation of c-Myc is an early response to either oestrogen or antioestrogen treatment of breast cancer cells [6]. Overexpression of c-Myc reverses or attenuates antioestrogen 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 antioestrogens [9].

In their recent article, Mukherjee and Conrad provide further mechanistic insight into the potential role of c-Myc in resistance to antioestrogens 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 antioestrogen-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 antioestrogens. 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 $p21^{WAF1/CIP1}$ (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 antioestrogen-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 antioestrogens [18,19] and increased p21 expression inhibited the ability of oestrogen to overcome antioestrogen-mediated arrest [20]. Similarly, when Mukherjee and Conrad overexpressed p21 using a recombinant adenovirus, c-Myc could not promote proliferation in antioestrogen-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/antioestrogen-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 antioestrogen 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 antioestrogen 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/antioestrogen 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

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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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References

1. Osborne CK, Shou J, Massarweh S, Schiff R. Crosstalk between estrogen receptor and growth factor receptor pathways as a cause for endocrine therapy resistance in breast cancer. *Clin Cancer Res* 2005; **11**: 865s–870s.
2. Nicholson RI, Staka C, Boyns F, Hutcheson IR, Gee JM. Growth factor-driven mechanisms associated with resistance to estrogen deprivation in breast cancer: new opportunities for therapy. *Endocr Relat Cancer* 2004; **11**: 623–641.
3. Adhikary S, Eilers M. Transcriptional regulation and transformation by Myc proteins. *Nat Rev Mol Cell Biol* 2005; **6**: 635–645.
4. Deming SL, Nass SJ, Dickson RB, Trock BJ. C-myc amplification in breast cancer: a meta-analysis of its occurrence and prognostic relevance. *Br J Cancer* 2000; **83**: 1688–1695.
5. Blancato J, Singh B, Liu A, *et al*. Correlation of amplification and overexpression of the c-myc oncogene in high-grade breast cancer: FISH, in situ hybridisation and immunohistochemical analyses. *Br J Cancer* 2004; **90**: 1612–1619.
6. Foster JS, Henley DC, Ahamed S, Wimalasena J. Estrogens and cell-cycle regulation in breast cancer. *Trend Endocrinol Metab* 2001; **12**: 320–327.
7. Prall OW, Rogan EM, Musgrove EA, Watts CK, Sutherland RL. c-Myc or cyclin D1 mimics estrogen effects on cyclin E-Cdk2 activation and cell cycle reentry. *Mol Cell Biol* 1998; **18**: 4499–4508.
8. Venditti M, Iwasiow B, Orr FW, Shiu RP. C-myc gene expression alone is sufficient to confer resistance to antiestrogen in human breast cancer cells. *Int J Cancer* 2002; **99**: 35–42.
9. Carroll JS, Swarbrick A, Musgrove EA, Sutherland RL. Mechanisms of growth arrest by c-myc antisense oligonucleotides in MCF-7 breast cancer cells: implications for the antiproliferative effects of antiestrogens. *Cancer Res* 2002; **62**: 3126–3131.
10. Mukherjee S, Conrad SE. c-Myc suppresses p21WAF1/CIP1 expression during estrogen signaling and antiestrogen resistance in human breast cancer cells. *J Biol Chem* 2005; **208**:17617–17625.
11. Coller HA, Grandori C, Tamayo P, *et al*. Expression analysis with oligonucleotide microarrays reveals that MYC regulates genes involved in growth, cell cycle, signaling, and adhesion. *Proc Natl Acad Sci USA* 2000; **97**: 3260–3265.
12. Gartel AL, Ye X, Goufman E, *et al*. Myc represses the p21(WAF1/CIP1) promoter and interacts with Sp1/Sp3. *Proc Natl Acad Sci USA* 2001; **98**: 4510–4515.
13. Seoane J, Le HV, Massague J. Myc suppression of the p21(Cip1) Cdk inhibitor influences the outcome of the

https://doi.org/10.1017/S1470903106004925 Published online by Cambridge University Press

p53 response to DNA damage. *Nature* 2002; **419**: 729–734.
14. Planas-Silva MD, Weinberg RA. Estrogen-dependent cyclin E-cdk2 activation through p21 redistribution. *Mol Cell Biol* 1997; **17**: 4059–4069.
15. Prall OW, Carroll JS, Sutherland RL. A low abundance pool of nascent p21WAF1/Cip1 is targeted by estrogen to activate cyclin E*Cdk2. *J Biol Chem* 2001; **276**: 45433–45442.
16. Prall OW, Sarcevic B, Musgrove EA, Watts CK, Sutherland RL. 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. *J Biol Chem* 1997; **272**: 10882–10894.
17. Skildum AJ, Mukherjee S, Conrad SE. The cyclin-dependent kinase inhibitor p21WAF1/Cip1 is an antiestrogen-regulated inhibitor of Cdk4 in human breast cancer cells. *J Biol Chem* 2002; **277**: 5145–5152.
18. Cariou S, Donovan JC, Flanagan WM, Milic A, Bhattacharya N, Slingerland JM. Down-regulation of p21WAF1/CIP1 or p27Kip1 abrogates antiestrogen-mediated cell cycle arrest in human breast cancer cells. *Proc Natl Acad Sci USA* 2000; **97**: 9042–9046.
19. Carroll JS, Prall OW, Musgrove EA, Sutherland RL. A pure estrogen antagonist inhibits cyclin E-Cdk2 activity in MCF-7 breast cancer cells and induces accumulation of p130-E2F4 complexes characteristic of quiescence. *J Biol Chem* 2000; **275**: 38221–38229.
20. Lai A, Sarcevic B, Prall OW, Sutherland RL. Insulin/insulin-like growth factor-I and estrogen cooperate to stimulate cyclin E-Cdk2 activation and cell cycle progression in MCF-7 breast cancer cells through differential regulation of cyclin E and p21(WAF1/Cip1). *J Biol Chem* 2001; **276**: 25823–25833.
21. Varshochi R, Halim F, Sunters A, Alao JP, Madureira PA, Hart SM, *et al*. ICI182,780 induces p21Waf1 gene transcription through releasing histone deacetylase 1 and estrogen receptor alpha from Sp1 sites to induce cell cycle arrest in MCF-7 breast cancer cell line. *J Biol Chem* 2005; **280**: 3185–3196.

https://doi.org/10.1017/S1470903106004925 Published online by Cambridge University Press