Clinical Oncology - Science in focus - Editorial

TITLE:
Understanding oestrogen receptor function in breast cancer, and its interaction with the progesterone receptor. New preclinical findings and their clinical implications.

AUTHORS:
Richard D Baird¹, Jason S Carroll²

¹ Department of Oncology, University of Cambridge
² Cancer Research UK Cambridge Institute

Correspondence to: richard.baird@medschl.cam.ac.uk or jason.carroll@cruk.cam.ac.uk.

Background
Oestrogen antagonists have been used for decades for the treatment of patients with oestrogen receptor- (ER-) positive breast cancers, to the benefit of many millions of women worldwide. However, the clinical outcomes of these women vary considerably, something that has been an important focus for research, but an unresolved issue. One key observation has been that patients with ER-positive and progesterone receptor- (PR-) positive breast cancers tend to have better clinical outcomes than those with ER-positive, PR-negative tumours.

PR is an oestrogen regulated target gene and for many years, the accepted explanation was that PR-positivity was a passive marker of a functional oestrogen receptor (Figure 1 A). ER-
positive, PR-positive tumours were more likely to be sensitive to treatment with ER antagonists, and therefore have a better outcome. Conversely, ER-positive, PR-negative tumours were thought to have a "non-functional" oestrogen receptor, and therefore are less likely to respond to anti-oestrogens.

However, recently published preclinical data suggest an alternative explanation, namely that progesterone receptor activity can change where ER binds to DNA, directly modulating ER function and thereby potentially improving the tumour response to ER antagonists (Figure 1 B). This is important, since such combination endocrine therapies might lead to improved clinical outcomes. This new preclinical data sheds new light on where ER makes contact with DNA and the importance of parallel pathways that can influence the DNA binding profile and ultimately, activity of the ER complex in breast cancer.

**Where does ER make contact with DNA?**

It might be thought that after stimulation by oestrogen, ER binds to DNA and regulates the transcription of genes encoded immediately adjacent to the ER binding site. In fact, transcription factor mapping techniques (eg. Chromatin Immunoprecipitation-sequencing (ChIP-seq)) have shown that in most cases, ER regulates its target genes from considerable distances. ER and its associated proteins (termed co-factors) bind to ER binding sites and subsequently, DNA loops form to bring this ER complex adjacent to its distant target genes. Interestingly, when ER+ PR+ (good outcome) tumours are compared with ER+ PR- (poor outcome) tumours, the ER DNA binding sites are distinct, with different genes being switch on and off as a result.
How does ER make contact with DNA? The role of ER-interacting proteins.

ER function is heavily influenced by ER-binding proteins, which can make the DNA more accessible for the ER complex, or assist in stabilisation of the ER-DNA interaction. A comprehensive list of these proteins can be obtained using unbiased techniques like RIME (Rapid Immunoprecipitation Mass Spectrometry of Endogenous protein), in which the protein of interest is pulled out of cell lysates with an antibody, and mass spectrometry used to discover which other proteins it physically associates with. Using this technique, over 100 ER-binding proteins have been identified, including FoxA1 and GATA3, as would be expected[^3]. Out of these, the pioneer factor FoxA1 has been found to be essential for ER function, by stabilising ER-DNA interactions. In the absence of FoxA1, ER-positive breast cancer cells stop growing[^4], revealing a dependence on FoxA1 for ER functioning. These findings make FoxA1 itself a major focus of research, including understanding the structural interactions between ER and FoxA1, the role of potential chemical FoxA1 post-translational modifications and the discovery of chemical inhibitors that block FoxA1 function.

Interestingly, in addition to the known ER interacting protein, PR was purified as an ER associated protein, suggesting a putative functional role for PR in the ER complex.

What happens when a progesterone agonist is added?

When breast cancer cells are treated *in vitro* with a progesterone agonist, the same RIME technique can be used to examine changes in protein binding under these new conditions. Firstly focusing on changes in PR interaction, there is an increase in a number of proteins, including ER and the ER-binding proteins FoxA1 and GATA3. However, when the focus is on ER interactors, the only progesterone-induced change observed is an increase in PR itself[^1]. Furthermore, the addition of progesterone causes a rapid redistribution of ER binding sites,
with ER binding reprogrammed to thousands of new DNA sites within 3 hours of treatment. This new list of binding sites is highly reproducible, and the same effect is seen whether endogenous or synthetic progestogens are used. The progesterone induced ER-DNA binding sites are mediated by PR, such that ER becomes sequestered by PR to new locations in the genome. The net result of this is inhibition of ER gene expression activity and consequent decreases in cellular growth. In support of this observation, primary tumour samples cultivated ex vivo for up to two weeks showed a reduction in tumour cell growth in response to progesterone treatment and a reversal of the oestrogen-induced growth. In vivo experiments have also now shown that the addition of progesterone to tamoxifen therapy of breast cancer cell line xenografts both inhibits proliferation as measured by Ki67, but also prevents tumour growth more effectively than tamoxifen alone\textsuperscript{1}.

**An alternative explanation to the "non-functional" ER theory**

For many years, the "non-functional" ER theory has been used to explain why ER+ PR-breast cancers have a worse clinical outcome, however there are now multiple reasons to think that this theory might be too simplistic. Firstly, in patients with metastatic ER+ breast cancer, resistance to one endocrine therapy does not necessarily mean resistance to another endocrine therapy; indeed, it is standard of care for patients to be managed with sequential endocrine therapy in the absence of rapidly progressive visceral disease. Second, we know from preclinical oestrogen-responsive element (ERE) reporter experiments that in endocrine therapy-resistant tumours, there is still evidence of ER-associated transcriptional activity. Furthermore, we know from such models that continued tumour proliferation is dependent on the ER-binding protein FoxA1, implying maintenance of a functional ER complex. In support of this, recent discoveries have revealed that ER is frequently mutated
in metastases that arise from ER+ breast cancers\textsuperscript{5–7} and the mutations occur in a predictably part of ER that renders ER independent of oestrogen. These new findings support a role for a functional, albeit constitutive, ER complex in endocrine resistant patients. In fact the non-functional ER theory has been challenged before\textsuperscript{8,9}, but perhaps it is only now that we are able to propose a plausible alternative; namely that progesterone receptor expression is not just a passive consequence of an active oestrogen receptor; but that PR can actively reprogram ER binding to alternative sites and PR negativity might actually contribute to an altered, but still functional, ER complex. The loss of PR can be the cause, not the consequence of altered ER activity and tumour progression. An alternative explanation for low PR levels has been identified, namely a frequent deletion in the genomic regions that encodes the PR gene (PGR), essentially removing the PR ‘molecular handbrake’ that can sequester and inactivate ER. This raises the possibility that we can activate this ‘molecular handbrake’ by modulating PR with existing compounds with potential clinical benefit.

\textbf{Potential clinical significance}

The potential clinical significance of these findings is clear. First, the addition of a progesterone agonist might enhance the anti-proliferative activity of anti-oestrogen therapies, and therefore prove a more effective combination therapy. Clinical data already exist which suggest that the PR agonist megestrol acetate ("Megace") may help control tumour growth in patients with ER-positive metastatic breast cancer after aromatase inhibitor treatment failure. In a single-arm phase II study, 48 postmenopausal "hormone sensitive" patients were treated with megestrol acetate at the dose of 160 mg daily. The treatment was reasonably well tolerated, and yielded a clinical benefit rate of 40%, with a median duration for this benefit of 10 months\textsuperscript{10}. 
It should also be noted that there is a body of evidence supporting the use of low-dose PR agonists as supportive therapies to help ameliorate the hot flashes associated with anti-oestrogen therapy\textsuperscript{11}. A second important motive for such combination therapy is therefore to improve the quality of life for women taking anti-oestrogens. This is even more important at a time when the intensity\textsuperscript{12} and / or duration\textsuperscript{13} of adjuvant endocrine therapy is being increased for many patients.

As the preclinical findings described here are translated into the clinic, a number of key questions remain. Firstly, can we confirm in the clinic that the addition of a PR agonist enhances the anti-proliferative activity of anti-oestrogen therapy? Second, what is the lowest dose of progesterone therapy which can achieve a significant biological effect with an acceptable side effect profile? Third, and most importantly, can the combination of PR agonist with ER antagonist improve clinical outcomes for both metastatic and early stage breast cancer patients. Clinical trials are currently being set up to test these and other questions, in an attempt to improve the outcome for millions of patients who are diagnosed with ER-positive breast cancer every year.

**References**


3. Mohammed, H. *et al.* Endogenous purification reveals GREB1 as a key estrogen


Figure 1. Our changing understanding of the interaction between oestrogen receptor (ER) and progesterone receptor (PR) function.

A) **Old Model**

PR status as a passive consequence of ER function

B) **New Model**

PR actively influences ER binding sites and function

i) ER function in presence of oestrogen alone

ii) ER function in presence of oestrogen plus progesterone