Viewpoint

Understanding endocrine resistance: the critical need for sequential samples from clinical breast cancer and novel *in vitro* models

Julia MW Gee and Iain R Hutcheson

Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, UK

Corresponding author: Julia MW Gee, gee@cardiff.ac.uk

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Introduction

Although the mechanisms that underlie endocrine resistance in breast cancer are complex, their investigation has potential to reveal new targets to treat or even prevent this undesirable state. Of particular interest are growth factor receptor kinase cascades; there are experimental data implicating increased signalling through these pathways in both *de novo* and acquired resistance to tamoxifen. Various estrogen receptor (ER)-negative and ER-positive *de novo* tamoxifen resistant cell models have elevated epidermal growth factor receptor (EGFR)/HER2 signalling. In acquired tamoxifen resistance, models such as TAMR are equally persuasive in implicating EGFR, HER2 and insulin-like growth factor-1 receptor, and activity of extracellular signal-regulated kinase (ERK)1/2 mitogen-activated protein kinase (MAPK) and phosphoinositol 3-kinase (PI3K)/AKT [1]. Signal transduction inhibitors (STIs) such as the EGFR selective tyrosine kinase inhibitor gefitinib and agents that target the increased downstream intracellular kinases can be growth inhibitory in both resistance settings. As such, clinical trials are underway with STI monotherapy in recurrent tamoxifen resistant, advanced/metastatic and ER-negative breast cancer.

Further experimental studies have demonstrated that the growth factor receptor kinases, in addition to their direct stimulation of proliferation/survival signals, interplay with ER to promote tamoxifen resistant growth. Both genomic and nongenomic mechanisms of crosstalk have been implicated. In the acquired TAMR model, EGFR/HER2 primed MAPK and AKT phosphorylate nuclear ER AF-1 residues, allowing coactivator recruitment that enhances the transcriptional activity of the tamoxifen–ER complex and promotes growth [1]. In MCF-7/HER2-18 cells [2], tamoxifen activates plasma membrane ER, triggering HER2/MAPK activity, phosphorylation of nuclear ER and activation of its coactivator AIB1. Such close interplay has provided considerable rationale for further clinical trials employing STIs in combination with various antihormonal strategies [3]. However, several burning issues remain.

Are growth factor receptor kinases prominent in clinical acquired resistance to tamoxifen, and is there any evidence of crosstalk with ER?

If STIs are to be valuable in patients, then it is critical that model system data are underpinned by equivalent demonstration of the target signalling pathways in clinical breast cancer. Consistent with experimental observations, ER-negative tumours do commonly exhibit enhanced EGFR/HER2 expression and activation of ERK1/2 MAPK and AKT, whereas (more controversially) ER-positive patients who are *de novo* resistant to tamoxifen can also exhibit such signalling increases versus responsive disease [1]. However, deciphering growth factor receptor kinase pathways at the time of acquisition of tamoxifen resistance has proved notoriously problematic in clinical material because of the difficulty in obtaining paired samples before tamoxifen administration and subsequently on relapse. Some but not all of the limited data available are compatible with *in vitro* observations demonstrating growth factor receptor kinase pathway functionality in acquired resistance. Increases in several components of EGFR/MAPK signalling, the alternative MAPK Jun kinase [4] and HER2 [5] have been shown on progression, including studies using sequential breast cancer samples taken during tamoxifen treatment of ER-positive primary elderly patients [1]. However, such increases can be quite modest, requiring sensitive assays for accurate measurement both of expression and signalling activity. Not surprisingly, therefore, the magnitude of the pathways in clinical acquired resistance remains highly controversial.

EGFR = epidermal growth factor receptor; ER = estrogen receptor; ERK = extracellular signal-regulated kinase; MAPK = mitogen-activated protein kinase; PI3K = phosphoinositol 3-kinase; STI = signal transduction inhibitor.

A recent report from Guttierez and coworkers [6] is important because it assembles further supportive clinical evidence of prominent HER2/kinase signalling in acquired resistance using paired breast cancer samples taken before adjuvant tamoxifen treatment and on progression. In tumours retaining ER, HER2 overexpression and/or amplification occurred at the time of tamoxifen relapse in 11% of HER2-negative tumours. Increased signalling through the stress-activated MAPK p38 and ERK1/2 MAPK were also apparent on recurrence. Increases in HER2 and p38 activity were similarly noted in acquired tamoxifen resistant xenografts. Interestingly, there were correlations between ER and activity of the two kinases in the clinical relapse material, supportive of ER–kinase crosstalk and hence targeting of the contributory pathways following acquisition of tamoxifen resistance.

The mechanism of kinase interplay with ER now requires deciphering, using antibodies specific for ER phosphorylation and coactivators. However, there remains an urgent requirement for access to sequential clinical tumour material during treatment and at relapse if this quest to decipher growth factor receptor-kinases and their cross-talk with ER is to continue. Collection of circulating tumour cells may provide a useful resource in this regard. As proof of principle, Meng and coworkers [7] were able to use this approach to reveal modest HER2 gene amplification acquired by some HER2-negative patients during progression on chemotherapy, with subsequent responses to herceptin-containing therapy.

Is growth factor receptor kinase crosstalk with ER contributory in acquired resistance to oestrogen deprivation and can it be therapeutically targeted?

In antihormone responsive models, STIs improve the actions of endocrine strategies and delay development of resistance. STIs also enhance the inhibitory effects of endocrine agents in acquired tamoxifen resistant TAMR and ER positive *de novo* tamoxifen resistant MCF-7/HER2-18 cell models [1]. Indeed, in the latter model STIs can recover tamoxifen sensitivity [2]. Such observations provide a rationale for clinical studies exploring combination treatment of STIs with antioestrogens or aromatase inhibitors [3]. However, given that aromatase inhibitors are increasingly a frontline treatment in the management of ER-positive postmenopausal breast cancer, a key issue is whether the value of combination treatment extends to cells that are refractory *de novo* or have acquired resistance to oestrogen deprivation. The detail of growth factor receptor kinase–ER interplay remains controversial in such cells, although it seems likely that there are subtle differences in pathway detail versus tamoxifen resistant states.

A new report by Sabnis and coworkers [8] is important not only because it further adds to our knowledge of growth factor receptor kinase–ER signalling in experimental acquired resistance to oestrogen deprivation, but also because it has successfully demonstrated that targeting with STIs can indeed enhance the inhibitory effects of endocrine agents in such cells. In the few long-term oestrogen deprived models examined to date, increases in HER2, insulin-like growth factor-1 receptor (with less evidence for EGFR) and downstream MAPK/PI3K signalling have been implicated. Such pathways interact with ER in a genomic or nongenomic manner, and together with adaptive increases in ER can promote oestrogen hypersensitivity [9-11]. STIs targeting MAPK kinase (MEK), PI3K and mammalian target of rapamycin (mTOR); gefitinib at dosages that block HER2; and the ER downregulator fulvestrant are growth inhibitory.

Sabnis and coworkers [8] now confirm that HER2 and PI3K/AKT signalling (but not MAPK) is upregulated and growth contributory in a new model (UMB-1Ca) of acquired resistance to oestrogen depletion generated from ER-positive aromatase-transfected MCF-7Ca cells *in vitro*. Although UMB-1Ca cells are apparently refractory to oestrogen, tamoxifen and anastrazole, they contain elevated ER that appears functional because cell growth can be inhibited by high fulvestrant concentrations. In addition to direct promotion of cell survival by AKT, immunoprecipitation studies indicate crosstalk with ER in UMB-1Ca cells. Excitingly, targeting of PI3K/AKT signalling or HER2 (using wortmannin or gefitinib, respectively) restores the antiproliferative activity of anastrazole or tamoxifen in UMB-1Ca cells, and wortmannin also improves the inhibitory effects of fulvestrant. In total, these new data are highly supportive of examining STIs and their combination with endocrine agents in patients, including those refractory to oestrogen deprivation.

Conclusion

Although the preclinical and, in general, clinical data available indicate that growth factor receptor kinase pathways are important in several forms of antihormone resistant breast cancer, a number of key issues remain. There are questions regarding the prominence of these pathways in clinical acquired resistance, and deciphering of the elusive mechanisms of interplay with ER signalling is required across the various endocrine resistant states. Clarification of these issues through clinical and preclinical studies, like those reported by Guttierez [6] and Sabnis [8] and their groups, is essential if we are to interpret adequately the various ongoing STI trials in clinical breast cancer, allow design of improved targeting strategies, and better select patients for treatment by revealing appropriate predictive biomarkers. Because in parallel with a role in growth these signalling pathways also appear able to promote invasiveness of endocrine resistant cells, including UMB-1Ca [8,12], such knowledge may reveal strategies that ultimately confer major improvements in patient prognosis.

Competing interests

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