Viewpoint

Extreme growth factor signalling can promote oestrogen receptor-α loss: therapeutic implications in breast cancer

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Introduction

Most breast cancers overexpress the oestrogen receptor (ER)-α, and the ERα+ phenotype is relatively stable during endocrine treatment and on subsequent treatment failure. However, approximately 30% of tumours are ERα– at diagnosis, while a proportion of tumours, which are initially ERα+, lack the receptor at the time of tamoxifen relapse in the adjuvant or metastatic setting. The mechanisms underlying *de novo* and acquired ERα negativity remain poorly-defined, although their elucidation is of obvious therapeutic interest since the ERα– phenotype is associated with endocrine resistance, aggressive tumour biology and poor prognosis. Somatic mutations in the ERα gene are quite rare and are therefore unlikely to explain the frequency of ERα negativity. Epigenetic mechanisms, notably CpG island methylation and histone deacetylation, may contribute by silencing ERα gene transcription in approximately 25% of *de novo* ERα– cancers [1]. Interestingly, ERα expression and function can be partially recovered in ERα– models using the DNA methyl transferase (DNMT) inhibitor aza-2-deoxycytidine, DNMT1 antisense, or the histone deacetylase inhibitor trichostatin [2]. However, there is also emerging evidence that sustained, exaggerated growth factor pathways [notably those hyperactivating extracellular signal-regulated kinases (ERK)1/2 mitogen activated protein kinase (MAPK)] may promote substantial decline, and even total loss, of ERα. A new article by Holloway and colleagues [3] sheds light on this mechanism of ERα negativity, revealing that exaggerated ERK1/2 MAPK signalling promotes ERα downregulation via its impact on cytoplasmic substrates that include the transcription factor nuclear factor kappa B (NFκB).

Hyperactivation of ERK1/2 MAPK promotes oestrogen receptor loss via cytoplasmic substrates including NFκB

Growth factor pathways can enhance ERα phosphorylation, transcriptional activity and cell growth in the absence of ERα ligand. Paradoxically, a decline in ERα expression may also be a possible outcome when growth factor signalling is extreme, reminiscent of the ERα downregulation that occurs during chronic receptor activation by oestrogen [4]. Supportive evidence are drawn from stable transfection studies where growth factor signalling elements, notably those comprising the epidermal growth factor receptor (EGFR)/HER2 pathway, promote ERα loss when overexpressed in ERα+ breast cancer cells. Oh and colleagues noted precipitous decreases in ERα mRNA and protein in MCF-7 cells transfected with constitutively active HER2, mitogen activated kinase kinase 1 (MEK1), Raf-1 kinase (Raf1) or ligand-activatable EGFR, all of which hyperactivate ERK1/2 MAPK [5]. They also noted a loss of oestrogen-mediated gene expression and oestrogen response element (ERE) activity, and acquisition of endocrine resistance. Interestingly, the phenomenon was reversible, since abrogation of hyperactivated ERK1/2 MAPK restored ERα expression and activity. Extending these studies, Holloway and colleagues have now begun to decipher the mechanism of MAPK-mediated ERα loss in these various transfected models. Using dominant negative constructs, they show that hyperactivated ERK1/2 MAPK downregulate ERα via a common substrate. Use of an ERK2 deletion construct to prevent nuclear MAPK activity reveals this substrate is cytoplasmic. By examining potential MAPK substrates

DNMT = DNA methyl transferase, EGFR = epidermal growth factor receptor, ER = oestrogen receptor; ERK = extracellular signal regulated kinase, MAPK = mitogen activated protein kinase, STI = signal transduction inhibitor.

(again using appropriate dominant negatives), they demonstrate that activator protein-1 (AP-1) and 90 kDa ribsosomal S6 kinase 1 (RSK1) are not responsible for the ER downregulation promoted by MAPK hyperactivation. However, there does appear to be some importance for NFκB. This growth-promoting transcription factor can be cytoplasmically-activated (prior to its nuclear translocation) via MAPK-mediated induction of autocrine growth factors (e.g. heparin-binding EGF). Holloway and colleagues demonstrate gross elevation of NFκB activity in the various models exhibiting MAPK hyperactivation. This NFκB activity is inhibited by abrogating ERK1/2 MAPK signalling. Importantly, blockade of NFκB activity (e.g. using Parthenolide) restores ERα expression and activity in parallel, although interestingly there was only partial recovery indicating existence of additional cytoplasmic substrates.

Conclusions

These new data obtained by Holloway and colleagues in stable transfected cells are important in that they provide proof of principle that extreme growth factor signalling, resulting in ERK1/2 MAPK hyperactivation and recruitment of cytoplasmic substrates including NFκB, is capable of promoting ERα loss. Further molecular detail of the receptor downregulation mechanism, determination of growth factor signalling thresholds required to instigate ERα loss, and the impact of hyperactivation of additional signalling cascades (e.g. phosphatidylinositol 3′ kinase [6]) is now required. Questions clearly remain regarding relevance to ERα negativity *de novo* or acquired during therapy. Significant, however, is the inverse ERα/EGFR association in clinical disease and elevated ERK1/2 MAPK and NFκB signalling observed in ERα– cells [7,8]. Moreover, ERα transrepresses proinvasive genes and so growth factor-mediated ERα loss may underlie poor prognosis in ERα negative disease [6]. Finally, modestly increased growth factor signalling activates ERα in acquired tamoxifen resistance, explaining subsequent antihormone response; however, more extreme/prolonged signalling might promote ERα negative endocrine insensitivity in some patients during sequential endocrine challenge [7].

While future studies are clearly required, the data from Holloway and colleagues do have exciting ramifications for possible therapeutic approaches in ERα– disease. Manipulation of growth factor pathways (in particular ERK1/2 MAPK and potentially NFκB signalling) with signal transduction inhibitors (STIs) might feasibly recover ERα positivity in ERα– cells, hence restoring sensitivity to antioestrogen if used in combination. Importantly, the group demonstrate that *in vitro* pharmacological or dominant negative blockade of ERK1/2 MAPK signalling does re-instate physiological levels of ERα expression and function in their stable-transfected cells. Of course, we must await future experimental consolidation and appropriate clinical examination, but a compelling preliminary study demonstrates that reversion of ERα negativity and re-instatement of endocrine responsiveness occurs in a proportion of advanced HER2+ breast cancer patients using Herceptin to inhibit growth factor signalling [9]. Since this mechanism may only be applicable in tumours without epigenetic ERα silencing, combination therapy of STIs plus appropriate strategies to abrogate ER methylation/deacetylation could prove worthy of future exploration. However, DNMT1 can be growth factor-regulated and so perhaps sustained increases in growth factor signalling ultimately culminate in ERα promoter silencing. If so, STIs might also prove effective in restoring ERα where there is ERα promoter hypermethylation. Intriguingly, pharmacological inhibition of Ras signalling does reverse gene methylation events in other cancer models via downregulating DNMT1 [10].

Competing interests

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