Title: Estrogen receptor mutations in breast cancer – new focus on an old target

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The authors have no conflicts of interest

Running Title: Estrogen receptor mutations in breast cancer

(key words: ESR1 mutations, metastatic, resistance, breast cancer)

Acknowledgements:

We thank the Mary-Jean Mitchell Green Foundation and Breakthrough Breast Cancer for funding. We acknowledge NHS funding to the NIHR Royal Marsden Hospital and Institute of Cancer Research Biomedical Research Centre.

Abstract

Recent studies have provided strong evidence for the emergence of substantial numbers of constitutively active ESR1 mutations in estrogen-receptor positive metastatic breast cancer that are undetected in primary disease. Some of these mutants remain partially sensitive to current anti-estrogen therapies but effective therapeutics targeted at them may require new approaches.
Main text

In this issue of *Clinical Cancer Research*, Jeselsohn and colleagues (1) report an overall 12% frequency of mutations in the coding sequence of *ESR1* in metastatic ER positive (ER+) breast cancers but the lack of their detection in primary disease.

About 80% of primary breast cancers express estrogen receptor α (ERα), the product of the *ESR1* gene, and are described as ER+. Estrogen is the primary stimulant of the development and continued growth of ER+ tumours: withdrawal of estrogen from primary ER+ breast tumours decreases proliferation in >90%, although this reduction may be modest in some (2). The mainstay of treatment of ER+ breast cancer is endocrine therapy, in the form of drugs that antagonise the ER (eg, tamoxifen), therapies that result in estrogen deprivation (eg, aromatase inhibitor (AIs)) or fulvestrant, a drug that destabilises and antagonises the receptor. Most patients receive endocrine therapy for five years starting shortly after surgery (adjuvant therapy).

Endocrine therapy for breast cancer has resulted in markedly reduced recurrence and mortality rates however a significant proportion of patients relapse with metastatic disease. If the recurrence has not arisen within a few months of starting adjuvant therapy, these patients will be treated with an alternative endocrine treatment (first-line metastatic treatment). Resultant shrinkage or stasis of metastatic lesions is achieved in many patients and those showing a response have a good chance of responding to subsequent lines of endocrine treatments: patients whose cancer has progressed on tamoxifen often respond to fulvestrant or an AI and *vice versa* (3). The lack of cross-resistance suggests that the mechanisms by which this occurs may vary depending upon the prior treatment. Eventually pan-endocrine resistance develops.

Many mechanisms have been elucidated in model systems of resistance to tamoxifen, and more recently to estrogen deprivation and fulvestrant. These include altered expression of growth factor receptors, such as HER2, or aberrant expression of ER co-regulators leading to either ER-independent growth or ER dependent/ligand independent growth (4). However, there has been...
limited confirmation of these findings in the clinic. Whilst some patients with acquired resistance to tamoxifen show loss of ER in metastases, an obvious route to loss of sensitivity, the majority continue to express ER and there is less evidence for ER loss with resistance to AIs (4, 5). Thus resistance mechanisms remain incompletely explained and there is little biomarker-related guidance of therapy for metastatic disease.

Despite the central role of estrogens in the development of breast cancer, ERα mutations in primary disease have thus far proved rare (Figure 1). For example no ESR1 mutations were identified in a study of 390 ER+ primary tumours (6). In metastatic disease, the presence of an ESR1 mutation unique to the metastases was first noted 20 years ago (7) but until very recently surprisingly few further data accumulated. However, three groups have recently reported substantial numbers of ESR1 mutations in metastatic breast cancer (8-10). The current work of Jeselsohn and colleagues (1) is consistent with and extends these reports. They sequenced the coding region of ESR1, and 182 additional cancer-related genes, in 58 treatment-naïve primary and 76 metastatic ER+ HER2- breast tumours and set out to describe better the functional relevance of the observed ESR1 mutations. None of the primaries contained detectable ESR1 mutations but 11 (14.5%) of the metastatic samples contained previously reported mutations (8, 9), clustered in the ligand-binding-domain (LBD), predominantly at amino acids 537 or 538, as well as a novel 344insC mutation. Notably, a direct correlation was apparent between the number of endocrine treatments and mutation frequency, which was 5/25 (25%) in tumours from patients receiving an average of seven lines of treatment. All of the other 182 cancer-related genes sequenced exhibited similar mutation frequencies between the primary and metastasis.

In the largest of the other recent reports, Toy et al. (8) found nine LBD mutations in 36 cases enrolled in a metastatic collection program. In the two cases where they could also test the primary tumour, the mutation was absent. They also found that 5/44 cases from the BOLERO-2 clinical trial of letrozole ± everolimus harboured ESR1 mutations with the frequency enriched in cases with a
long duration of endocrine therapy (8). Similarly, Robinson et al. (9) and Merenbakh-Lamin et al. (10) reported that 6/11 and 5/13 cases, respectively, of breast cancer metastases contained LBD-localised ESR1. In each of these reports mutations at 537 and 538 were the most frequent but others at 534 or 536 were also noted. Given the consistent finding of mutations in the LBD of ERα, elucidation of their functional significance and susceptibility to therapeutic targeting is of paramount importance.

Functional studies by Jeselsohn and colleagues (1) and others (8-12) provide evidence that many of the mutants are strong promoters of ER signalling and, importantly, are constitutively active in the absence of estradiol and therefore likely to elicit resistance to estrogen deprivation strategies. They stimulate increased expression of ER-regulated genes, and increase proliferation and migration in vitro compared with wildtype ESR1. The ESR1 mutants have also generally been found to confer reduced sensitivity to anti-estrogens compared with wildtype ESR1. Although signalling is reduced by 4-hydroxytamoxifen and fulvestrant, much higher doses are required to reach a similar level of inhibition observed in the wildtype (1, 8-12). These in vitro findings are supported by molecular modelling that indicate that the mutants favour the agonist conformation and mediate their effects through influence of co-activator or co-repressor binding (8). It is also notable that in ER-Y537N mutant cells, fulvestrant induces much more modest ER degradation compared with wildtype (1) and therefore reduced ability to inhibit ligand-independent activity of ER. This relative resistance to degradation seems less apparent with ER-Y537S (11).

Interestingly, while it has been difficult to sustain patient derived xenografts (PDX) from ER+ breast cancer tissues, aberrations in the ESR1 gene were found in 4/5 luminal breast cancer metastases. This included one case with ER-Y537S that was found in a further PDX in which the ER status was not available. A fusion gene (ESR1/YAPI) was also discovered in an ER+ PDX that, like the point mutations, induced estradiol-independent growth. It is plausible that the drive provided by certain constitutively active ESR1 alterations is a major advantage in promoting survival and growth in the
low estrogen environment of the PDX system. These PDXs are likely to be particularly valuable in elucidation of the best therapeutic approaches for tumours with specific $ESR1$ alterations.

In summary, there is now substantial evidence for the emergence of functionally aberrant $ESR1$ mutations, particularly in the LBD, in metastatic ER+ breast cancer that are undetectable in primary tumours. Correlative evidence is consistent with this emergence being due to selective pressure of multiple endocrine therapies. The genomic instability associated with advanced disease may contribute to the prevalence of the mutations, for example through self-perpetuating defects in DNA repair mechanisms. $ESR1$ mutations might also promote the success of the metastatic process and be apparently enriched as a result, although this hypothesis requires their presence below the detection limits of the high-depth sequencing conducted to date. To confirm the role of the mutations as resistance mechanisms and their potential as targets for new therapies requires careful prospective study of metastatic disease aligned with comprehensive genomic and functional analyses.
Figure 1. Schematic of ESR1 mutations identified in primary and metastatic advanced ER+ breast cancer and factors that may influence their development. The majority of the mutations affect the ligand binding domain in helix 12 (H12). Collated from (1, 6-12).

* Total number of patients with mutations in primary disease (7/674). 0/58 Ref (1), 0/390 Ref (6), 1/35 Ref (7), 6/183 Ref (8), 0/3 Ref (9), 0/5 Ref (10).

§ Total number of patients with mutations in metastatic disease (43/222). 11/76 Ref (1), 2/5 Ref (7), 14/80 Ref (8), 6/11 Ref (9) 5/13 Ref (10), 2/7 Ref (11), 3/30 [NB ER+ status unconfirmed] Ref (12).

References

Figure 1:

Primary disease-frequency 1%*

Potential factors
Time, treatment, metastasis, mutational drivers

Metastatic disease-frequency 19% §
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Clin Cancer Res Published OnlineFirst February 28, 2014.

Updated version
Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-14-0067

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