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The search for *ESR1* mutations in breast cancer

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Strap-line

Two new studies report identification of activating *ESR1* gene mutations in aromatase inhibitor-resistant metastatic breast cancers. This insight into therapeutic resistance suggests new approaches that may be useful for management of endocrine-resistant breast cancer.

Blockade of estrogen receptor α (ER) action has been a successful treatment strategy for women whose cancers express ER for nearly four decades. Sadly, *de novo* or acquired resistance to endocrine therapy is common. Mutations that inactivate ER were proposed as a possible mechanism of resistance; however, *ESR1* mutations were found to be infrequent in primary breast cancer. Indeed The Cancer Genome Atlas sequenced 390 ER-positive (ER+) primary cancers and did not identify any *ESR1* mutations [1]. Given the relevance of ER for development and progression of breast cancer, the mechanism of resistance to endocrine therapy is an important problem to solve. A clue surfaced recently in a paper by Matthew Ellis and colleagues [2] that reported identification of *ESR1* mutations in xenografts derived from aggressive treatment-resistant primary or metastatic breast cancers. Now, two independent studies by Chinnaiyan and colleagues [3], and Chandarlapaty and colleagues [4] in this issue of Nature Genetics show that activating *ESR1* mutations are relatively frequent events in advanced ER+ hormone-resistant breast cancer, particularly in metastatic lesions from women who took estrogen-lowering drugs such as aromatase inhibitors (AI).

ESR1 mutations in metastases

Robinson et al [3] and Toy et al [4] identified somatic *ESR1* mutations in 6 out of 11 (55%), and 9 out of 36 (25%) ER+ metastatic breast tumors, respectively. Further, Toy et al [4] identified somatic *ESR1* mutations in 5/44 (11%) ER+ metastatic breast cancers obtained from participants in the BOLERO-2 clinical trial whose disease had progressed during AI treatment [5]. Combining the results from these two studies leads to an estimated 22% (20/91 cases) rate of *ESR1* mutations in advanced breast cancer. In a subset of cases with *ESR1* mutation, both groups sequenced primary tumors obtained before therapy, and showed that the *ESR1* mutation is not present in the treatment-naïve cancer. Also Robinson et al did not find *ESR1* mutations in 80 breast cancers that lack ER and progesterone receptor expression, and a previously reported study of 46 ER+ pre-treatment tumor biopsies did not identify any *ESR1* mutations [6]. Additionally, Toy et al. report an analysis of 183 pretreatment tumor biopsies from BOLERO-2 trial participants and identified *ESR1* mutations in only 3% of cases. Most of these studies were performed using deep sequencing

approaches, which should have allowed the identification of *de novo* mutations even if they are present only in minor cell populations in heterogeneous breast cancers. Thus *ESR1* mutations are rare in newly diagnosed untreated breast cancers but appear to be frequently acquired during progression to hormone resistance, especially in the context of estrogen-deprivation therapy.

ESR1 mutations and resistance

The mutations identified in both studies cluster in the ligand binding domain (LBD) of ER, at amino acids 534-538. Toy et al also identified mutations in Ser463 [4], and Li et al. found an additional mutation in Glu380 [2]. Previous studies have shown that mutations in the LBD of ER α result in ligand-independent activity [7–11] and both Toy et al. [4] and Robinson et al. [3] showed that the ER LBD mutations they identified cause constitutive, estrogen-independent receptor activity. Toy et al. also showed that the mutant ER induces expression of novel target genes and performed elegant molecular modeling of the mutant ER proteins, showing that these changes lead to stabilization of an agonist conformation, especially of the coactivator-recruiting region at helix 12, through formation of hydrogen bonds between Ser537 (and Gly538) and Asp351. Previous studies showed that LBD mutants demonstrate increased interaction with the coactivator AIB1, and increased phosphorylation of Ser118, known to be important for both ligand-dependent and ligand-independent activity of ER α [12–14]. Thus, taken together, these studies show that mutations in the *ESR1* LBD result in receptors that are highly active in the absence of ligand and could cause resistance to AI therapy.

Do ESR1 mutations matter?

These studies raise questions about potential clinical implications of *ESR1* mutations in advanced treatment resistant ER+ disease. Toy et al. [4] report that in individuals enrolled in the BOLERO-2 trial, there was no obvious difference in response to AI for tumors with wild type or mutant ER, but few samples were studied and all were derived from patients whose tumors had already progressed on AI therapy. Future studies will be required to address this important question. Another critical question is whether detection of these mutations can guide selection of therapy. Both Toy et al. and Robinson et al. show that mutant ER protein can still bind antiestrogens such as tamoxifen and fulvestrant, although higher doses are required to inhibit the mutant ER. Thus, it is possible that the doses needed to achieve potent ER α inhibition in *ESR1* mutant tumors may exceed steady state, intratumoral levels that are routinely achieved in the clinic. This raises the possibility that altered dosing or development of more potent and/or selective ER antagonists may inhibit residual ER activity and thus overcome resistance. Toy et al. suggest that the ER α mutant proteins may have a different conformation, which provides the exciting opportunity to screen for drugs specific for mutant ER α proteins, as has been done for BCR-Abl [15]. Should we routinely biopsy metastatic tumors or monitor patients for development of *ESR1* mutations? If confirmed, these results are likely to stimulate the development of tests designed to monitor patients on AI therapy for the development of *ESR1* mutations, perhaps via sequencing of circulating free DNA, so that changes in therapy can be made.

In summary, the studies by the Chinnaiyan and Chandarlapaty groups show that *ESR1* mutations are rare in primary breast cancer, seldom arise under the pressure of treatment with selective estrogen receptor modulators like tamoxifen, and are most likely to be important in acquired resistance to estrogen deprivation such as AI therapy (Fig 1). The finding that these mutations cluster in the ER LBD, resulting in a constitutively active receptor with a different conformation, is both mechanistically pleasing and potentially tractable for the development of new targeted strategies.

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Figure 1. Schematic representation of identification of *ESR1* mutations in metastatic breast cancer

The structural domains of ER α are activation function 1 (AF1), DNA binding domain (DBD), and AF2, which includes the ligand-binding domain (LBD). The mutations (small arrows) identified in refs 3 and 4 are depicted below AF2/LBD, and an additional *ESR1* mutation recently identified [in ref 2] is illustrated on top.