**Abstract**  
Estrogen and estrogen receptor-alpha (ER) signaling are important factors in the pathogenesis and treatment of ER-positive breast cancers. Therefore targeted therapies against ER, known as endocrine therapies, are widely used in the treatment of ER-positive breast cancers. While these therapies have shown great success, de novo and acquired resistance are common. The approach to the problem of endocrine therapy resistance is twofold: identify the mechanisms of resistance and develop alternative treatments to overcome these mechanisms. This review focuses on the progress and integration of these two aspects of resolving endocrine therapy resistance in ER-positive breast cancer patients.

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**Introduction**

The estrogen receptor-alpha (ER), encoded by the gene *ESR1*, is a nuclear steroid hormone receptor which acts as a transcription factor to regulate proliferation and differentiation of cells in which it is expressed. Estrogen signaling via ER plays a major role in elements of the female reproductive cycle such as ovulation and mammary gland development, and ER is highly expressed in corresponding tissues such as the ovaries and breasts and has been linked to carcinogenesis in those tissues. Despite the understanding that all cancers are genetic diseases, genetic alterations in *ESR1* are rarely found in primary breast tumors. This led to the notion that *ESR1* gene alterations are not involved with breast carcinogenesis despite the importance of ER signaling for therapeutic intervention in the majority of breast cancer patients. However, more contemporary studies have suggested that mutations in *ESR1* are now known to be mediators of resistance to endocrine therapies. Thus, although *ESR1* mutations are not likely to be involved with the development of breast cancers, the
recognition of ESR1 mutations in metastatic disease may lead to newer therapies that can overcome resistance for therapeutic benefit.

ER signaling

In "classical" ER signaling, binding of the ligand estrogen to ER induces dimerization and activation of the receptor complex (Fig. 1). In addition to a ligand-binding domain (LBD), ER contains a DNA-binding domain which recognizes estrogen response elements (EREs) in the genome and a transactivation domain which induces changes in transcriptional activity at EREs upon ligand-dependent receptor activation and DNA binding. A number of co-activators and co-repressors have been shown to form complexes with ER in order to modulate its activity—the balance of co-activator/co-repressor expression in different tissues and under different cellular conditions is thought to be a major factor in ER functionality and even tissue-specific responses to endocrine therapies targeting ER signaling in the cancer setting.1,2

In "non-classical" ER signaling, ER can alternatively interact with non-ERE genomic loci and/or become activated under ligand-independent conditions. Transcription factors such as AP-1, Sp1, and NF-κB can interact with ER, conferring the ability to recognize non-traditional response elements for transcriptional activation.3 Additionally, post-translational modifications to ER that occur downstream of certain receptor tyrosine kinases and G-protein coupled receptors can induce ligand-independent ER signaling. This includes modulating three well-characterized phosphorylation sites of ER which can be acted upon by important additional kinases such as MAP kinase, Akt, protein kinase A, and HER2, respectively.4,5 The integration of these well-described pathways with ER activity has profound implications for normal ER function, as well as its role in cancer and resistance to endocrine therapies. For example, phosphorylation of ER by HER2 and other kinases may circumvent estrogen-dependence of ER activation, contributing to resistance to anti-estrogen endocrine therapies.6 ER is also capable of non-genomic signaling activities, and has been described to have non-nuclear interactions via interactions with a number of important cellular signaling factors, most notably proteins within the MAP kinase signaling pathway.7

ER in breast cancer pathology

The complex but compelling role of ER in breast cancer has long been recognized. ER is expressed in approximately 70% of breast cancers, usually along with other markers that are often found in luminal breast epithelial cells; ER-expressing (ER+) breast cancers have thus been deemed luminal type. This group has been further divided into luminal A and luminal B subtypes, distinguished by significant expression of genes such as HER2 or Ki67 in luminal B tumors. Luminal A type breast cancer is generally associated with a better prognosis than the other breast cancer subtypes due to a combination of factors: it tends to be more differentiated and less aggressive than other subtypes, the rate of recurrence is lower and slower, and there are ever-increasing numbers of targeted therapies against ER and ER-related signaling being developed and approved for this disease subtype. Generally speaking, most but not

**Fig. 1**  Estrogen receptor-alpha (ER) signaling and related targeted therapies. ER signaling results in both genomic and non-genomic responses which contribute to the behavior of the cell. Therapies targeting ER and key proteins in interacting networks, such as mTOR and MAPK, can mitigate tumorigenic properties resulting from ER signaling.
all luminal A type breast cancers are ER+ and HER2− for receptor expression.

Endocrine therapies

The difficult-to-tolerate side effects of systemic chemotherapy have led to efforts to identify and develop targeted cancer therapies wherever possible. The crucial role of ER in luminal breast cancer has led to wide use of three broad classes of endocrine therapies against ER signaling in the breast.

A class of drugs deemed selective estrogen receptor modulators (SERMs) confers a change in ER activity based on conformational change and/or co-repressor binding. All SERMs are partial ER agonists, exhibiting anti-proliferative activity in breast tissue but estrogenic activity in other peripheral tissues such as endometrium and bone, depending on the particular SERM.1 In randomized clinical trials, the first-generation SERM tamoxifen has performed well and subsequently been approved for prevention of breast cancer in high-risk patients, as an adjuvant treatment for early disease in pre- and postmenopausal women, and as first-line therapy in metastatic breast cancer. However, tamoxifen use is associated with increased risk of endometrial cancer and thromboembolic events.Raloxifene, a second-generation SERM, is also used for prevention of breast cancer and has shown a reduction of side effects, notably endometrial cancer, when compared with tamoxifen in breast cancer clinical trials (although raloxifene’s primary use is in the treatment of osteoporosis).4 Importantly, raloxifene is not used for the treatment of breast cancer. Several third-generation SERMs are currently under investigation in clinical trials, including toremifene, which has been used successfully in the early adjuvant and metastatic settings but has a safety profile similar to that of tamoxifen and bazedoxifene.

Selective estrogen receptor down-regulators (SERDs) such as fulvestrant reduce ER activity and induce its proteasomal degradation. Unlike SERMs, fulvestrant exhibits full antagonistic activity of ER in all tissues. Fulvestrant is currently a second-line endocrine therapy given to post-menopausal ER+ breast cancer patients with advanced disease, and promising results from recent clinical trials indicate that it may be used in other settings as well, such as first-line therapy and in premenopausal patients.9,10 In addition to fulvestrant, two candidate SERDs with improved bioavailability are being evaluated in Phase I clinical trials.11,12 These latter two SERDs have the significant advantage of oral formulations, compared to fulvestrant which requires monthly gluteal intramuscular injections.

Aromatase inhibitors (AIs) greatly reduce production of estrogen in the body, thus removing the main source of ER activation. This class includes the steroidal AI exemestane, as well as the non-steroidal AIs letrozole and anastrozole. AIs can only be used in post-menopausal women or premenopausal women who no longer have ovarian production of estrogen, either through surgical removal of the ovaries or from luteinizing releasing hormone agonists, such as goserelin. This is because ovarian production of estrogen does not rely upon aromatase, an enzyme that is a cytochrome P450 family member. In the post-menopausal state, aromatase is the major if not exclusive source of estrogen production. For post-menopausal breast cancer patients, AIs have been proven to be superior to tamoxifen in the majority of trials. Thus, these drugs constitute the standard first-line adjuvant and metastatic endocrine therapies for post-menopausal ER+/HER2− breast cancer patients.

Endocrine therapy resistance

Although all three drug classes are used effectively in patients with ER+/HER2− breast cancer, resistance to these therapies has become a major hurdle.5 Almost half of ER+/HER2− patients do not respond to endocrine therapies, termed primary or de novo resistance. In addition, the majority of metastatic patients who initially respond to endocrine therapies will eventually progress or relapse, termed secondary or acquired resistance. The mechanisms for resistance have not been fully elucidated, but depend on estrogen-independent survival and proliferation of the tumor cells.

Combination therapies

Due to challenges with resistance to endocrine therapies, additional options for the treatment of ER+/HER2− breast cancer have emerged and others are under investigation. As in other cancers, it is thought that combination therapies, by halting multiple signaling pathways at once, will prevent ER+/HER2− tumors from evolving compensatory mechanisms to bypass ER signaling. Therefore, a number of non-endocrine targeted agents have been tested in combination with endocrine therapies and more studies are underway. Several promising agents have been identified and are now approved therapies for ER+/HER2− disease.

Complex crosstalk between PI3 kinase/mTOR and ER signaling pathways suggests that targeting of both pathways could be detrimental to ER+ tumor growth.13 For this reason, the BOLERO trials tested the hypothesis that the mTOR inhibitor everolimus in combination with current endocrine therapies could improve outcomes for metastatic ER+ patients with endocrine therapy resistant disease. Improved outcomes demonstrated by the BOLERO-2 trial have resulted in approval of the combination of everolimus and exemestane for clinical use in advanced ER+ breast cancers.14 Further BOLERO studies will test everolimus in combination with other endocrine agents such as letrozole and in additional breast cancer settings such as premenopausal and/or endocrine-naive patients.15

Another hypothesis posits that cell cycle arrest could enhance endocrine therapy.16 The CDK 4/6 inhibitors palbociclib and ribociclib are therefore interesting candidates for combination therapy, and numerous trials have been initiated with these agents. Early results of the PALOMA trials have demonstrated improved outcomes with palbociclib in combination with certain endocrine therapies including letrozole and fulvestrant and further studies are underway.15,17 This has led to the FDA approval of letrozole and palbociclib for first-line therapy in metastatic ER+/HER2− breast cancer patients.

Finally, HDAC inhibitors such as entinostat may deregulate ESR1 transcription sufficiently to overcome endocrine
resistance. The ENCORE 301 study demonstrated improved outcomes with entinostat and exemestane combination therapy and confirmatory trials are underway.14

On the other hand, studies of several additional classes and combinations of targeted agents have met with limited success in ER\textsuperscript{+} breast cancers. Anti-angiogenic agents, multi-target kinase inhibitors, EGFR inhibitors, and polyendocrine therapy did not significantly improve outcomes in their respective trials but may benefit from the identification of biomarkers that can select for patient populations most likely to benefit from these therapies either singly or in combination.

Amplification of ESR1

Although ER is highly expressed in a large proportion of breast cancers, it appears that this is largely not a product of ESR1 amplification. This issue was controversial after a 2007 fluorescent in situ hybridization study indicated that significant ESR1 amplification was present in over 20% of breast cancers.18 Later studies failed to replicate this rate of amplification and it was eventually suggested that accumulation of ESR1 transcripts in the nucleus may have been responsible for the perceived amplification and its association with strongly ER\textsuperscript{+} breast cancer.19 However, another recent study found that amplification of ESR1 occurred in MCF7 cells (a widely used breast cancer cell line) after long-term estrogen deprivation, which may model the paradoxical clinical phenomenon in which long-term endocrine therapy in a small subset of patients leads to estradiol hypersensitivity.20 In this group, estradiol may be useful as a breast cancer therapy which causes apoptosis in hypersensitive cells, thus stabilizing the tumor.

ER variants

As an important clinical target in breast cancer, ER seems to be a clear candidate to carry mutations that drive endocrine therapy resistance. Early in vitro mutagenesis studies of ESR1 identified dominant-negative and ligand-independent forms of the ER protein\textsuperscript{11} and in vivo and clinical studies identified acquired mutations which mediate the effects of tamoxifen,\textsuperscript{22,23} but large clinical surveys have shown, somewhat surprisingly, that ER variants are rare in primary breast cancer.24 However, it has been suggested that ER variants play a role in advanced breast cancer\textsuperscript{25} and indeed, recent studies have identified rearrangements and recurrent somatic mutations of ESR1 in metastatic hormone-resistant disease.\textsuperscript{20,26–29} Each of these studies has detected a significant rate of ESR1 mutation or variation (Table 1). In various forms, the authors of these studies suggest that the low-estrogen conditions achieved by the endocrine therapies favor somatic variation of ESR1 in order for tumor cells to adapt and thrive.

Notably, there appears to be a mutational hot spot region in the ER LBD at residues 536–538, which includes a tyrosine phosphorylation site at residue 537 (Fig. 2). Mutagenesis studies have identified the critical role of these amino acids in regulation of ER activity.30,31 A number of different substitutions within this hot spot have been shown to confer constitutive ligand-independent activation of ER, suggesting that these naturally-occurring mutations likely play a role in acquired resistance to endocrine therapies.\textsuperscript{20,26–29} These residues lie within helix 12 of the LBD, which is responsible for closing the LBD pocket upon ligand binding and creating a surface with which corepressors can interact. As shown in structural modeling of one particular LBD mutation, D538G, changes to helix 12 can result in a conformational change that, even in the absence of ligand, mimics the ligand-bound form of ER.29 This not only precludes binding of ligands such as estradiol, tamoxifen, and fulvestrant but also allows ligand-independent co-activator recruitment. Finally, this ligand-

| Study               | ER variant types detected | n | Variant rate |
|---------------------|---------------------------|---|--------------|
| Li et al 2013       | Amplification, fusion, mutation | 7 | 57%          |
| Merenbach-Lamin et al 2013 | Mutation               | 13| 38%          |
| Robinson et al 2013 | Mutation                  | 11| 55%          |
| Toy et al 2013      | Mutation                  | 80| 18%          |
| Jeselsohn et al 2014| Mutation                  | 76| 12%          |

Fig. 2  Schematic diagram of estrogen receptor-alpha (ER). The ESR1 gene encodes the nuclear receptor protein ER, containing domains for DNA binding, transactivation functions 1 and 2, and ligand binding. The most common ESR1 mutations result in variation of the ligand binding domain at residues 536, 537, and 538. These residues are part of Helix 12 of the ligand binding domain, which is known to regulate ligand binding and recruitment of co-repressors and co-activators, therefore constituting the basis for ligand-dependent activation of the ER.
independent ER has constitutive transcriptional activity at EREs and may contribute to enhanced cell proliferation and migration.²⁹

A study of patient-derived breast cancer xenografts, which also detected LBD hot spot mutations, noted one chromosomal translocation between the coding regions of *ESR1* and *YAP1* leading to an in-frame, expressed fusion protein.²⁰ Much like two previously identified naturally-occurring ER fusion proteins, the N-terminus of ER containing the DNA-binding domain and AF1 transactivation domain was preserved but the C-terminus was replaced by that of YAP1—thus losing the LBD and AF2 transactivation domain of ER. Although the physical genetic properties are quite different from the LBD mutations discussed above, this fusion protein similarly gained ligand-independence while preserving DNA-binding and transactivation function. However, while LBD point mutations will alter ligand affinities and contribute to resistance to particular endocrine therapies, complete loss of the LBD as in these fusion proteins leads to an "intrinsic and universal endocrine-therapy resistance" which will need to be addressed with different clinical strategies.²⁰ While clearly occurring at a low frequency, the recurrence of this type of fusion protein across three studies indicates that the effect is robust and almost certainly drives endocrine therapy resistance in this small subset of patients.

It is likely that ER variants can be categorized according to the type of endocrine therapy that they resist: SERM and SERD resistance depends upon changes to the protein’s interaction with the drug, while AI resistance depends upon estrogen-independent activity of the receptor or of the cell itself. Since ER varies widely in its affinity for various ligands, it may be that each individual LBD mutation will confer a different effect on each individual receptor–ligand interaction, which will have profound effects on endocrine therapy decisions for patients with these mutations.

Finally, it cannot be ignored that these mutations were identified only after the recent practice of obtaining metastatic biopsies for sequencing. Although some studies have suggested that these LDB mutations are in primary tumors,²⁸ most studies have not validated these results. We now understand, based on these studies and others, that a single biopsy from the primary tumor is usually not representative of a patient’s heterogeneous and ever-evolving tumor cell population. For example, some of the aforementioned studies of *ESR1* mutations identified mutations in one site of disease but not other metastatic sites within the same patient.²⁸,²⁹ In this respect, we have demonstrated that a "liquid biopsy" technique, which is representative of all disease sites in the body including micrometastatic disease, can detect low-frequency mutations including those in *ESR1*.³² We speculate that in the future this will provide additional information to make the best treatment decisions. Thus, the lesson learned from cancer genome sequencing studies over the past decade is that profiling a patient’s primary tumor may be inadequate to truly understand the molecular evolution of metastatic disease. In this regard, liquid biopsies may allow a more comprehensive profiling of a patient’s total disease burden, and for ER positive disease, further characterization of *ESR1* variants. This, along with ongoing drug development, has brought about a positive outlook for ER+/HER2− breast cancer patients.

**Conclusions**

The characterization of clinically relevant *ESR1* variants and their sensitivity to various endocrine therapies will be crucial for optimal treatment for this subset of ER+ breast cancers. The advent of molecular profiling and precision medicine brings the opportunity to account for these *ESR1* variants and treat patients accordingly. While new therapies may be needed for certain variants, higher doses of already approved therapies may afford near term benefit, a testable hypothesis for clinical trials.²⁹ In addition, the continued development of newer SERMs and SERDs, along with novel therapeutic combinations, may allow for improved outcomes for patients with metastatic ER+/HER2− disease.

**Conflict of interest**

None of the funding sources influenced the design, interpretation or submission of this manuscript.

**Disclosures**

B.H.P. is a member of the scientific advisory boards for Horizon Discovery, LTD and Loxo Oncology, and has research contracts with Genomic Health, Inc. and Foundation Medicine, Inc. Under separate licensing agreements between Horizon Discovery, LTD and The Johns Hopkins University, B.H.P. is entitled to a share of royalties received by the University on sales of products. The terms of this arrangement are being managed by the Johns Hopkins University, in accordance with its conflict of interest policies. B.H.P. also has ownership interest in Loxo Oncology. B.B. declares no potential conflicts.

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