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Original contribution

ESR1 genetic alterations and their association with clinicopathologic characteristics in advanced breast cancer: a single academic institution experience☆

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Summary Estrogen receptor (ER) alpha, a ligand-dependent nuclear transcription factor encoded by the *ESR1* gene, is expressed in 70% of breast carcinomas (BCs) and is used as a target for endocrine-based therapies. However, some patients develop resistance to endocrine-based therapies due to *ESR1* mutation, which leads to constitutive activation in the absence of ligand. We retrospectively analyzed 223 clinically advanced BCs using the FoundationOne CDX assay and found 13.9% (31/223) of cases had *ESR1* genetic alterations (26 mutations and 5 amplifications). All *ESR1* mutations occurred within the ligand binding domain, with the most prevalent being Y537S (42.3%) and D538G (38.5%), and all *ESR1*-mutated cases had a history of aromatase inhibitor use. No significant difference in clinicopathologic features was identified between *ESR1*-mutated and *ESR1*-amplified cases except higher frequency of HER2 positivity and *TP53* mutations in *ESR1*-amplified cases. The prevalence of *ESR1* mutations in ER-positive BCs was 19.1% (26/136). In comparison to *ESR1*-nonmutated ER-positive cases, *ESR1*-mutated cases demonstrated significantly higher percentage of tumor cells with ER and progesterone receptor expression, an increased tendency for overall distant metastasis and liver metastasis, higher frequency of *FGF3/4/19* mutations, lower frequency of *TP53* mutation, but no difference in overall survival and metastatic/recurrent intervals. In conclusion, our findings suggest that development of *ESR1* mutations are selected for under the influence of estrogen deprivation, and a positive correlation between *ESR1* mutations and ER protein expression may exist.

☆ Disclosure: None.

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1. Introduction

Estrogen receptor (ER) alpha, encoded by the *ESR1* gene, is expressed in approximately 70% of breast carcinomas (BCs) [1]. ER functions as a ligand-dependent nuclear transcription factor important for physiologic processes involving carcinogenesis, tumor progression, and mediation of other signaling pathways [2−4]. Given the importance of ER and its common expression in BC, endocrine-related therapies are a cornerstone treatment modality [5,6]. Common endocrine therapies include aromatase inhibitors, which decease production of endogenous estrogen; selective ER modulators, such as tamoxifen, which compete with estrogen for binding to ER and have antiproliferative effects in breast tissue; and selective ER degraders, which inhibit the ER signaling pathway and lead to degradation of receptor, the most notable of which is fulvestrant [7−10].

Despite the effectiveness of endocrine therapy in both prevention and treatment of BC, approximately 25% of patients with ER-positive early-stage BC will develop resistance to such therapies within the first 10 years after initial diagnosis [11]. In addition, in the metastatic setting, endocrine therapy will lead to tumor regression in only 30% of patients, with resistance developing in almost all patients [1]. One mechanism of endocrine resistance involves mutations of the ligand binding domain (LBD) of the *ESR1* gene leading to ligand-independent constitutive activation of ER. The prevalence of such mutations has been reported in close to 20% of metastatic BCs, the vast majority of which occurred in patients who had received endocrine therapy [12−14]. In addition, these mutations have been detected both in paraffin-embedded tissue and circulating tumor DNA [15]. The most common mutations include D538G and Y537S [16]. Overall, these patients exhibit resistance to standard endocrine therapy and a worse overall survival [17].

Although *ESR1* mutations have been associated with use of aromatase inhibitors, not all patients treated with aromatase inhibitor develop *ESR1* mutations. In this study, we aimed to identify clinicopathologic characteristics which may be associated with *ESR1* mutations and to expand upon the currently available data through exploring an institutional cohort of BCs harboring *ESR1* genetic alterations and comparing with those without *ESR1* alterations.

2. Materials and methods

2.1. Study cohort

This study was approved by the Ohio State University Institutional Review Board. At our institution, most cases of clinically advanced BC are sent to Foundation Medicine (Cambridge, MA) for analysis of genetic alterations using the FoundationOne CDx next-generation sequencing (NGS) assay as described before [18]. In our institution, although most patients with advanced disease were tested to find potential targeted therapy, a small portion of these patients (up to 20%) were not tested due to lack of insurance coverage, outside consultation, and so on. In this study, we retrospectively analyzed all cases of BC which had accompanying FoundationOne CDx NGS reports from our institution between January 2014 and June 2018, including 223 clinically advanced BCs (66 locally recurrent and 157 metastatic). Clinicopathologic characteristics were collected from the electronic medical record and surgical pathology reports, including BC biomarker results [(ER), progesterone receptor (PR), and HER2]. Biomarker status had been previously evaluated by breast pathologists at our institution by manual quantification of immunohistochemical (IHC) stains (ER: clone SP1 [Spring, Pleasanton, CA], PR: clone PgR 636 [DAKO, Carpinteria, CA], HER2: 4B5 clone [Ventana, Tucson AZ]). Representative images are demonstrated in Fig..

2.2. Genetic analysis

Genetic alterations for all cases had been previously detected via the FoundationOne CDX NGS assay performed at Foundation Medicine, which evaluates for 315 cancer-related genes and 28 selected rearrangements of which have implications in cancer biology, prognosis, and options for targeted therapy. The assay was performed using DNA extracted from formalin-fixed paraffin-embedded tumor tissue. For this study, data were extracted from the corresponding reports provided by Foundation Medicine. The genetic alterations extracted from the FoundationOne CDx reports were organized by frequency and percentage for each group.

2.3. Statistical analysis

All variables were summarized categorically or numerically with frequency and percentage in each group. Fisher's exact test was used to compare each variable between different groups. An adjusted p-value of <0.05 was considered significant. Data analysis were completed using SAS 9.4 (SAS, Cary, NC).

3. Results

3.1. Clinicopathologic characteristics of 31 BCs with ESR1 alterations

Two hundred twenty three total cases of BC diagnosed at The Ohio State University Wexner Medical Center between 2014 and 2018 were analyzed, including 136 ER-positive and 87 ER-negative tumors. *ESR1* genetic alterations were detected in 31 cases (13.9%), including 26 mutations (11.7%) and 5 amplifications (2.2%). The median patient age of those with *ESR1* genetic alterations was 53 (range: 36−73). Both invasive carcinomas, no special type (27/31, 87.1%) and invasive lobular carcinomas (4/31; 12.9%)

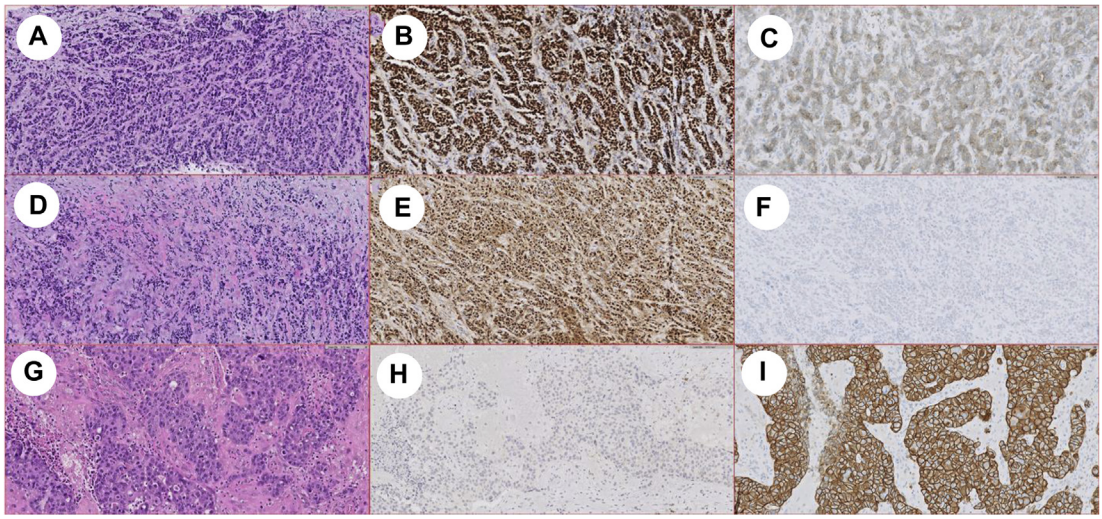

Fig.1 Representative images from cases with *ESR1* genetic alterations. (A-C) A metastatic case with *ESR1* mutation. (A) H&E stain; (B) strongly positive ER staining; (C) equivocal 2+ HER2 staining. (D-F) A metastatic case with *ESR1* amplification. (D) H&E stain; (E) strongly positive ER staining; (F) negative HER2 staining. (G-I) A metastatic case with *ESR1* amplification. (G) H&E stain; (H) negative ER staining; (I) 3+ HER2 staining. Magnification: 200×.

harbored these mutations, with a majority of cases coming from distant metastatic sites (27/31, 87.1%). There were no significant differences in age, histologic type, location (local recurrence vs. distant metastasis), or stage with regards to *ESR1* mutation versus amplification status (Table 1).

As expected, the majority of cases with *ESR1* genetic alterations were positive for ER by immunohistochemistry (96.8%), with an average of 84% cellular staining and only 1 negative case which showed *ESR1* amplification. Interestingly, HER2 was positive by immunohistochemistry in 4 cases (12.9%), 3 of which exhibited amplification of *ESR1* and 1 exhibiting *ESR1* mutation ($p = 0.0006$). There were no statistically significant differences in hormone receptor expression (ER/PR) between cases with *ESR1* amplification versus mutation. Nearly all patients with *ESR1* genetic alterations had received hormonal therapy with an aromatase inhibitor during the course of their treatment. The one exception was an *ESR1*-amplified breast cancer which was also ER-negative by immunohistochemistry, in addition to PR- and HER2-negative (triple negative). All BCs with *ESR1* mutations had been previously treated with an aromatase inhibitor (Table 1).

Table 1 Clinicopathologic characteristics of 31 breast carcinomas with *ESR1* genetic alterations.

Clinicopathologic parameter	Sub-categories	Total *ESR1* genetic alterations (n = 31)		*ESR1* amplification (n = 5)		*ESR1* mutations (n = 26)		*p* value
Age		53	36–73	59.4	50–70	51.9	36–73	NS
Histologic type	IC, NST	27	87.1%	4	80%	23	88.5%	NS
	ILC	4	12.9%	1	20%	3	11.5%	
Biomarkers	ER+	30	96.8%	4	80.0%	26	100.0%	NS
	ER%	84%	0–100%	77%	0–99%	90%	50–100%	
	PR+	23	74.2%	2	40.0%	21	80.8%	NS
	PR%	41%	0–100%	14%	0–50%	50%	0–100%	
	HER2+	4	12.9%	3	60.0%	1	3.8%	0.0006
Location	Local recurrence	4	12.9%	1	20.0%	3	11.5%	NS
	Distant metastasis	27	87.1%	4	80.0%	23	88.5%	
Stage	II	1	3.2%	0	0	1	3.8%	NS
	III	3	9.7%	1	20.0%	2	7.7%	
	IV	27	87.1%	4	80.0%	23	88.5%	
Aromatase inhibitor		30	96.8%	4	80.0%	26	100%	NS

ER, estrogen receptor; IC, NST, invasive carcinoma, no special type; ILC, invasive lobular carcinoma; NA, not applicable; NS, not significant; PR, progesterone receptor.

Table 2 *ESR1* mutation types and frequency.

ESR1 mutation type	Case #	%
Y537S	11	42.3%
D538G*	10	38.5%
L536H	2	7.7%
L536Q	2	7.7%
Y537C	2	7.7%
Y537N	1	3.8%
V534E	1	3.8%

Note: *Three cases with double mutations: D538G/Y537N, D538G/L536Q, D538G/Y537S.

3.2. ESR1 genetic mutation types and frequencies

Of 31 cases with *ESR1* genetic alterations, 26 cases showed single nucleotide variants (SNVs), and the other 5 showed gene amplification. There was a total of 7 unique SNVs detected, all of which occurred in the *ESR1* LBD. The most common SNVs encountered in this cohort were Y537S (11/26, 42.3%) and D538G (10/26, 38.5%). Other SNVs included L536H, L536Q, Y537C, Y537N, and V534E. Interestingly, three cases harbored two SNVs, all of which included D538G, in addition to L536Q, Y537N, and Y537S (Table 2).

3.3. Other concurrent gene mutations in beast carcinoma with ESR1 genetic alterations

The most common gene mutations other than *ESR1* in these 31 BCs included *PIK3CA* (35.5%), *FGF3/4/19* (35.5%), *FGFR1/3* (29%), *ZNF703* (29%), and *TP53* (25.8%), with no significant clinicopathologic differences between BCs with *ESR1* mutation and BCs with *ESR1* amplification. However, *TP53* mutations occurred more frequently in *ESR1*-amplified BCs than in *ESR1*-mutated BCs (80% vs. 15.4%, $p = 0.0025$). (Table 3).

3.3.1. Comparison of clinicopathologic features, genetic alterations, and survival between ESR1-mutated and ESR1-nonmutated BCs.

Then, we examined any difference in clinicopathologic features, genetic alterations, and survival between *ESR1*-mutated and *ESR1*-nonmutated BCs. The prevalence of *ESR1* mutations in the total cohort of ER-positive BCs was 19.1% (26/136). Four *ESR1*-amplified ER-positive cases were included in the cohort of 110 *ESR1*-nonmutated cases for analysis. *ESR1*-mutated cases demonstrated significantly higher percentage of tumor cells with ER (90% vs 71%) and PR (50% vs 20%) IHC staining. *ESR1*-mutated cases also showed an increased tendency for distant metastasis (88.5% vs 73.5%) and liver metastasis (53.8% vs 24.5%). Regarding other concurrent gene mutations, *ESR1*-mutated cases demonstrated higher frequency of *FGF3/4/19* but lower frequency of *TP53*. Both overall survival and metastatic/recurrent intervals were analyzed, but no difference was detected between *ESR1*-mutated and *ESR1*-nonmutated cases (Table 4).

4. Discussion

Endocrine therapy is the standard of care for patients with hormone receptor−positive BC. Despite its effectiveness, resistance and progression still occur with almost 25% of ER-positive BCs recurring within 10 years and tumor regression occurring in only 30% of patients with metastatic BC [1]. Although there are a multitude of etiologies for recurrence and progression of BC, one that has been getting attention recently are mutations in the LBD of ER.

In this cohort of 223 BCs, over a period of 6 years at our institution who underwent targeted NGS, 31 harbored *ESR1* genetic alterations with 26 mutations and 5 amplifications. All 26 mutations occurred in ER-positive BCs with an overall prevalence of 19.1% within the ER-positive cohort. *ESR1* mutations were far more likely to be found in distant metastatic tumors (23 of the 26 cases with mutations) with an overall prevalence of 23% in ER-positive distant metastatic BCs from this cohort. These findings are in keeping with previous large studies showing *ESR1* mutation prevalence between 14% and 17.5% in metastatic BCs [12−14]. Studies with smaller cohorts varied significantly in the reported prevalence of such mutations ranging from 14% up to 55% in one study of 11 patients with metastatic ER-positive BC [19−21]. In contrast to metastatic BCs, primary tumors are less likely to harbor *ESR1* LBD mutations. Previous studies have demonstrated *ESR1* mutations at a frequency of 0% up to 3.5% in primary and/or treatment

Table 3 Other concurrent mutations in breast carcinomas with *ESR1* genetic alterations.

Other genetic alteration	Cases with *ESR1* genetic alterations (n = 31)		*ESR1* amplification (n = 5)		*ESR1* mutations (n = 26)		*p* value
PIK3CA	11	35.5%	1	20.0%	10	38.5%	NS
FGF3/4/19	11	35.5%	1	20.0%	10	38.5%	NS
FGFR1/3	9	29.0%	3	60.0%	6	23.1%	NS
ZNF703	9	29.0%	3	60.0%	6	23.1%	NS
TP53	8	25.8%	4	80.0%	4	15.4%	0.0025

Table 4 Comparison of clinicopathologic features between *ESR1*-mutated and nonmutated ER + breast cancers.

Clinicopathologic Parameter	Sub-categories	All ER-positive cases		*ESR1*-nonmutated cases		*ESR1*-mutated cases		*p* value
Case #		136		110		26		
Age		51.5	26–75	51.3	26–75	51.9	36–73	NS
Histologic type	IC, NST	125	91.9%	102	92.7%	23	88.5%	NS
	ILC	21	15.4%	18	16.4%	3	11.5%	
Biomarkers	ER+	136	100.0%	110	100.0%	26	100.0%	NS
	ER%	74%	1–100%	71%	1–100%	90%	50–100%	0.0075
	PR+	75	55.1%	54	49.1%	21	80.8%	0.0055
	PR%	26%	0–100%	20%	0–100%	50%	0–100%	0.0002
	HER2+	11	8.1%	10	9.1%	1	3.8%	NS
Location	Local recurrence	36	26.5%	33	30.0%	3	11.5%	0.0519
	Distant metastasis	100	73.5%	77	70.0%	23	88.5%	
Distant metastatic site	Bone	21	15.4%	19	17.3%	2	7.7%	NS
	Lung	6	4.4%	6	5.5%	0	0.0%	NS
	Brain	2	1.5%	2	1.8%	0	0.0%	NS
	Liver	41	30.1%	27	24.5%	14	53.8%	0.0089
	Lymph node	12	8.8%	10	9.1%	2	7.7%	NS
	Other	18	13.2%	13	11.8%	5	19.2%	NS
Other mutations	PIK3CA	52	38.2%	42	38.2%	10	38.5%	NS
	FGF3/4/19	27	19.9%	17	15.5%	10	38.5%	0.0213
	FGFR1/3	23	16.9%	17	15.5%	6	23.1%	NS
	ZNF703	28	20.6%	22	20.0%	6	23.1%	NS
	TP53	45	33.1%	41	37.3%	4	15.4%	0.0432
Aromatase inhibitor		125	91.9%	99	90.0%	26	100.0%	NS
Overall survival (months)		107.3	15.6–411.8	104.2	15.6–404.3	119	32.5–411.8	NS
Metastatic/recurrent interval (months)		59.0	0.2–342.6	56.1	0.2–170.4	67.7	0.3–342.6	NS

ER, estrogen receptor; IC NST, invasive carcinoma no special type; ILC, invasive lobular carcinoma; NS, not significant; PR, progesterone receptor.
**ESR1*-nonmutatedcases included 4 *ESR1*-amplified ER-positive cases.

naïve BCs [12–14,19,20,22], suggesting these mutations are likely selected for in the setting of estrogen deprivation therapy.

The most common mutations identified in our study were Y537S and D538G, similar to previous studies [12,13,19,20]. These mutations have been shown to result in constitutive activation of ER in the absence of ligand [12,19,20]. In addition, these mutations have been found to result in reduced inhibition from both tamoxifen and fulvestrant, requiring higher concentrations [12,16]. This has been postulated to be selected for in the setting of estrogen deprivation therapy. Results from the BOLERO-2 trial showed that patients with these specific *ESR1* mutations had a lower median overall survival than those without these mutations. However, in our study, we could not detect any difference in overall survival or metastatic/recurrent interval between *ESR1*-mutated and *ESR1*-nonmutated ER-positive BC patients. The discrepancy may result from the difference in the patient composition of the study cohort as the present study cohort included a small number of cases and a potential selection bias. Our study cohort included only clinically advanced patients with BC, most of which had a poor prognosis. In addition, patients with both Y537S and D538G mutations had an even lower overall survival [17]. Of note, we found 3 cases harboring double mutations in our cohort, all of which included D538G. However, due to this limited number of patients with double mutations, no statistical analysis was performed in our study.

Five total cases from our cohort showed *ESR1* amplification (2.2%), and three of them were positive for HER2 overexpression by IHC. The significance of *ESR1* amplification has been debatable in the literature, with early studies showing high prevalence and favorable outcomes [23,24]. However, later studies using NGS to detect mutations found a similar prevalence of *ESR1* amplification (2%) in both primary and metastatic tumors, suggesting that it may not play a significant role in the development of endocrine resistance [14].

Previous studies have shown that *ESR1* LBD mutations typically arise in the metastatic setting in patients who had been previously treated with estrogen deprivation therapy, most often aromatase inhibitors [16]. Primary BCs rarely exhibit *ESR1* mutations, adding to the evidence that these mutations are likely selected for in the setting of estrogen

deprivation therapy. In our study, all patients with ER-positive BC and *ESR1* LBD mutations had been treated with an aromatase inhibitor at some point during their course of treatment. All ER-positive premenopausal patients were offered aromatase inhibitor treatment at our institution, including nonmutated cases. However, very few patients did not receive this therapy due to side effects or other issues. Besides aromatase inhibitors, some patients in our study cohort also received other types of hormonal therapy, such as tamoxifen, including 46% of patients with ER-positive BC and *ESR1* mutations having been treated with both tamoxifen and aromatase inhibitors. These findings add further evidence that these mutations do indeed arise in the setting of previous endocrine deprivation therapy and more specifically treatment with aromatase inhibitors.

The most common mutations occurring in *ESR1*-mutated and *ESR1*-amplified tumors from our cohort included *PIK3CA*, *FGF3/4/19*, *FGFR1/3*, *ZNF703,* and *TP53*, although we found no clinicopathologic differences among *ESR1*-mutated and *ESR1*-amplified tumors harboring these mutations. However, the majority of *ESR1*-amplified tumors also harbored mutations in *TP53*, the clinical significance of which is unclear, although the data are from a small group of only five patients. In comparison to *ESR1*-nonmutated BCs, *ESR1*-mutated cases demonstrated higher frequency of *FGF3/4/19* but lower frequency of *TP53*. The overall data suggest that *ESR1* mutation status alone will not be sufficient to predict clinical outcome in patients with ER-positive BC, as many tumors harbor other driver mutations that likely play a significant role in therapeutic resistance and disease progression [16].

Finally, a recent study showed that previously treated BCs harboring *ESR1* LBD mutations show no significant differences in expression of ER by IHC compared to primary untreated tumors [15]. They showed that the average tumor percentage staining for ER was 90% in untreated primary tumors and 95% in treated tumors. In our study, the average tumor nuclei stained by IHC for ER was 90% (range: 50−100%) and for PR was 50% (range: 0−100%) in *ESR1*-mutated BCs. We also compared the expression of ER and PR between *ESR1*-mutated BCs and *ESR1*-nonmutated BCs and found that *ESR1*-mutated BCs had significantly higher percentage of ER-positive and PR-positive tumor cells. These findings suggest that although *ESR1* mutations do not negatively impact ER IHC⧹ staining, they may occur more frequently in BCs with strong ER IHC staining.

Some limitations of this study include retrospectivity and the small cohort size of *ESR1*-mutated cases. However, we were able to detect some significant differences between *ESR1*-mutated and *ESR1*-nonmutated cases.

In conclusion, *ESR1* LBD mutations are found in a significant percentage of ER-positive BCs, predominantly in the setting of metastatic disease and previous aromatase inhibitor therapy, suggesting that the development of *ESR1* mutations are selected for under the influence of estrogen deprivation and are a contributing factor to clinical progression. Other driver mutations are found in BCs with *ESR1* genetic alterations, suggesting that the clinical significance of *ESR1* genetic alterations should not necessarily be considered in isolation. Finally, *ESR1*-mutated ER-positive BCs expressed more ER protein than *ESR1*-nonmutated ER-positive BCs, suggesting a potential positive correlation between *ESR1* mutations and ER protein expression.

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