ESR1 mutations, ESR1 fusions and co-occurring alterations assessed in breast cancer tumors

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Abstract

Background: ESR1 mutations and fusions arise in hormone receptor positive (ER+ and/or PR+) breast cancer (HR+ BC) patients after aromatase inhibitor (AI) therapy (low estrogen states), to become constitutively active in a ligand-independent manner. Patients with tumors harboring ESR1 D538G/Y537 mutations (by digital droplet PCR of ctDNA) exhibit worse prognosis and outcome with no particular predictive role in determining benefit of chemotherapy vs tamoxifen treatment after progression on AI (Augusto, et al. ASCO 2016). A retrospective analysis of the ESR1 mutation frequency and co-occurring alterations that could guide subsequent therapy approaches was investigated.

Methods: Molecular profiles of 416 breast tumors [HR+ (n=237), HER2+ (n=29) and TNBC (n=139)] were assessed. Protein expression (IHC) and gene amplification (ISH) were performed. Genomic testing included 592-gene hybrid-capture NGS [NextSeq Illumina platforms] and ArcherDx fusion assay based on anchored multiplex PCR (AMP) FusionPlex Solid Tumor. ESR1 variant (ESR1var) (mutation/fusion) profiles and HR+ BC patients lacking genomic ESR1 alterations (ESR1 WT) were compared; Pearson's chi-squared test was used to test for significant differences.

Results: An ESR1 mutation (point mutations, insertion-deletions [n=49] and fusions [n=4]) was detected in 13% (53 /416) of the specimens, and this constitutes 21% (50/237) of all HR+ breast cancers. Two TNBC patients exhibited ESR1 variants (H398Y in exon 7 and A491S in exon 9, both are classified as variants of unknown significance). ESR1 mutations were not detected in HER2+ BC. Seventy-seven percent of patients with ESR1 mutations were detected in metastatic specimens (p=0.03), with liver (19/53 or 36%) and bone (8/53 or 15%) specimens as the most frequent sources for ESR1 variant (ESR1var) positivity. The most common alleles detected were: D538G (24%), Y537S (18%), E380Q (14%) and L536H (4%); 15 other additional alleles were detected (each 2%). ESR1 fusions were detected in 4 ESR1 WT patients: ESR1-ATP2B2, ESR1-MKL1/ESR1-TNRC6B, ESR1-ARNT2 and ESR1-C6ORRF211. We next compared ESR1var (mutation/fusion) profiles to HR+ breast patients lacking genomic ESR1 alterations (ESR1 WT). ER expression was present in 100% and 96% of ESR1var and ESR1 WT BC, respectively, however expression of PR was negative in 22% and 38% of ESR1var and WT BC, respectively (p=0.05). Significantly higher rates of other gene amplification events observed in ESR1var vs. ESR1 WT BC included: c11orf30 [EMSY, BRCA2 interacting transcriptional repressor] (20% vs. 7%), CCND1 (51% vs.28%), CCND2 (6% vs. 0%), FGF3 (37% vs. 16%), FGF4 (40% vs. 12%) and FGF19 (40% vs. 15%), whereas cMYC was more frequently amplified in ESR1 WT BC (17% vs. 2%); all p-values <0.05. KRAS mutations were higher in ESR1var vs WT BC (4% vs. 0%; p=.004). Alterations in the PIK3CA pathway were common in both ESR1var and ESR1 WT BC: mutations in PIK3CA, AKT and PTEN were observed in 27% and 26%; 4% and 7%; 6% and 5%, respectively, and PTEN loss by IHC in 29% and 26%.

Conclusions: ESR1 mutations and fusions are detected in 21% of HR+ BC, the majority of which are in metastatic sites. Amplifications of genes involved in downstream regulatory pathways were present and may contribute to the poor prognosis of ESR1var HR+ BC. Correlation with antecedent therapy is currently underway.

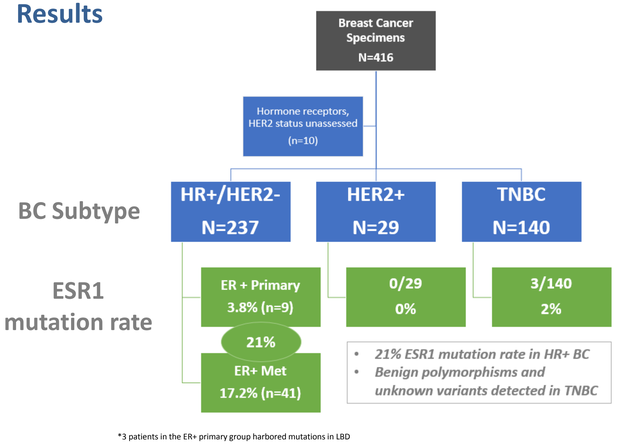
Background

- Aromatase inhibitors, which suppress estrogen biosynthesis and lower levels of circulating estrogen, play an integral role in the treatment of hormone receptor positive breast cancer, in both the adjuvant and metastatic settings.[1,2]
- Studies suggest ESR1 mutations in the ligand-binding domain are acquired to adapt to a low estrogen state, inducing ER signaling through a ligand-independent mechanism.[2]
- ESR1 mutations are found mostly in ER-positive tumors that are metastatic, and have had prior endocrine therapy, including aromatase inhibitors. ESR1 mutations are acquired in approximately 20-30% of patients with endocrine resistance.
- Combination strategies are now available for targeting endocrine resistance and include the use of mTOR inhibition or cyclin-dependent kinase inhibition with endocrine therapy, including aromatase inhibitors and selective estrogen receptor down-regulators.

Methods

416 consecutive breast tumors were centrally tested at a CLIA-approved laboratory with (1) 592-gene hybrid-capture NGS, (2) immunohistochemistry, (3) in situ hybridization and (4) Archer Dx fusion (anchored multiplex PCR (AMP) solid tumor panel). Retrospective analysis of these samples was performed to study rate of ESR1 mutations and fusions, and for co-occurring alterations. For comparison of ESR1 variant (ESR1var) positive and those HR+ BC patients lacking ESR1 alterations (ESR1 WT), Pearson's chi-squared test was used; p<0.05 was considered statistically significant. IHC thresholds utilized for ER, PR and AR (intensity, % cell staining): 1+1%, 1+1% and 1+10%, respectively.

Results



*3 patients in the ER+ primary group harbored mutations in LBD

Results

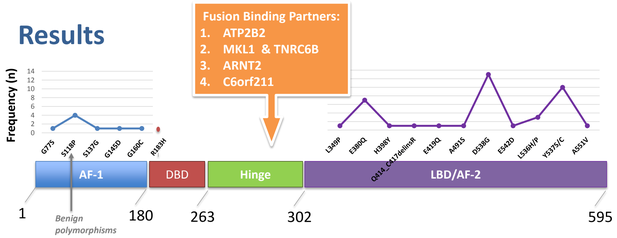


Figure 1. Distribution of ESR1 variants detected by NGS. All variants were found in ER+ patients, with the exception of three variants found in TNBC (S118P, A491S, H398Y). Most variants (82%) identified occur in the ligand binding domain (LBD). Orange inset describes the binding partners for ESR1 in the four patients with ESR1 fusion events.

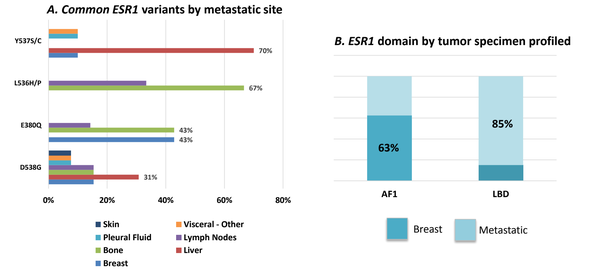


Figure 2. ESR1 variant or domain by tumor specimen site profiled. (A) The most common ESR1 variants and the frequency of specimen site for which the mutation was detected. (B) Although the majority of LBD variants are detected in metastatic sites profiled, the variant is detectable in breast specimens as well.

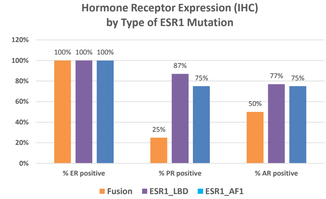


Figure 3. Hormone receptor expression by IHC according to type of ESR1 mutation. ER positivity is retained in most ESR1-var patients, whereas PR expression is more varied and reduced in 75% of fusion-positive patients.

Table 1. Co-occurring events with ESR1 Variants

A.		VAR	WT	p-value
tumor or patient characteristic	Liver_specimen	37%	20%	ns
	Bone_specimen	17%	11%	ns
	Breast_specimen	20%	34%	ns
	Metastatic	80%	67%	0.06
	Age_20-29	0%	1%	ns
	Age_30-39	4%	8%	ns
	Age_40-49	24%	22%	ns
	Age_50-59	33%	27%	ns
	Age_60-69	22%	26%	ns
	Age_70-79	13%	14%	ns
	Age_80-89	4%	2%	ns
	Age_ ≥90	0%	1%	ns
IHC or ISH	HER2_IHC	2%	10%	0.09
	HER2_CISH	5%	12%	ns
	ER_IHC	100%	96%	ns
	AR_IHC	78%	76%	ns
	PR_IHC	78%	62%	0.047
	TOP2A_CISH	3%	2%	ns
	PD1_IHC	31%	51%	ns
	PDL1_IHC	0%	2%	ns
	cMET_IHC	0%	4%	ns
	EGFR_IHC	0%	16%	ns
	ERCC1_IHC	34%	36%	ns
	MGMT_IHC	40%	70%	0.065
	PGP_IHC	0%	9%	ns
	PTEN loss_IHC	29%	26%	ns
	RRM1_IHC	16%	18%	ns
	TOPO1_IHC	59%	60%	ns
	TS_IHC	23%	29%	ns
	TUBB3_IHC	42%	47%	ns

B.		VAR	WT	p-value
CNV	CCND3	2%	1%	ns
	C11orf30	20%	7%	0.024
	CCND1	51%	28%	0.004
	CCND2	6%	0%	0.007
	FGF19	40%	15%	0.001
	FGF3	37%	16%	0.002
	FGF4	40%	12%	4.15021E-05
	cMYC	2%	17%	0.014
	CCNE1	2%	3%	ns
	CDKN2A	2%	3%	ns
	MDM2	2%	11%	0.09
NGS	AKT1(pathogenic)	4%	7%	ns
	ATR (all unclassified)	14%	4%	0.019
	BRIP1 (all unclassified)	15%	6%	0.031
	CAMTA1 (all unclassified)	16%	6%	0.029
	CREB3L1 (all unclassified)	24%	42%	0.024
	FANCA (all unclassified)	24%	7%	0.001
	GNAQ (all VUS)	7%	1%	0.02
	KRAS (pathogenic)	4%	0%	0.004
	LGR5 (all unclassified)	0%	8%	0.05
	MLLT6 (all unclassified)	26%	40%	0.08
	NTRK2 (all unclassified)	4%	1%	ns
	NTRK3 (all unclassified)	7%	0%	0.000393
	PDK1 (all unclassified)	4%	1%	ns
	PIK3CA (pathogenic)	27%	26%	ns
	RB1 (unclassified)	7%	3%	ns
	TP53 (pathogenic)	33%	41%	ns
	PTEN (pathogenic)	6%	5%	ns

Table 1. Comparison of ESR1var vs. ESR1 WT HR+ breast cancers: (A) tumor or patient characteristics, IHC and ISH, (B) CNV and NGS. Biomarker frequencies represent % positive, amplified or mutated in subgroups analyzed. Highlighted rows are comparisons with p≤0.05.

Conclusions

- ESR1 variants were detected in 21% of hormone receptor positive breast cancer specimens, consistent with what is reported in the literature.
- The majority of ligand-binding domain mutations which are associated with acquired resistance to aromatase inhibitor therapy were detected in metastatic specimens. Liver was the most frequent site for the Y537 and D538 hot spots.
- ER expression is retained in breast tumors harboring ESR1 variants. PR expression is more varied. Three of the four patients with ESR1 fusions lacked PR expression.
- Co-occurring alterations with ESR1 mutations may lend support to other novel combinatorial treatment strategies.

References

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