Kinome reprogramming as a therapeutic opportunity in ESR1 fusions driven breast cancer but not in gynecologic cancers

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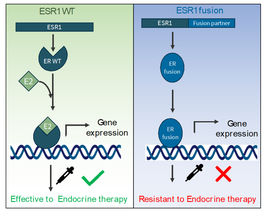
Abstract

Resistance to endocrine therapies can arise from prolonged exposure or inherent mechanisms pre-existing treatment. These mechanisms include heightened estrogen receptor (ER) activity due to estrogen receptor (ESR1) mutations, fusions or activation of ER-independent pathways promoting cell survival. Administering specific tyrosine-kinase inhibitors in kinase fusion positive cancers, like lung and pancreatic cancer, has significantly improved outcomes. Gene fusions involving ESR1 gene, a non-kinase, has previously been detected ~~have been observed~~ in breast and gynecologic cancers, including ovarian and uterine cancer, ~~they haven't yielded similar success~~.

Despite the longstanding success of ERa inhibitors in breast cancer, conventional ER signaling, reliant on estrogen binding to ER, hasn't been replicated in these cases. Treatment approaches involve aromatase inhibitors, selective ER modulators and selective ER degraders. However, most ESR1 fusion cases lack the ligand binding domain, rendering these therapies ineffective. To address this, we've characterized ESR1 fusions and proposed targeting a downstream surrogate as an effective approach for ESR1 fusion positive breast cancer.



Objectives

Women with breast cancer often have resistance to ER direct/indirect inhibition. ESR1 fusions have been speculated as a mechanism of resistance in this cohort of patients.

Determine the prognostic significance of ESR1 fusions in breast, ovarian and uterine cancers.

Delineate the effect of these fusions at a molecular level by delving deeper into structural characterization, co-occurring pathogenic variants, amplifications and expression profiles of these fusions in different indications.

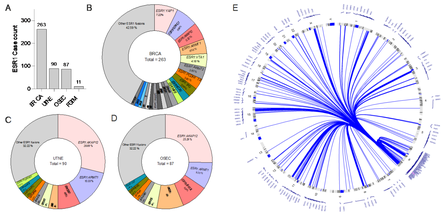
Validate downstream kinase signaling events that are differentially upregulated in ERa+ cancer cases (ESR fusions included) that can be therapeutically targeted.

Methods

We retrospectively analyzed pan cancer samples (N= 216,176) for ESR1 gene fusions from the Caris dataset. Methods briefly: For samples tested February 2019 and later, gene fusion detection was performed on mRNA isolated from a FFPE tumor sample (n = 216176) using the Illumina NovaSeq platform (Illumina, Inc., San Diego, CA) and Agilent SureSelect Human All Exon V7 bait panel (Agilent Technologies, Santa Clara, CA). FFPE specimens underwent pathology assessment to determine tumor content and size, requiring a minimum of 10% tumor content for RNA extraction. RNA extraction utilized the Qiagen RNA FFPE tissue extraction kit, with quality and quantity assessed using the Agilent TapeStation. Biotinylated RNA baits hybridized to cDNA targets, followed by post-capture PCR amplification. Resulting libraries were quantified, normalized, pooled, denatured, diluted, and sequenced using the GRCh37/hg19 reference genome. For Fusions, raw data were demultiplexed using the Illumina DRAGEN FFPE accelerator. FASTQ files were aligned with STAR aligner (Alex Dobin, release 2.7.4a github). A full 22,948-gene dataset of expression data were produced by the Salmon, which provides fast and bias-aware quantification of transcript expression. BAM files from STAR aligner were further processed for RNA variants using a custom detection pipeline. The reference genome used was GRCh37/hg19 and analytical validation of this test demonstrated ≥ 97% Positive Percent Agreement (PPA), ≥ 99% Negative Percent Agreement (NPA) and ≥ 99% Overall Percent Agreement (OPA) with a validated comparator method.

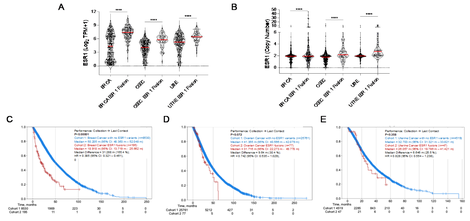
Results

Figure1: Frequency of ESR1 fusions



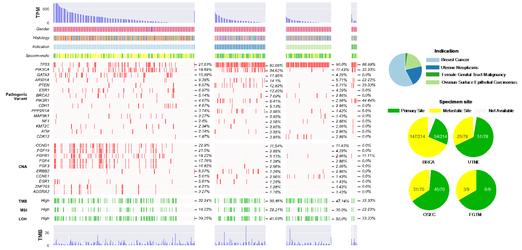
Statistics of overall patient cases harboring ESR1 fusions across cancer indications (A). Frequency and case counts of patients with ESR1 fusions in Breast cancer (B) Uterine cancer and (C) and Ovarian cancer (D) respectively. E) Circos plot mapping ESR1 and its corresponding gene fusion partners across chromosomes.

Figure 2: Expression profile and KM curves for patients with and without ESR1 fusions



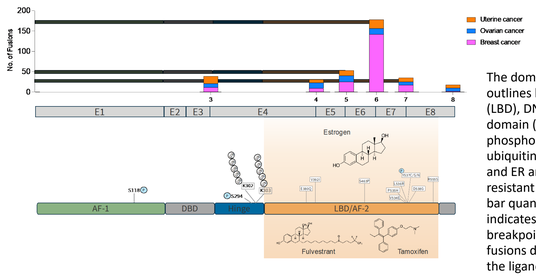
(A&B) Expression and copy number profiles of breast, ovarian and uterine cancer with and without ESR1 fusions. Kaplan-Meier survival curves for the overall survival of patients with ESR1 fusions vs patients without ESR1 variants in breast cancer (C) Ovarian cancer (D) and Uterine cancer (E).

Figure 3: Pathogenic variants in patients with ESR1 fusions



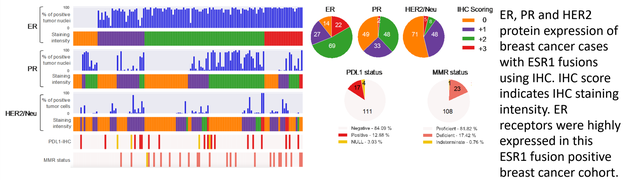
Genomic landscape of ESR1 fusions across cancer indications: Genetic alterations (co-occurring pathogenic mutations, copy number alterations and fusions) identified by whole- exome and RNA sequencing. Frequencies of other alterations in 451 analyzed samples are indicated at the end of each row. The top panel gives us an insight into the indication of histology and RNA expression (TPM) of each patient. The lower panel offers insight into the tumor mutational burden status as characterized by MSI and LOH.

Figure 4: Structural organization of ESR fusions



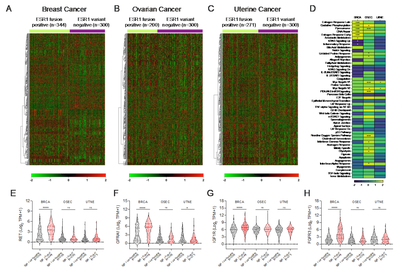
The domain mapping outlines ligand domain (LBD), DNA binding domain (DBD), phosphorylation and ubiquitination sites, and ER antagonist resistant sites. Stacked bar quantitively indicates that most breakpoints of ESR1 fusions do not contain the ligand binding domain

Figure 5: ER,PR & HER2 in ESR1 fusion breast cancer cases



ER, PR and HER2 protein expression of breast cancer cases with ESR1 fusions using IHC. IHC score indicates IHC staining intensity. ER receptors were highly expressed in this ESR1 fusion positive breast cancer cohort.

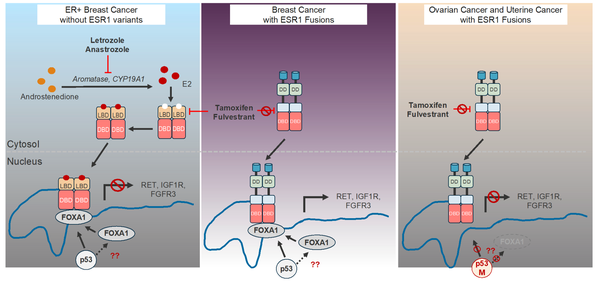
Figure 6: Heatmap indicating 500 most variable genes across control group and ESR1 gene fusion group



Heatmap depicting hierarchical clustering of the 500 most variable genes across control group lacking ESR1 variants and ESR1 gene fusion group in Breast (A) , Ovarian (B), Uterine (C) cancers. KEGG pathway analysis of differentially regulated genes of ESR1 fusions positive cohort vs control cohort(D) Oncogenic kinase genes including RET(E), IGF1R (G) FGFR3(H), and GFRA1 (RET co-receptor) (F), are upregulated in the ESR1 fusion positive cohort compared to the control cohort.

Study Highlights

Mechanism of action and resistance in estrogen receptor (ER) targeted therapy in breast cancer without ESR1 variants, breast cancer with ESR1 fusions, ovarian cancer and uterine cancer with ESR1 fusions. Breast cancer with ESR1 fusions rely heavily on the TP53- FOXA1- ER alpha transcriptional axis as compared to gynecologic indications that do not depend on this axis.



Conclusions

- In the Caris cohort, recurrent ESR1 fusions were detected in Breast cancer (263 cases), Uterine cancer (90 cases) and Ovarian cancer (87 cases).
- ESR1 fusions are ligand independent, hyperactive and physiologically stable with ~~greater half life~~ compared to ERα and may be sufficient for endocrine Tx resistance.
- Expression and copy number of ESR1 was significantly higher in the ESR1 fusion positive cohort compared to the patients without ESR1 variants. This was consistent across all indications including breast cancer, ovarian cancer and uterine cancer
- Patients with ESR1 fusions had significant poor prognosis in all indications compared to the control cohort .
- ESR1 fusions upregulate oncogenic kinase signaling in breast cancer observed by upregulation of RET, IGF1R and FGFR3.
- Similar upregulation of oncogenic signaling was not observed in ESR1 fusion cases of ovarian and endometrial cancer potentially due to the lack of the TP53-FOXA1- ER axis.
- Targeting oncogenic kinase signaling may be a promising approach in ESR1 fusion positive breast cancer.

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