ESR1 fusions drive endocrine therapy resistance and metastasis in breast cancer

Jonathan T. Lei, Xuxu Gou, and Matthew J. Ellis

Interdepartmental Graduate Program in Translational Biology & Molecular Medicine, Baylor College of Medicine, Houston, TX, USA; Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX, USA; Departments of Medicine and Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA

ABSTRACT

Estrogen receptor alpha gene (ESR1) fusion transcripts have been identified in breast cancer but their role in breast cancer is not completely understood. Here, we report a causal role for ESR1 fusions in driving both endocrine therapy resistance and metastasis, and describe a therapeutic strategy to target ESR1 fusion-induced growth.

The majority of breast cancers express the nuclear hormone receptor, estrogen receptor alpha (ER) and therefore are fueled by estrogen and downstream ER signaling. Despite the tremendous success endocrine therapies, resistance and the development of lethal metastatic disease is common and a major clinical problem.

Dysregulation of the estrogen receptor alpha gene (ESR1) is an established mechanism of inducing endocrine therapy resistance in ER positive (ER+) breast cancer. Recurrent point mutations clustering around the ligand binding domain (LBD) of ESR1 that cause single amino acid residue changes have been found in up to 40% of treatment-refractory, metastatic ER+ breast cancer patients (reviewed in 1). These activating ESR1 point mutations confer constitutive, hormone-independent activity of ER and are often enriched in breast tumors after aromatase inhibitor (AI) treatment.

Emerging evidence now suggests genomic rearrangement events involving ESR1 producing ESR1 fusion genes are another class of somatic mutation that is associated with endocrine therapy resistance. One class of recurrent ESR1 fusion transcripts have been found in a subset of Luminal B primary breast tumors that retain the first two non-coding exons of ESR1 (ESR1-e2) fused to various sequences from a nearby gene, coiled-coil domain containing 170, CCDC170 (ESR1-e2>CCDC170), potentially generated by tandem-duplication, based on the observed orientation of the fusion sequences.2 This fusion gene forms a promoter trap driving aberrant expression of CCDC170 since this gene is now in the context of the ESR1 promoter, producing stable truncated forms of CCDC170 protein (ACCDC170). Specific forms of ACCDC170 leads to reduced tamoxifen sensitivity in experimental models.2 Recently, an additional ESR1-e2 fusion with the acidic residue methyltransferase 1 gene, C6orf211 (ESR1-e2>C6orf211) fusion as well as ESR1-e2>CCDC170 fusions have been identified in AI resistant breast tumors, but more studies are required to test whether these ESR1-e2 fusions produce pathogenic proteins.3 Taken together, these ESR1 fusion events have the potential to generate pathogenic, truncated forms of fusion partner proteins, but does not produce ESR1 fusion proteins (Figure 1B).

Another mechanism that can produce ESR1 fusions is inter- or intra-chromosomal translocation events where the ESR1 gene is fused to more distant locations in the genome. Our group described the first stable and functional ESR1 fusion protein produced by a fusion gene involving the first six exons of ESR1 (ESR1-e6) fused in-frame to C-terminal sequences of the yes associated protein 1 gene, YAP1 (ESR1-e6>YAP1) provided by an inter-chromosomal translocation event.4 This was identified in a patient with endocrine therapy-resistant, metastatic ER+ disease and in a matched patient-derived xenograft (PDX). A recent study described additional ESR1-e6 in-frame, inter-chromosomal translocation events involving the disabled homolog 2 gene, DAB2, and the glycosgenin 1 gene, GYG1 (ESR1-e6>DAB2 and ESR1-e6>GYG1) producing stable in-frame ESR1 fusion proteins at metastatic sites in endocrine therapy refractory ER+ breast cancer patients.5 All of these ESR1-e6 fusions retain the N-terminus of ESR1 encoding the DNA binding and nuclear localization domains, suggesting some functionality. However, detailed functional characterization and studies demonstrating a causal role for ESR1 fusions in endocrine therapy resistance and metastasis has been lacking (Figure 1B).

Our current study investigated the role of ESR1 fusion genes in driving therapeutic resistance and metastasis in ER+ breast cancer.6 We identified a variety of in-frame and out-of-frame translocations involving ESR1 from RNA-seq analysis of primary and metastatic ER+ breast samples. In-frame fusions included inter-chromosomal ESR1 translocations with the YAP1 gene (ESR1-e6>YAP1) as previously described,7 the protocadherin 11 X-linked gene, PCDH11X (ESR1-e6>PCDH11X) and the nucleolar protein 2 homolog gene, NOP2 (ESR1-e6>NOP2), and two intra-chromosomal translocations with the...
A-kinase anchoring protein 12 gene, AKAP12 (ESR1-e6>AKAP12) and the DNA polymerase eta gene, POLH (ESR1-e7>POLH). However, only inter-chromosomal ESR1 fusions, ESR1-e6>YAP1, ESR1-e6>PCDH11X, and ESR1-e6>NOP2, produced stable in-frame ESR1 fusion proteins in vitro. Of these, the two ESR1 fusions identified from endocrine therapy-refractory, ER+ metastatic disease, ESR1-e6>YAP1 and ESR1-e6>PCDH11X, drove endocrine therapy resistant proliferation in experimental models, while ESR1-e6>NOP2 from an endocrine therapy naïve primary tumor did not. In addition, none of the out-of-frame ESR1-e3, ESR1-e4, ESR1-e5, and ESR1-e6 containing fusions nor even an in-frame ESR1-e7 fusion, all identified in primary tumors, were able to drive growth under estrogen-deprived conditions (Figure 1B).

To explore transcriptional properties of the ESR1 fusions that produced stable ESR1 fusion proteins, chromatin immunoprecipitation followed by next-generation sequencing (ChIP-seq) was performed along with RNA-seq in T47D cell lines to examine regulation of ESR1 fusion bound genes. The ESR1-e6>YAP1 and ESR1-e6>PCDH11X drove constitutive expression of these ER target genes in the absence of estrogen, demonstrating strong estrogen-independent transcriptional activation. The ESR1-e6>NOP2 bound relatively few sites compared to the other ESR1 fusions, potentially explaining the weak functional activity in proliferation assays. Interestingly, a cluster of genes was found to be strongly and uniquely up-regulated by the ESR1-e6>YAP1 and ESR1-e6>PCDH11X. Pathway analysis revealed enrichment of epithelial-to-mesenchymal transition (EMT) genes, including induction of an established EMT gene, snail family transcriptional repressor 1, SNAI1. Subsequent functional studies showed the transcriptionally active ESR1-e6>YAP1 and ESR1-e6>PCDH11X fusions induced cell motility in vitro and drove metastasis to the lung in xenograft models.

Since the formation of these ESR1 fusion proteins lacks the LBD of ESR1, all known endocrine therapies that target the LBD are likely to be ineffective. Therefore, we targeted downstream ER signaling events by using a United States Food and Drug Administration approved cyclin-dependent kinase (CDK) 4/6 inhibitor for metastatic ER+ breast cancer, palbociclib, based on our previous observation that palbociclib antagonized tumor growth driven by ESR1 point activating mutations. Palbociclib suppressed growth driven by ESR1-e6>YAP1 and ESR1-e6>PCDH11X in vitro, and at primary and metastatic sites in a PDX model naturally harboring the ESR1-e6>YAP1 fusion.

Taken together, these results further our understanding of the mechanisms underlying endocrine therapy resistance and metastasis in ER+ breast cancer. Transcriptionally active in-frame ESR1-e6 fusions such as ESR1-e6>YAP1 and ESR1-e6>PCDH11X constitutively drives expression of not only ER target genes leading to endocrine therapy-resistant
proliferation but also induces EMT genes leading to metastasis (Figure 1B). Therefore, formation of ESR1 fusion genes links these two processes together, potentially explaining the lethal outcomes in the patients these fusions were identified.

These findings also have important therapeutic and diagnostic implications. Since ESR1 fusion driven growth remained sensitive to CDK4/6 inhibition, the presence of an in-frame ESR1 fusion could be used as a biomarker to stratify patients for CDK4/6 inhibitor therapy. Also, a pattern of ESR1 fusions is emerging in metastatic ER+ breast cancer, in which the first six exons of ESR1 are fused in-frame to C-terminal partner sequences provided by the partner gene. This finding could potentially drive targeted sequencing approaches to efficiently identify additional active ESR1 fusions by using a 3’ exon sequence against ESR1 exon 6 as bait.

In conclusion, ESR1 fusions are a new class of recurrent somatic mutations that drive endocrine therapy resistance and metastasis in ER+ breast cancer. This study adds to the catalog of actionable ESR1 alterations and furthers our understanding of how ER+ breast cancer gives rise to lethal metastatic disease.

Disclosure statement
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ORCID
Jonathan T. Lei http://orcid.org/0000-0002-0209-8051
Xuxu Gou http://orcid.org/0000-0003-2318-778X
Matthew J. Ellis http://orcid.org/0000-0002-8467-8534

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