The *ESR1* Mutations: From Bedside to Bench to Bedside

Francisco Hermida-Prado[1,2] and Rinath Jeselsohn[1,2,3]

The *ESR1* ligand–binding mutations were unveiled a number of years ago and are the most common genetic mechanism of acquired resistance to endocrine treatment, particularly, to aromatase inhibitors. The discovery of these mutations was enabled after advancements in sequencing technologies and when metastatic tissue samples were interrogated. The *ESR1* ligand–binding domain mutations are activating mutations that lead to constitutive ligand-independent activity, which explains the emergence of these mutations under the selective pressure of aromatase inhibitors. Arnesen and colleagues have generated new models of the *ESR1* mutations using CRISPR technology to generate single-cell–derived clones in which the *ESR1* ligand–binding mutations were "knocked-in" and expressed under the endogenous promoter of estrogen receptor. The authors have extensively characterized these models and have shed new light on the functional consequences *ESR1* mutations.

See related article by Arnesen et al., p. 539

Estrogen receptor (ER)-positive breast cancer accounts for the majority of breast cancer cases and the majority of breast cancer mortality. Despite the efficacy of endocrine treatment, resistance remains a major clinical challenge. As we are increasingly sequencing endocrine-resistant tumors, we are uncovering multiple genomic aberrations enriched after endocrine treatment that are likely drivers of endocrine resistance. These include alterations in the MAPK signaling pathway, such as loss-of-function mutations in *NF1* and gain-of-function mutations in *ERBB2*, and alterations related to transcription, consisting of *MYC* amplifications and hotspot mutations of *CTCF* and *FOXA1* (1). Although the frequencies of these alterations are increased after endocrine treatment, their prevalence remains relatively low and the most common mutations acquired after endocrine treatment are those affecting the *ESR1* ligand–binding domain. In metastatic ER-positive breast cancer, *ESR1* mutations are detected in 30%–40% of patients, and of these, the Y537S and D538G mutations are the most common (reviewed in ref. 2). In addition, a number of less prevalent missense hotspot *ESR1* mutations were identified. These low-prevalence *ESR1* mutations have not been as extensively characterized, but they appear to possess diminished phenotypes compared with Y537S and D538G (3). *ESR1* mutations are rare in primary disease and evolve under the selective pressure of aromatase inhibitor treatment in metastatic disease. Although to a lesser extent, a number of studies have now shown that the *ESR1* mutations are also enriched after adjuvant endocrine treatment (4, 5).

The discovery of the *ESR1* mutations in breast cancer metastatic tumors and the subsequent preclinical and clinical studies underscore the importance of bedside-to-bench-to bedside translational research. The initial preclinical studies of the *ESR1* mutations showed the mutations engender ligand-independent transcriptional activity, enhanced growth in hormone-deprived conditions, and relative resistance to tamoxifen and fulvestrant. The structure of the Y537S and D538G mutations was solved and provided mechanistic insights to the constitutive properties of these mutations. In addition, biophysical studies revealed that the mutations have decreased affinity to tamoxifen and fulvestrant, thus explaining their relative resistance to these agents (summarized in ref. 2). In clinical studies, plasma cell-free DNA (cfDNA) analysis of *ESR1* mutations in patients with ER-positive metastatic breast cancer demonstrated that these mutations lead to aromatase inhibitor resistance and relative resistance to fulvestrant (6). However, when comparing aromatase inhibitors with fulvestrant in the presence of *ESR1* mutations, fulvestrant was shown to have superior activity. Most recently, the initial report of the PADA-1 phase III clinical trial was presented at the 2020 American Society of Clinical Oncology (ASCO) Meeting. The PADA-1 trial, one of the first studies to prospectively study *ESR1* mutations in metastatic ER-positive breast cancer, revealed that the presence of *ESR1* mutations significantly decreased the median progression-free survival in first-line treatment with an aromatase inhibitor and the CDK4/6 inhibitor, palbociclib [median progression-free survival was 11 months in the presence of an *ESR1* mutation and 26.7 months with wild-type (WT) *ESR1*]. In addition, baseline and end-of-treatment analysis of *ESR1* mutations in the PALOMA-3 phase III clinical trial, in which patients with metastatic breast cancer who progressed on an aromatase inhibitor were randomized to palbociclib in combination with fulvestrant versus fulvestrant alone, showed an increase in Y537S mutations after treatment with fulvestrant or fulvestrant in combination with palbociclib. Both studies suggest that the *ESR1* mutations have a role in the resistance to the combination of endocrine treatment plus palbociclib. ER acts upstream of cyclin D1–CDK4/6–Rb1 axis, which can explain the role of the activating *ESR1* mutations in resistance to endocrine treatment in combination with CDK4/6 inhibition. *ESR1* mutations are also a prognostic marker of poor overall survival in metastatic breast cancer. This was demonstrated for both the Y537S and D538G as monoclonal and polyclonal mutations.

A number of groups have generated stable cell lines expressing the ER mutations. Various methods have been employed to develop these models, including models in which the mutations were knocked-in by applying gene editing technologies and homologous recombination and models with stable inducible expression of *ESR1* mutations. Despite the differences between these models, these studies all validated the ligand-independent transcriptional activity of the mutations and also showed that the *ESR1* mutations regulate the transcription of genes that are not estrogen dependent in *ESR1* WT cells (7, 8). In the study by Arnesen and colleagues, the unique ER mutant–regulated

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts. [2]Center for Functional Cancer Epigenetics, Dana-Farber Cancer Institute, Boston, Massachusetts. [3]Susan F. Smith Center for Women's Cancers, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts.

Corresponding Author: Rinath Jeselsohn, Dana-Farber Cancer Institute, 450 Brookline Ave., Boston, MA 02215 D728. Phone: 617-632-1900; Fax: 617-632-1980; E-mail: Rinath_jeselsohn@dfci.harvard.edu

Cancer Res 2021;81:537–8

doi: 10.1158/0008-5472.CAN-20-4037

genes were enriched in genes involved in cell migration and motility. Similarly, in our study, in which we investigated doxycycline-inducible cell models of the *ESR1* mutations, the ER mutant–regulated genes were enriched in genes involved in metastasis. Furthermore, we showed that the expression of the ER mutations promotes metastasis in *in vivo* studies. Importantly, we showed that silencing of the ER mutations led to regression of metastases, which demonstrates the therapeutic potential of targeting *ESR1* mutations (8). The studies from the stable cell lines from the different research groups showed that the unique transcriptomes regulated by ER mutants are allele specific, with functional differences between the Y537S and D538G mutations. The differences between the Y537S and D538G were also borne out by the respective structures, pharmacology, and clinical outcomes of the *ESR1* Y537S and D538G mutants.

The study by Arnesen and colleagues (7) also highlights the variability between different cell models developed in different laboratories. As evidenced by the principal components analysis plots of the RNA sequencing of the MCF7 and T47D cell models in the article by Arnesen and colleagues, the cells strongly segregated on the basis of the laboratory of origin and not by mutational status or treatment condition. This is not surprising, as the cells have been maintained in different laboratories and the mutations were introduced by employing different methods. While the doxycycline-inducible models were generated from polyclonal cell populations and enable the evaluation of isogenic models, the models produced by Arnesen and colleagues were derived from single-cell clones. A landmark study demonstrated that cell lines are not clonal or genetically stable (9). Single-cell clones derived from the same MCF7 parental population differed in their mutational landscape, and the clones continued to evolve after multiple passages. Thus, the clonal nature of the cells described in the article (9) may also explain the differences in the transcriptional effects of the mutant ER cistrome observed in this article versus previous work. These differences illustrate the cell context–dependent functional consequences of the *ESR1* mutations, which are likely true for other mutations and have important clinical implications. As an example, *PIK3CA* mutations in early-stage ER-positive breast cancer are associated with good outcomes, whereas in metastatic ER-positive breast cancer, *PIK3CA* mutations are associated with decreased overall survival compared with WT PIK3CA. Moreover, in metastatic triple-negative breast cancers, *PIK3CA* mutations are associated with better outcomes (10).

In addition to the cell context–dependent functional consequences of *ESR1* mutations, the functional and clinical consequences of *ESR1* mutations may vary in the context of different treatment regimens. As aforementioned, there is clinical evidence that *ESR1* mutations contribute to and evolve during the onset of resistance to palbociclib and endocrine treatment. However, it is not known whether *ESR1* mutations result in unique therapeutic vulnerabilities after the acquisition of resistance to CDK4/6 inhibitors. Along these lines, we do not know whether and how *ESR1* mutations affect response to chemotherapy or novel targeted therapies in clinical development, such as AKT1 inhibitors. Importantly, we now have early evidence from the PADA-1 study that successful clearance of the cfDNA *ESR1* mutations in first-line treatment of metastatic disease with endocrine treatment and palbociclib was associated with improved outcomes (presented at ASCO 2020). However, it is not known why some patients receiving a certain treatment regimen will clear cfDNA *ESR1* mutations, whereas other patients receiving the same treatment do not. This may be due, in part, to the tumor-specific genetic or epigenetic background and/or to the allele frequency of the mutation, or the specific allelic mutation.

Taken together, the preclinical and clinical studies all point to the importance of targeting *ESR1* mutations in metastatic ER-positive breast cancer. In response, there are a number of new endocrine treatments in clinical development that will hopefully enable better targeting of ER mutations and improve patient outcomes.

Authors' Disclosures

R. Jeselsohn reports grants from Lilly and Pfizer and personal fees from Carrick Therapeutics and Luminex during the conduct of the study. No disclosures were reported by the other author.

Received December 3, 2020; accepted December 4, 2020; published first February 1, 2021.

Reference

1. Razavi P, Chang MT, Xu G, Bandlamudi C, Ross DS, Vasan N, et al. The genomic landscape of endocrine-resistant advanced breast cancers. Cancer Cell 2018;34:427–38.
2. Jeselsohn R, De Angelis C, Brown M, Schiff R. The evolving role of the estrogen receptor mutations in endocrine therapy-resistant breast cancer. Curr Oncol Rep 2017;19:35.
3. Toy W, Weir H, Razavi P, Lawson M, Goeppert AU, Mazzola AM, et al. Activating ESR1 mutations differentially affect the efficacy of ER antagonists. Cancer Discov 2017;7:277–87.
4. Kuang Y, Siddiqui B, Hu J, Pun M, Cornwell M, Buchwalter G, et al. Unraveling the clinicopathological features driving the emergence of ESR1 mutations in metastatic breast cancer. NPJ Breast Cancer 2018;4:22.
5. Zundelevich A, Dadiani M, Kahana-Edwin S, Itay A, Sella T, Gadot M, et al. ESR1 mutations are frequent in newly diagnosed metastatic and loco-regional recurrence of endocrine-treated breast cancer and carry worse prognosis. Breast Cancer Res 2020;22:16.
6. O'Leary B, Hrebien S, Morden JP, Beaney M, Fribbens C, Huang X, et al. Early circulating tumor DNA dynamics and clonal selection with palbociclib and fulvestrant for breast cancer. Nat Commun 2018;9:896.
7. Arnesen S, Blanchard Z, Williams MM, Berrett KC, Li Z, Oesterreich S, et al. Estrogen receptor alpha mutations in breast cancer cells cause gene expression changes through constant activity and secondary effects. Cancer Res 2021;81:539–51.
8. Jeselsohn R, Bergholz JS, Pun M, Cornwell M, Liu W, Nardone A, et al. Allele-specific chromatin recruitment and therapeutic vulnerabilities of ESR1 activating mutations. Cancer Cell 2018;33:173–86.
9. Ben-David U, Siranosian B, Ha G, Tang H, Oren Y, Hinohara K, et al. Genetic and transcriptional evolution alters cancer cell line drug response. Nature 2018;560:325–30.
10. Mosele F, Stefanovska B, Lusque A, Tran Dien A, Garberis I, Droin N, et al. Outcome and molecular landscape of patients with PIK3CA-mutated metastatic breast cancer. Ann Oncol 2020;31:377–86.