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Neomorphic ERα Mutations Drive Progression in Breast Cancer and Present a Challenge for New Drug Discovery

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In this issue of *Cancer Cell*, Jeselsohn et al. dissect the function of several of the most clinically important estrogen receptor alpha mutants associated with endocrine therapy resistance in breast cancer and demonstrate that they manifest disease-relevant neomorphic activities that likely contribute to tumor pathogenesis.

The majority of breast cancers express estrogen receptor alpha (ERα), a ligand-regulated transcription factor that enables cells to respond to the mitogenic actions of estrogens. Not surprisingly, inhibition of estrogen biosynthesis, using aromatase inhibitors, or direct inhibition of ERα activity, using the selective estrogen receptor modulator (SERM) tamoxifen, have been the cornerstone of therapeutic regimens for ER^+ breast cancer. However, *de novo* and acquired resistance remains an impediment to durable clinical responses to these interventions. In the case of tamoxifen, resistance likely occurs as a consequence of adaptive events that increase the weak partial agonist activity of this drug. This can result from the selection of cells that express a coregulator that recognizes the ERα-tamoxifen complex as "active" (Norris et al., 1999). In the case of aromatase inhibitors, resistance is associated with a dramatic increase in the sensitivity of cells to the mitogenic activities of estrogens secondary to increased activity and/or expression of receptor-associated coregulators. However, the identification of ERα mutations exhibiting altered ligand pharmacology in metastatic lesions from patients who have progressed while on tamoxifen or aromatase inhibitors has provided an alternative, unanticipated mechanism to explain resistance. Further, because these ERα mutants are rarely (if ever) found in primary tumors but are present in about 30% of metastatic biopsies taken from patients progressing on endocrine therapy, it has been suggested that they may contribute directly to disease pathogenesis (Jeselsohn et al., 2014).

Significant in the Jeselsohn study in this issue of *Cancer Cell* was the observation that the most commonly occurring mutations, Y537S and D538G, while regulating a similar cohort of genes as WT ERα, also exhibited mutant-specific transcriptional activities that result in the expression of genes encoding proteins that are associated with aggressive disease (metastasis) (Figure 1) (Jeselsohn et al., 2018). Demonstration that the "neomorphically expressed genes" associated with each mutant were also expressed in metastatic tumors from patients suggests that products of these genes may contribute directly to disease pathobiology. This position is supported by the results of studies showing that, when compared to cells expressing WT ERα, cells engineered to express Y537S or D538G exhibit substantially increased metastatic activity in mice. Mechanistic studies revealed that the chromatin-binding landscape of the ERα mutants differs significantly from WT ERα, an unexpected finding given that the mutations cluster in a region that is spatially and functionally distinct from the receptor's DNA-binding domain (DBD). This raises the question as to how mutations in the ligand-binding domain (LBD) influence DNA binding specificity. *De novo* motif analysis revealed that the ERα-interacting *cis*-elements within the neomorphic genes are enriched for estrogen response elements (EREs) that appear to be structurally indistinguishable from classical EREs. It was noted, however, that the DNA-binding activity of the ERα mutants at the gained ERα binding sites was less reliant on the pioneer factor FOXA1 than is wild-type (WT) ERα at previously defined EREs. This suggests that a different pioneer factor may be employed by these mutants at the gained DNA binding sites, although it is unclear how such a factor would distinguish between WT and mutant ERα. Informative in this regard are the results of a previous study demonstrating, in cellular models of endocrine-therapy-resistant prostate cancer, that phosphorylation of the transcriptional coregulator EZH2 shifted its binding partner preference from PRC1 to androgen receptor (AR), which resulted in a significant change in the AR-cistrome (Xu et al., 2012). Further, it has been shown that the ERα cistrome can be altered by activating other nuclear receptors (e.g., progesterone, glucocorticoid, androgen, or retinoic acid receptors) (Mohammed et al., 2015; Yang et al., 2017). When taken together, it seems likely that the binding partner preferences of WT and mutant ERα will be different and that these differences may explain the alterations in the chromatin-binding landscape noted. It is also possible, however, that the domain harboring the mutations directly influences the function of the DBD. The crystal structure of full-length ERα (WT or mutants) is not yet available. However, analysis of the structure of the full-length nuclear receptor PPARγ in the context of a PPARγ:RXRα complex has indicated that the LBD and DBD of this receptor interact to influence DNA-binding activity (Chandra et al., 2008). Similar intramolecular interactions in ERα, if they occur, could influence ERα-DNA binding preferences.

This and other studies of ERα mutants in breast cancer have raised the question of how to mitigate their negative impact on tumor biology. Jeselsohn et al. tackled

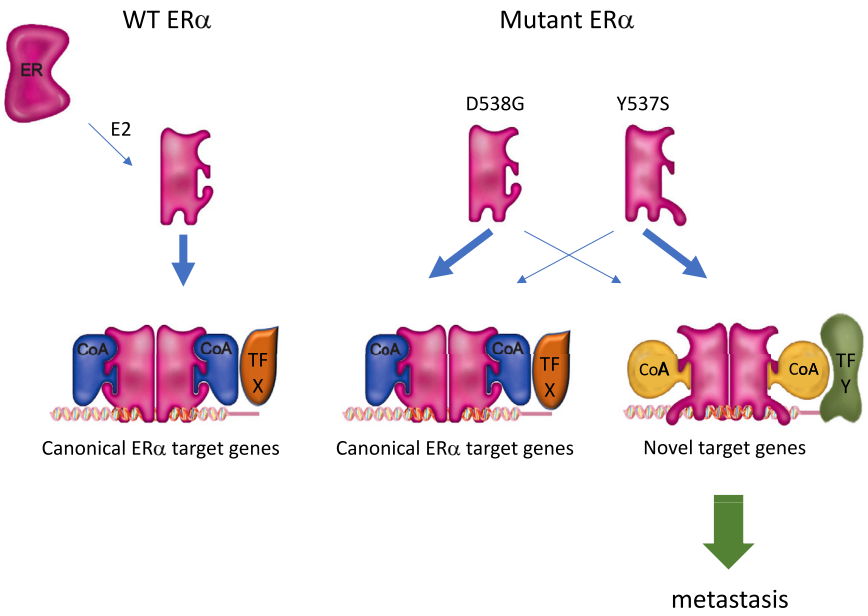

Figure 1. Disease-Relevant ERα Mutants Exhibit Neomorphic Transcriptional Activities
In the absence of hormone, wild-type (WT) ERα is maintained in an inactive state within cells. Upon binding estrogens, such as 17β-estradiol (E2), the receptor undergoes an activating conformational change that enables its spontaneous dimerization and association with specific estrogen response elements (EREs) located within the regulatory regions of target genes. The DNA-bound receptor subsequently facilitates the assembly of large multi-protein complexes, which include coactivators (CoAs), cooperating transcription factors (TFs), chromatin-modifying enzymes, and components of the general transcription machinery to drive the transcription of canonical ERα target genes. The ERα mutants D538G and Y537S (and Y537N) assume an active conformation in the absence of ligand and exhibit constitutive activity. Further, likely a consequence of differential coregulator binding, these mutations also exhibit non-identical neomorphic functions, inducing the expression of genes that encode proteins that likely contribute to disease pathobiology.

this problem using CRISPR technology to identify targetable proteins that were essential for the growth of cells expressing WT or mutant ERα. In this manner, they identified CDK7 as essential and showed that an inhibitor of this enzyme effectively inhibited the growth of all ERα-expressing breast cancer cells. A more immediate solution to the problem is to exploit our current understanding of ERα allostery to develop new, or repurpose existing, SERMs/antagonists that alter the conformation of the ER mutants (and WT ERα) in such a manner as to disengage their interaction with obligate coregulators/cooperating pioneer factors. The efficacy of such approaches, however, may be compromised by the fact that the "active" conformation is considerably stabilized by the Y537S mutation (Toy et al., 2013). This reinforces the importance of efforts to identify and develop selective estrogen receptor downregulators (SERDs), drugs that interact with ERα (and relevant mutants) and target it for degradation. The only SERD currently approved for clinical use is fulvestrant, and although it effectively degrades WT ERα, its affinity for the most common ERα mutants is significantly reduced. More promising are SERDs that are in development, such as GDC0927, AZ9496, LSZ102, RAD1901, and SERM/SERD hybrids like bazedoxifene (McDonnell et al., 2015). All of these compounds have been shown to interact with WT ERα and clinically relevant mutants, and some have been shown to inhibit the growth of patient-derived xenografts harboring relevant mutations. A new class of SERDs that employ PROTAC technology may have particular use in inhibiting ERα mutant function, because their efficacy would not likely be impacted by receptor conformation (Neklesa et al., 2017). Drugs of this type employ an ERα-interacting ligand (agonist or antagonist) tethered by a short linker to a small molecule that enables the engagement of specific E3 ligases. Notwithstanding some pharmaceutical limitations that may arise as a consequence of the large size of these molecules, it is likely that they will have a considerable impact on our ability to manage disease in patients whose tumors harbor ERα mutations.

Although disease-relevant ERα mutants in breast cancer were only discovered recently, it appears that there are already several drugs in development that may attenuate their impact on breast cancer pathology. Further, the discovery of genes/proteins whose expression is regulated by mutant but not WT ERα affords the opportunity to develop biomarkers that report on the efficacy of new interventions that target the mutant receptors. Such biomarkers may also in and of themselves serve as targets, the exploitation of which may yield new drugs that reinforce the activity of existing endocrine therapies or those under development.

DECLARATION OF INTERESTS

D.P.M. serves on the Scientific Advisory Board (SAB) of G1 Therapeutics and Zeno Pharmaceuticals and is a shareholder in these companies. In the past he has served on the SAB or was a consultant for Pfizer, Arvinas, Sermonix, and Radius Pharmaceuticals. J.D.N. is a consultant for G1 Therapeutics. C.C. has no relevant conflicts to disclose.

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Glycans Pave the Way for Immunotherapy in Triple-Negative Breast Cancer

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The clinical efficacy of therapies targeting the PD-1/PD-L1 pathway is still limited. In this issue of *Cancer Cell*, Li and colleagues identify a PD-L1 glycosylation-based mechanism in triple-negative breast cancer that fosters immunosuppression by enhancing interactions with PD-1. Targeting glycosylated PD-L1 with a drug-conjugated antibody opens new avenues for treatment.

Immune checkpoint inhibition represents a major breakthrough in the treatment of cancer. In particular, programmed death receptor-1 (PD-1) and its ligand PD-L1 are recognized as powerful targets to enhance tumor-directed T cell functions. PD-1 is an inhibitory receptor expressed on the surface of activated T cells, B cells, NK cells, monocytes, and dendritic cells, responsible for maintaining immune tolerance, blunting exuberant inflammation, and preventing autoimmune diseases. Engagement of PD-1 by its specific ligand PD-L1 conveys inhibitory signals that ultimately lead to T cell exhaustion and immunosuppression (Topalian et al., 2015). This inhibitory mechanism is co-opted by cancer cells, mainly through expression of PD-L1, to evade immune attack. Immunotherapy based on monoclonal antibodies targeting the PD-1/PD-L1 pathway has changed the treatment landscape for advanced melanoma, Hodgkin lymphoma, non-small-cell lung cancer, renal cell cancer, gastric cancer, and head and neck squamous cell carcinoma (Topalian et al., 2015). Moreover, PD-1 blockade has recently been shown to be active across a range of solid tumors with mismatch-repair deficiency (Le et al., 2017). However, only a small proportion of breast cancer patients respond to anti-PD-1/PD-L1 therapy (Katz and Alsharedi, 2017).

Triple-negative breast cancer (TNBC), clinically defined as breast tumors lacking expression of estrogen receptor, progesterone receptor, and the receptor tyrosine kinase ERBB2, is the molecular subtype with the worst prognosis and survival rates (Perou et al., 2000). At present, chemotherapy is the standard of care in the adjuvant, neoadjuvant, and metastatic settings. Among breast cancer types, TNBC exhibits the highest frequency of PD-L1-positive cells, highest mutational index, and most prominent immune infiltrate (Katz and Alsharedi, 2017). Although these parameters constitute key hallmarks of the so-called "cancer immunogram" and are positive predictors of immunotherapy responses (Blank et al., 2016), clinical studies in TNBC patients revealed low response rates to immune checkpoint blockade (Katz and Alsharedi, 2017). Thus, development of more effective immunotherapeutic modalities and validation of new biomarkers of treatment response are urgently needed.

In this issue of *Cancer Cell*, Li et al. identify a mechanism, based on PD-L1 glycosylation, that fosters immunosuppression in TNBC microenvironments (Li et al., 2018). The authors found that *N*-glycosylation of PD-L1 on TNBC cells is essential for its interaction with PD-1 receptor, enabling transmission of inhibitory signals and favoring T cell exhaustion. Remarkably, targeting glycosylated PD-L1 was highly effective in eradicating TNBC tumors (Figure 1).

In previous studies, the authors showed that glycogen synthase kinase 3β (GSK3β), an active protein kinase, induces phosphorylation-dependent proteasome degradation of non-glycosylated PD-L1. However, glycosylation driven by epidermal growth factor (EGF) signaling