HHS Public Access

Author manuscript

Breast. Author manuscript; available in PMC 2020 January 02.

Published in final edited form as:

Breast. 2019 November ; 48(Suppl 1): S26–S30. doi:10.1016/S0960-9776(19)31118-X.

Endocrine therapy resistance: new insights

Jonathan T. Lei[a], **Meenakshi Anurag**[a,b], **Svasti Haricharan**[c], **Xuxu Gou**[a,d], **Matthew J. Ellis**[a,b,d,*,†]

[a]Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX 77030, USA

[b]Department of Medicine, Baylor College of Medicine, Houston, TX 77030, USA

[c]Department of Tumor Microenvironment and Cancer Immunology, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037, USA

[d]Interdepartmental Graduate Program in Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, TX 77030, USA

Abstract

The estrogen receptor positive (ER+) subset is the dominant contributor to global deaths from breast cancer which now exceeds 500,000 deaths annually. Lethality is driven by endocrine resistance, which has been shown to be associated with high mutational rates and extreme subclonal diversity. Treatment forces subclonal selection until the patient eventually succumbs to metastatic treatment-resistant disease. Recently, we have been addressing several questions related to this process: What is the cause of the increased mutation rate in lethal ER+ breast cancer? Why is endocrine therapy resistance related to mutational load? What are the functions of the somatic mutations that are eventually selected in the treatment resistant and metastatic clones? These questions have provoked new mechanistic hypotheses that link resistance to endocrine agents to: (1) Specific defects in single strand break repair are associated with increased mortality from ER+ breast cancer [1,2]; (2) Loss/mutations of certain single strand break repair proteins that disrupt estrogen-regulated cell cycle control through the ATM, CHK2, CDK4 axis [1,2] thereby directly coupling endocrine therapy resistance to specific DNA repair defects; (3) Acquired mutations that drive metastasis include the generation of in-frame *ESR1* gene fusions that activate epithelial-to-mesenchymal transition (EMT) driven metastasis as well as endocrine drug-resistant proliferation [3].

Keywords

Breast cancer; DNA damage repair; Endocrine therapy resistance; ESR1 fusions; Metastasis; Cancer biomarkers

Introduction

ER+ breast cancer, while considered a more treatable and better prognosis subtype of breast cancer, is associated with a consistent annual relapse rate that persists beyond ten years after

*Corresponding author at: Baylor College of Medicine, Houston, TX 77030, USA. † mjellis@bcm.edu (M. J. Ellis).

diagnosis. This, along with the high incidence rate of the disease, ensures that an ER+ diagnosis remains the most common cause of breast cancer-related deaths [4]. While most ER+ breast cancer may initially respond to endocrine treatment, 15–20% of tumors are intrinsically resistant to treatment, and another 30–40% acquire resistance to treatment over a period of many years [5]. Resistance to treatment inevitably results in relapse and metastasis, leading to death. Understanding the underlying causes of treatment resistance has, therefore, been the focus of many studies to address this major clinical problem.

Years of research on endocrine therapy resistant ER+ breast cancer disease models identified activation of growth factor pathways as one mechanism of resistance. While clinical trials are still ongoing to test the possibility of targeting these pathways in endocrine treatment resistant patients, results so far have been mixed. It is possible that adaptive growth factor activation is a continuous process due to the selective pressure of drug treatment. Although targeting drivers of resistance arising in compensatory growth factor pathways may be initially effective, over time, therapeutic resistance is inevitable, as the tumor acquires a new driver. Identifying and targeting resistance drivers in these circumstances is challenging and remains a clinical problem. Therefore, it is critical to gain a more complete understanding of the underlying resistance mechanisms and elucidate targets for therapeutic intervention.

More recently, the mutational burden and clonal diversity of ER+ breast tumors have come under genomic scrutiny which has provided critical insights into the underlying causes of therapeutic resistance. Results of these studies suggest that over the course of tumor evolution, clonal subpopulations of ER+ breast tumors continuously arise during treatment that confer resistance to endocrine therapies [6]. Some clones may harbor defects in DNA single-strand break repair mechanisms that contribute to high somatic mutation load leading to intrinsic resistance in ER+ breast tumors [1,2]. Otherclones may harbor somatic mutations in *ESR1, NF1* or *DDR1* which is associated with poor prognosis in breast tumors treated with tamoxifen [7,8]. During disease progression and prolonged exposure to endocrine therapy, subclones harboring other forms of somatic mutations may be selected for and enriched in metastatic tumors. In addition to the emergence of well-established activating *ESR1* point mutations [9], formation of *ESR1* fusion genes have now been documented as another acquired mutational mechanism that drives both endocrine therapy resistance and metastasis in ER+ breast cancer [3].

Meanwhile, CDK4/6 inhibitors have emerged as one of a handful of targeted agents that can prove efficacious in the endocrine treatment resistant setting. However, CDK4/6 inhibitor use in the clinic is associated with some challenges: (a) this is an expensive intervention that has to be continued long-term to remain effective, (b) is associated side effects that include neutropenia, fatigue, and nausea and importantly, (c) not all endocrine-refractory tumors respond to CDK4/6 inhibition. Therefore, an emerging critical need in breast cancer research is to identify markers that predict both resistance to endocrine treatment and response to CDK4/6 inhibitors, in order to identify the precise population of patients who can benefit from this treatment strategy. In a sense, CDK4/6 inhibitor therapy is a good example of a targeted yet “biomarker-untargeted” therapy.

In an attempt to address this challenge, we have conducted extensive, “omics” screens in patient tumors, and have discovered several novel mechanisms that underlie endocrine treatment resistance and CDK4/6 inhibitor sensitivity, thereby identifying new biomarker candidates for predictive diagnostics.

Endocrine therapy resistance in primary breast cancer

DNA Damage Repair Deficiency

The discovery of a role for DNA damage repair defects in inducing endocrine treatment resistance is not unexpected but the details have remained obscure. TCGA analysis of >800 primary ER+ breast tumors opened the door for high-throughput data analysis in patient tumors. A reanalysis of TCGA data identified high somatic mutation load in ER+ tumors as associated with significantly worse patient survival [10]. This observation led to the hypothesis that underlying DNA damage repair defects that induce high mutation load may also directly contribute to endocrine treatment resistance. A screen to examine the frequency of DNA damage repair defects and association with poor prognostic metrics identified mismatch repair (MMR) as the primary pathway, that when defective, induces resistance to endocrine treatment. Notably, only the MutL complex of mismatch repair, i.e. MLH1, PMS1 and PMS2, contributed to this clinical phenotype (Figure 1A, B), while none of the other mismatch repair components appeared to have a similar effect [1]. Functional studies conducted in ER+ breast cancer cell lines *in vivo* and *in vitro* identified loss of MutL gene function as causal to endocrine treatment resistance in ER+ breast cancer cells [1]. In the same study, this causal link was supported by experiments using patient-derived xenograft (PDX) models.

Transcriptomic and proteomic screens using MutL deficient ($MutL^{-}$) ER+ breast cancer cells and PDX lines indicated that these cells lost the ability to activate Chk2 in response to endocrine treatment. Chk2 is a cell cycle checkpoint protein that is instrumental for inhibiting CDK4/6 activation, and thereby preventing transition from G1 to S phase of the cell cycle. One of the specific functions of the MutL complex during DNA repair is to activate Chk2. Therefore, defects in MutL genes result in impaired Chk2 activation, and a failure of cancer cells to arrest in response to endocrine treatments (Figure 1B). Functional experiments in multiple cell lines, and corroborative experiments in PDX lines confirmed this causal link.

This finding leads naturally to the hypothesis that loss of MutL, resulting in unrestrained CDK4/6 activation can be addressed by downstream inhibition of CDK4/6 (Figure 1D). Pharmacological targeting of CDK4/6 using *in vitro* and *in vivo* experimental models validated this hypothesis by demonstrating significant growth inhibition and even tumor regression of $MutL^{-}$ ER+ breast cancer cells and in a $MutL^{-}$ PDX model in response to endocrine treatment in combination with CDK4/6 inhibitors. These data were further verified by genomic analysis of the NeoPalAna clinical trial which measured neoadjuvant response of ER+ breast tumors to an aromatase inhibitor and CDK4/6 inhibitor treatment [11]. Together, these findings suggest that loss of MutL function can be considered a viable diagnostic strategy to predict endocrine treatment resistance and sensitivity to CDK4/6 inhibition.

An extension of these findings is to question whether other DNA damage repair pathways have similar effects on endocrine treatment resistance when defective. A second screen in patient tumor data identified key members of nucleotide excision and base excision repair (NER, BER) pathways, particularly CETN2, ERCC1 and NEIL2, as having a similar role (Figure 1B) [2]. Loss of these key genes lead to dysregulated G1-S transition, hence making the models more sensitive to CDK4/6 inhibitors (Figure 1D). These results further support that defects in single-strand break repair mechanisms play a role in driving endocrine therapy resistance. In addition, these results provide strong pre-clinical rationale to develop diagnostic approaches that can test for the loss of these pathways in ER+ breast tumors to guide treatment selection. More detailed studies of these pathways are currently in progress.

Low frequency genomic variants

Cancer-associated mutations can be broadly classified into two types: (1) Hereditary and (2) Somatic. While the role of former is widely studied in breast cancer *(BRCA1/2, ATM)* [12,13], somatic mutations are relatively less explored, especially in the context of ER+ tumors. *ESR1* and *ERBB2* (HER2) mutations are well-established examples of how mutations can associate with poor prognosis and inadequate response to therapy [14–16]. But there are other classes of uncommon genomic variants that have the potential to generate therapeutic hypotheses. When targeted DNA sequencing of 83 genes using samples from primary ER+ breast cancer patients (625 postmenopausal women treated uniformly with adjuvant tamoxifen, the “UBC-TAM cohort”), a set of previously unreported somatic mutations, along with the usual suspects, that associated with prognosis, were identified [7]. The significance of this study lies in the utilization of almost 20-year-old patient samples to understand the prognostic effects driven by the mutational landscape of ER+ disease. Using bioinformatics and statistical approaches that overcame the lack of matched normal DNA samples, somatic mutations were identified in each case. This study confirmed the prognostic effects of well-reported somatic mutations. For example, *MAP3K1* and *PIK3CA* mutations were observed in luminal A cases and associated with favorable prognosis (Figure 1B). Similarly, *TP53* mutations was observed primarily in luminal B cases and associated with poor prognosis. What was particularly interesting is identification of rare somatic mutations that strongly associated with poor prognosis, including *NF1* frame-shift/nonsense (FS/NS) mutations and *DDR1* missense mutations. In UBC-TAM, *NF1* FS/NS mutations were a poor outcome driver that was further validated in independent patient cohort (METABRIC) (Figure 1B). Similarly, *DDR1* mutations were strongly associated with poor prognosis in UBC-TAM after false discovery correction (Figure 1B). Because of lack of mutation data on *DDR1* gene status in METABRIC, a secondary validation could not be performed. The prognostic effects of other mutations in *ERBB2, ERBB4, JAK1, LTK, MAP3K4, MET, PDGFRA, RB1, RELN,* and *TGFB2* also await further study in larger sample sizes. Data from the Arimidex, Tramoxifen, Alone or in Combination (ATAC) trial should be available soon and will likely provide additional insights regarding the impact of mutations in ER+ breast tumors treated with endocrine therapy.

These findings suggest that the long tail of low frequency somatic mutation events in luminal breast cancer may provide molecular explanations for poor clinical outcomes that requires a series of collaborative efforts to thoroughly screen thousands of properly

annotated ER+ cases. Additionally, extensive functional studies should be performed to understand the biological effects of low frequency somatic mutations that associate with poor clinical outcomes in patient datasets. It would also be important to investigate whether mutations in these key genes can create a therapeutic vulnerability that can be exploited to benefit endocrine-therapy resistant cases.

The role of *ESR1* fusions in driving endocrine therapy resistance and metastasis

Metastasis of breast cancer to distant organs is the leading cause of death from the disease. It is estimated that over 150,000 women are living with metastatic breast cancer in the US [17] and approximately 60% of metastatic breast tumors are ER+ [18]. While early stage breast cancer patients may benefit initially from endocrine therapies, many experience progression with metastatic disease due to acquired resistance.

ESR1 point mutations that cluster around the ligand-binding domain (LBD) is a well-established mechanism of acquired endocrine therapy resistance. They have been found in up to 40% of endocrine-refractory ER+ metastatic breast tumors [9]. The most common of these mutants, ESR1-Y537S and ESR1-D538G, confer constitutive, hormone-independent activation of ER, leading to endocrine therapy resistance and upregulation of metastasis-associated genes (Figure 1C) [19].

More recently, as RNA sequencing (RNA-seq) has become more widely implemented as part of precision oncology programs, detection of in-frame *ESR1* fusion genes that encode functional *ESR1* fusion proteins are increasingly being detected in metastatic ER+ breast tumors (Figure 1C) [3,20,21]. The first functional and stable *ESR1* fusion protein, ESR1-YAP1, arising from a fusion transcript between the first 6 exons of *ESR1* fused in-frame to C-terminal sequences from the Hippo pathway coactivator, *YAP1*, was found in a patient with pan-endocrine therapy resistant, metastatic ER+ breast cancer [20]. ESR1-YAP1 lacks the LBD of *ESR1* and is therefore resistant to all endocrine therapies that target the LBD, such as tamoxifen and fulvestrant. A follow-up study characterized a variety of out-of-frame and in-frame *ESR1* fusions from both primary and endocrine therapy-resistant, metastatic ER+ disease [3]. A second fusion was identified, ESR1-PCDH11X, that has the same fusion pattern as ESR1-YAP1 (i.e. the first 6 exons of ESR1 fused in-frame to C-terminal sequences from PCDH11X and consequently also lacks the LBD of *ESR1*). ESR1-PCDH11X was also identified in an endocrine-refractory metastatic breast tumor and encoded a stable, functional fusion protein. Results from experimental models, including a PDX model naturally harboring that ESR1-YAP1, showed that these two *ESR1* fusions drove profound endocrine therapy resistance [3].

Interestingly, a role for *ESR1* fusions in the metastatic process was also discovered [3]. Both ESR1-YAP1 and ESR1-PCDH11X fusions altered the ER cistrome, activating epithelial-to-mesenchymal transition (EMT) genes leading to increased cellular motility and metastatic capacity to the lung. This potentially explains the underlying mechanism associated with fatal disease progression in the metastatic patients from which these two driver *ESR1* fusions were identified in.

An additional in-frame *ESR1* fusion that also produced stable fusion protein, ESR1-NOP2, was discovered in a treatment-naïve primary tumor, but was transcriptionally inactive and did not induce endocrine therapy resistance nor promote metastasis-associated biology [3]. Collectively, these results suggest that formation of transcriptionally active *ESR1* fusions is, like *ESR1* point mutations, a mechanism of acquired resistance that not only alters response to endocrine therapy, but also triggers metastasis, linking these two lethal processes together. The same study was also the first to report a therapeutic strategy to treat *ESR1* fusion-driven growth. Transcriptionally active *ESR1* fusions were found to activate the CDK4/6/pRb axis, and subsequent pharmacological targeting with a CDK4/6 inhibitor, palbociclib, suppressed growth driven by ESR1-YAP1 and ESR1-PCDH11X (Figure 1D). This suggests that the diagnosis of an *ESR1* fusion could aid in stratifying patients to receive CDK4/6 inhibitor therapy as an alternative to chemotherapy, even after the development of profound endocrine therapy resistance.

In another study to better understand the mechanism underlying transcriptional activation and therapeutic vulnerabilities of *ESR1* fusions, *ESR1* fusion interacting proteins were examined [22]. Results showed that recruitment of the 26S proteasome is enhanced to ESR1-YAP1 transcriptional complexes compared to WT-ER and *ESR1* LBD point mutants. Subsequent pharmacological targeting with a proteasomal inhibitor, MG-132, blocked ESR1-YAP1 induced transcriptional activity *in vitro*. Furthermore, growth of ESR1-YAP1 fusion expressing breast cancer cells was suppressed by bortezomib, a specific 26S proteasome inhibitor. Bortezomib is approved for use in multiple myeloma and is in phase II clinical testing in combination with fulvestrant for patients with endocrine-refractory, metastatic ER+ breast cancer [23]. This proteasomal inhibition strategy targeting specific co-factors that are recruited to *ESR1* fusion complexes potentially represents an additional therapeutic strategy to target *ESR1* fusion-driven breast tumors.

A recent study reported additional in-frame *ESR1* fusion transcripts identified in endocrine-refractory, metastatic ER+ breast tumors that produced stable *ESR1* fusion proteins, ESR1-DAB2 and ESR1-GYG1 [21]. Despite limited functional studies with these two fusions, they both follow a similar fusion pattern as ESR1-YAP1 and ESR1-PCDH11X. This suggests that a highly recurrent fusion pattern consisting of the first 6 exons of *ESR1* fused in-frame to C-termini of diverse gene partners may be linked to endocrine-refractory metastatic ER+ disease.

The number of functionally significant *ESR1* fusions identified to date remains very few and it is estimated that *ESR1* fusions account for at least 1% of metastatic breast cancer cases [21]. Further studies examining more examples of *ESR1* fusions from endocrine-refractory and metastatic disease are required to better understand the incidence of *ESR1* fusions in late-stage breast cancer. Studies investigating structure function relationships and preferred gene partners of *ESR1* fusions are also needed. In addition, more research is needed to better understand the functional and therapeutic significance of transcriptionally active *ESR1* fusions. Whether all such fusions activate similar transcriptional programs and whether growth promoted by all these fusions can be suppressed with CDK4/6 inhibitors is unknown. Finally, a better understanding of targets for therapeutic intervention is needed for *ESR1* fusion driven tumors. Although CDK4/6 inhibition suppressed *ESR1* fusion-driven growth,

it did not cause tumor regression [3]. Therapeutic strategies to treat *ESR1* fusion-driven tumors beyond CDK4/6 and 26S proteasome inhibitors remains unexplored.

Conclusions and future directions

The major cause of death from ER+ breast cancer is driven by endocrine therapy resistance and metastasis. Collectively, the studies described herein have deepened our understanding of the contributing factors that trigger drug resistance and metastasis. Key to these seminal findings have been the analysis of next generation sequencing of breast tumors from both early and late-stage disease. Results of these analysis have identified loss of function in single-strand DNA repair as a new class of endocrine therapy resistance drivers in ER+ breast tumors. Defects in MMR, NER, and BER pathways directly deregulates estrogen-regulated cell cycle activity through CDK4 thereby inducing resistance. Another critical factor in identifying mutational drivers of therapeutic resistance was the generation and analysis of sequencing data from large-scale ER+ breast cancer cohorts that were treated uniformly and with long-term follow-up to link the genomic information to clinical phenotypes and outcomes. By refining laboratory techniques to extract genomic material from samples over 20 years old in combination with informatics approaches to deal with the lack of matched normal DNA samples, rare somatic mutations in *NF1* and *DDR1* were identified that were associated with poor prognosis, in addition to well-reported *PIK3CA* and *MAP3K1* mutations associated with a favorable prognosis in ER+ breast cancer. Sequencing studies from late-stage ER+ breast tumors identified the formation of *ESR1* fusions that have a role in endocrine therapy resistance mediated by estrogen-independent activation of CDK4/6 activity and also a role in driving the metastatic process by activating EMT genes. These studies have contributed to establishing the role of *ESR1* fusion genes as a mechanism underlying treatment-resistance and metastasis in ER+ breast cancer.

Taken together, these results potentially provide clues explaining the clonal diversity observed in ER+ breast tumors that contribute to drug resistance [6]. Subclonal populations may arise partly driven by defects in DNA repair mechanisms that confer endocrine therapy resistance while also increasing mutational load thereby potentially driving clonal diversity while other rare populations may harbor mutations in *NF1* or *DDR1* that are associated with poor prognosis (Figure 1A, B). Exactly how *NF1* missense/truncating and *DDR1* mutational events drive treatment resistance remains unclear and is the focus of ongoing investigation by our group. After the development of drug resistance, additional mutational mechanisms may be acquired during disease progression such as the generation of in-frame *ESR1* fusion genes observed in the metastatic setting (Figure 1C).

More large-scale sequencing studies using samples with detailed clinical information and follow-up will be required to further validate the responses of tumors with single-strand break defects to CDK4/6 inhibitor treatment. In tumors that are MutL proficient, it is still unclear whether or not other aberrant DNA repair pathways may contribute to resistance and warrants further investigation. Additional sequencing data on relapsed samples will also be required to better understand the incidence of *ESR1* fusions in the metastatic setting. The two active *ESR1* fusions, ESR1-YAP1 and ESR1-PCDH11X, were identified in a small-cohort of late-stage ER+ cases. Larger sample sizes from endocrine-refractory, metastatic

biopsy specimens will be required to better understand the landscape of *ESR1* fusions in metastatic disease.

These studies also have important therapeutic and diagnostic implications. The finding that loss of single-strand DNA repair mechanisms and *ESR1* fusions induce sensitivity to CDK4/6 inhibitors suggests that a clinical diagnosis of either mechanisms could be used to stratify patients to CDK4/6 inhibitor treatment to treat both intrinsic and acquired endocrine therapy resistance (Figure 1D). These findings may ultimately drive clinical trials and therefore have significant impact to improve survival in ER+ breast cancer.

Acknowledgements

This work was supported by a Susan G. Komen Promise Grant (PG12220321 to MJE); a Cancer Prevention Institute of Texas (CPRIT) Recruitment of Established Investigators Award (RR140033 to MJE); a Breast Cancer Research Foundation Grant (BCRF ELFF-16-003 to MJE); by a National Institutes of Health (NIH) National Cancer Institute Transition Career Development Award (K22-CA229613-01 to SH); and by a NIH Training Grant (T32-CA203690-01 to JTL). Dr. Matthew J. Ellis is a CPRIT Scholar in Cancer Research, McNair Medical Foundation Fellow, and a Susan G. Komen Scholar.

This article was published as part of a supplement sponsored by St. Gallen Oncology Conferences.

Conflict of Interest Statement

MJE received consulting fees from Abbvie, Sermonix, Pfizer, AstraZeneca, Celgene, NanoString, Puma, and Novartis, and is an equity stockholder, consultant, and Board Director member of BioClassifier, and inventor on a patent for the Breast PAM50 assay.

References

[1]. Haricharan S, et al. Loss of MutL disrupts CHK2-dependent cell-cycle control through CDK4/6 to promote intrinsic endocrine therapy resistance in primary breast cancer. Cancer Discov 2017;7:1168–1183. [PubMed: 28801307]

[2]. Anurag M, et al. Comprehensive profiling of DNA repair defects in breast cancer identifies a novel class of endocrine therapy resistance drivers. Clin Cancer Res 2018;24:4887–4899. [PubMed: 29793947]

[3]. Lei JT, et al. Functional annotation of ESR1 gene fusions in estrogen receptor-positive breast cancer. Cell Rep 2018;24:1434–1444 e1437. [PubMed: 30089255]

[4]. World Cancer Report. International Agency for Research on Cancer, 2014.

[5]. Anurag M, Ellis MJ, Haricharan S. DNA damage repair defects as a new class of endocrine treatment resistance driver. Oncotarget 2018;9:36252–36253. [PubMed: 30555626]

[6]. Miller CA, et al. Aromatase inhibition remodels the clonal architecture of estrogen-receptor-positive breast cancers. Nat Commun 2016;7:12498. [PubMed: 27502118]

[7]. Griffith OL, et al. The prognostic effects of somatic mutations in ER-positive breast cancer. Nat Commun 2018;9:3476. [PubMed: 30181556]

[8]. Razavi P, et al. The genomic landscape of endocrine-resistant advanced breast cancers. Cancer Cell 2018;34:427–438 e426. [PubMed: 30205045]

[9]. Pejerrey SM, et al. The impact of ESR1 mutations on the treatment of metastatic breast cancer. Horm Cancer 2018;9:215–228. [PubMed: 29736566]

[10]. Haricharan S, Bainbridge MN, Scheet P, Brown PH. Somatic mutation load of estrogen receptor-positive breast tumors predicts overall survival: an analysis of genome sequence data. Breast Cancer Res Treat 2014;146:211–220. [PubMed: 24839032]

[11]. Ma CX, et al. A Phase II trial of neoadjuvant MK-2206, an AKT inhibitor, with anastrozole in Clinical Stage II or III PIK3CA-mutant ER-positive and HER2-negative breast cancer. Clin Cancer Res 2017;23:6823–6832. [PubMed: 28874413]

[12]. King MC, Marks JH, Mandell JB, New York Breast Cancer Study, G. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003;302:643–646. [PubMed: 14576434]

[13]. Swift M, Morrell D, Massey RB, Chase CL. Incidence of cancer in 161 families affected by ataxia-telangiectasia. N Engl J Med 1991;325:1831–1836. [PubMed: 1961222]

[14]. Bose R, et al. Activating HER2 mutations in HER2 gene amplification negative breast cancer. Cancer Discov 2013;3:224–237. [PubMed: 23220880]

[15]. Fuqua SA, Gu G, Rechoum Y. Estrogen receptor (ER) alpha mutations in breast cancer: hidden in plain sight. Breast Cancer Res Treat 2014;144:11–19. [PubMed: 24487689]

[16]. Fuqua SA. The role of estrogen receptors in breast cancer metastasis. J Mammary Gland Biol Neoplasia 2001;6:407–417. [PubMed: 12013530]

[17]. Mariotto AB, Etzioni R, Hurlbert M, Penberthy L, Mayer M. Estimation of the number of women living with metastatic breast cancer in the United States. Cancer Epidemiol Biomarkers Prev 2017;26:809–815. [PubMed: 28522448]

[18]. Cejalvo JM, et al. Intrinsic subtypes and gene expression profiles in primary and metastatic breast cancer. Cancer Res 2017;77:2213–2221. [PubMed: 28249905]

[19]. Jeselsohn R, et al. Allele-specific chromatin recruitment and therapeutic vulnerabilities of ESR1 activating mutations. Cancer Cell 2018;33:173–186 e175. [PubMed: 29438694]

[20]. Li S, et al. Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. Cell Rep 2013;4:1116–1130. [PubMed: 24055055]

[21]. Hartmaier RJ, et al. Recurrent hyperactive ESR1 fusion proteins in endocrine therapy-resistant breast cancer. Ann Oncol 2018;29:872–880. [PubMed: 29360925]

[22]. Gates LA, et al. Proteomic profiling identifies key coactivators utilized by mutant ERalpha proteins as potential new therapeutic targets. Oncogene 2018;37:4581. [PubMed: 29748621]

[23]. Adelson K, et al. Randomized phase II trial of fulvestrant alone or in combination with bortezomib in hormone receptor-positive metastatic breast cancer resistant to aromatase inhibitors: a New York Cancer Consortium trial. NPJ Breast Cancer 2016;2:16037. [PubMed: 28721390]

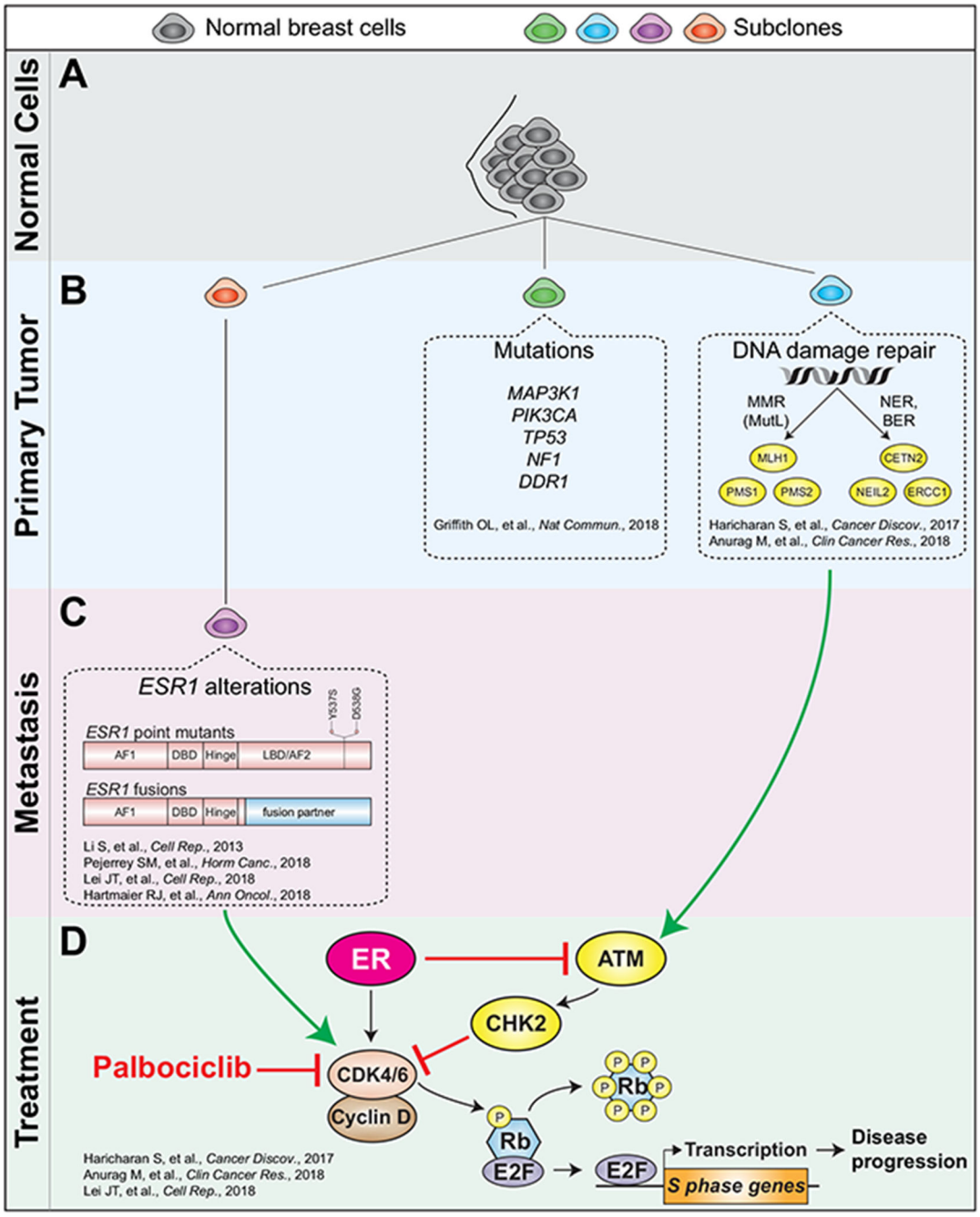

Fig. 1. Mechanisms of endocrine therapy resistance in ER+ breast cancer and treatment strategy.

(**A,B**). Normal breast cancer cells can give rise to primary ER+ breast tumors that include clonal subpopulations that harbor genomic alterations. Some of these clones may harbor loss of components in the MutL complex (MLH1, PMS1, PMS2) involved in the mismatch repair pathway (MMR) or loss of CETN2, ERCC1, and NEIL2 which are critical components in directing nucleotide excision and base excision repair (NER, BER). Other clones may contain individual prognostic mutations such as *MAP3K1* or *PIK3CA* mutations which are associated with favorable prognosis, or contain *TP53*, or increasingly rare mutations in *NF1*

(non-sense/frameshift mutations) and *DDR1*, all three of which have been associated with poor prognosis. It is still unclear how *NF1* and *DDR1* mutations impinge on treatment resistance. (**C**) During disease progression, acquired *ESR1* alterations have been identified in endocrine-refractory, metastatic tumors. These include *ESR1* point mutations, the most common of which cluster in the LBD of *ESR1* (Y537S and D538G) and also include the generation of in-frame *ESR1* fusions. These alterations produce constitutively active mutant proteins that are able to drive endocrine therapy resistance. (**D**) Loss of single-strand DNA damage repair pathways leads to uncoupling of ER-dependent activation of CDK4/6 through ATM and CHK2, while *ESR1* mutant proteins constitutively activate the CDK4/6/Rb/E2F axis which leads to de-regulation of the cell cycle at the G1-S checkpoint. CDK4/6 inhibition with palbociclib suppresses endocrine therapy resistant proliferation driven by both loss of single strand break repair mechanisms and *ESR1* alterations. Activation function 1, AF1; DNA-binding domain, DBD; ligand-binding domain, LBD; activation function 2, AF2.