REVIEW

Overcoming oncogene addiction in breast and prostate cancers: a comparative mechanistic overview

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Abstract

Prostate cancer (PCa) and breast cancer (BCa) are both hormone-dependent cancers that require the androgen receptor (AR) and estrogen receptor (ER, ESR1) for growth and proliferation, respectively. Endocrine therapies that target these nuclear receptors (NRs) provide significant clinical benefit for metastatic patients. However, these therapeutic strategies are seldom curative and therapy resistance is prevalent. Because the vast majority of therapy-resistant PCa and BCa remain dependent on the augmented activity of their primary NR driver, common mechanisms of resistance involve enhanced NR signaling through overexpression, mutation, or alternative splicing of the receptor, coregulator alterations, and increased intracrine hormonal synthesis. In addition, a significant subset of endocrine therapy-resistant tumors become independent of their primary NR and switch to alternative NR or transcriptional drivers. While these hormone-dependent cancers generally employ similar mechanisms of endocrine therapy resistance, distinct differences between the two tumor types have been observed. In this review, we compare and contrast the most frequent mechanisms of antiandrogen and antiestrogen resistance, and provide potential therapeutic strategies for targeting both advanced PCa and BCa.

Key Words

- endocrine therapy resistance
- androgen receptor
- estrogen receptor
- oncogene

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Introduction

The vast majority of prostate cancers (PCa) and breast cancers (BCa) are hormone-dependent malignancies that grow when stimulated by androgens and estrogens, respectively. These hormones bind and activate their cognate nuclear receptors (NRs), androgen receptor (AR) and estrogen receptor (ER, ESR1), which then drive pro-proliferative transcriptional programs in cancer cells (Fig. 1). A number of antiandrogens targeting AR (enzalutamide, bicalutamide, flutamide, apalutamide, darolutamide) and antiestrogens targeting ER (tamoxifen, fulvestrant) have shown efficacy in and improved survival for patients. However, these drugs are rarely curative and endocrine therapy resistance is common, as cancer cells evolve mechanisms to maintain their dependence on their primary oncogenic drivers – their respective NRs. In this review, we will assess the similarities and differences in the mechanisms of endocrine therapy resistance to the representative antiandrogen, enzalutamide, and the canonical antiestrogen, tamoxifen, in PCa and BCa. Since the differences in the mechanisms

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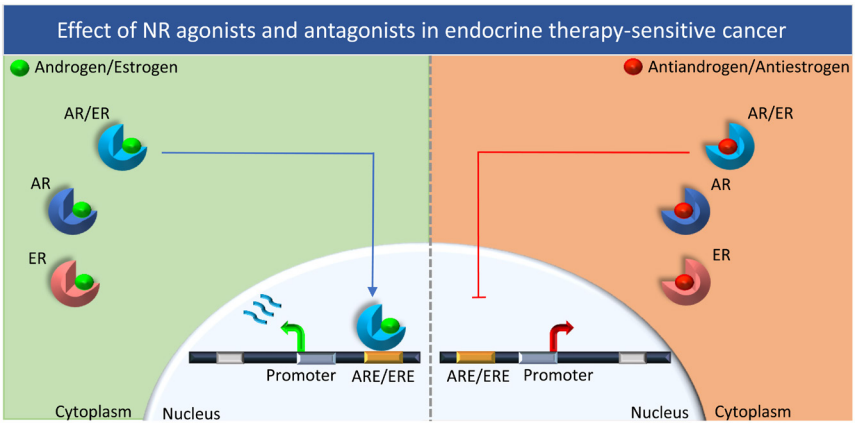

Figure 1
Effect of NR agonists and antagonists. Nuclear receptor (NR) agonists, such as androgens and estrogens, bind to the NR in the cytoplasm and cause translocation to the nucleus, where they bind their cognate NR-response elements on the DNA (ARE/ERE), and drive transcription of NR-regulated genes (green box). In contrast, NR antagonists bind to the NR, prevent nuclear translocation, DNA binding, and transcription of NR-regulated genes. As a consequence, NR antagonists shut down NR-driven gene expression (red box). A full color version of this figure is available at https://doi.org/10.1530/ERC-20-0272.

of therapy resistance may be in part due to the antagonist used, we will first explore the salient features of both enzalutamide and tamoxifen.

Enzalutamide

Enzalutamide is a highly selective AR antagonist, possessing an increased (eight-fold) affinity for AR, compared to first-generation antiandrogens, including bicalutamide. However, enzalutamide still has a lower affinity (two-fold) for AR than the AR agonist dihydroxytestosterone (DHT) (Tran *et al.* 2009). Clinically, enzalutamide is approved for the treatment of non-metastatic and metastatic castration-resistant PCa (CRPC). In non-metastatic CRPC patients, enzalutamide delays the onset of metastasis by 36 months, while in newly diagnosed metastatic CRPC patients, it delays disease progression by 15 months (Hussain *et al.* 2018, Komura *et al.* 2019). Durable long-term responses to enzalutamide are rare, and disease progression is common.

Tamoxifen

Tamoxifen is a highly selective estrogen receptor modulator (SERM) with predominantly antagonistic properties in breast tissue. Tamoxifen binds to ER with equal or greater affinity than the ER agonist estradiol (E2) (Fabian *et al.* 1981, Katzenellenbogen *et al.* 1984). Clinically, tamoxifen is approved as an adjuvant treatment in primary breast cancer, and in this setting, increases overall and disease-free survival by more than 96 months (Delozier *et al.* 2000). Tamoxifen is also used in the metastatic setting, primarily in premenopausal women. Since clinical intervention with tamoxifen usually occurs earlier in the progression of BCa than with enzalutamide in PCa, the effects on survival are enhanced. However, as with enzalutamide-treated patients, disease recurrence remains prevalent and may occur after significant time (>10 years) on tamoxifen therapy.

Mechanisms of therapy resistance

The majority of enzalutamide-resistant prostate cancer (EnzR-PCa) and tamoxifen-resistant breast cancer (TamR-BCa) maintain AR or ER signaling, respectively. To maintain an addiction to their cognate NR, most EnzR-PCa (70%) and TamR-BCa (60%) either amplify the expression of their oncogenic NR or modify the NR protein with alternative splicing, gene fusions, or point mutations to enable therapy resistance. In contrast, some EnzR-PCa (10–15%) and TamR-BCa (15–30%) are marked by a loss of NR expression (and consequently their NR addiction), and rely on alternative molecular drivers (Johnston *et al.* 1995, Robinson *et al.* 2015, Abida *et al.* 2019).

In this review, we will assess eight distinct potential mechanisms of antiandrogen and antiestrogen resistance based on four criteria: (1) enrichment of mechanistic drivers in therapy-resistant patient tumors, (2) the ability of the mechanistic drivers to independently mediate therapy resistance, (3) capability of pharmacological inhibition or genetic ablation of these drivers in restoring drug (enzalutamide or tamoxifen) sensitivity, and (4) the clinical utility of drugs targeting the mechanistic drivers in therapy-resistant patients.

NR overexpression

NR overexpression alters the stoichiometric ratio of the receptor to the antagonist (enzalutamide or tamoxifen), potentially overwhelming the antagonist's ability to effectively neutralize NR activity (Fig. 2). As a result, cancer cells display hypersensitivity to agonists even under treatment conditions (Chen *et al.* 2004, Tran *et al.*

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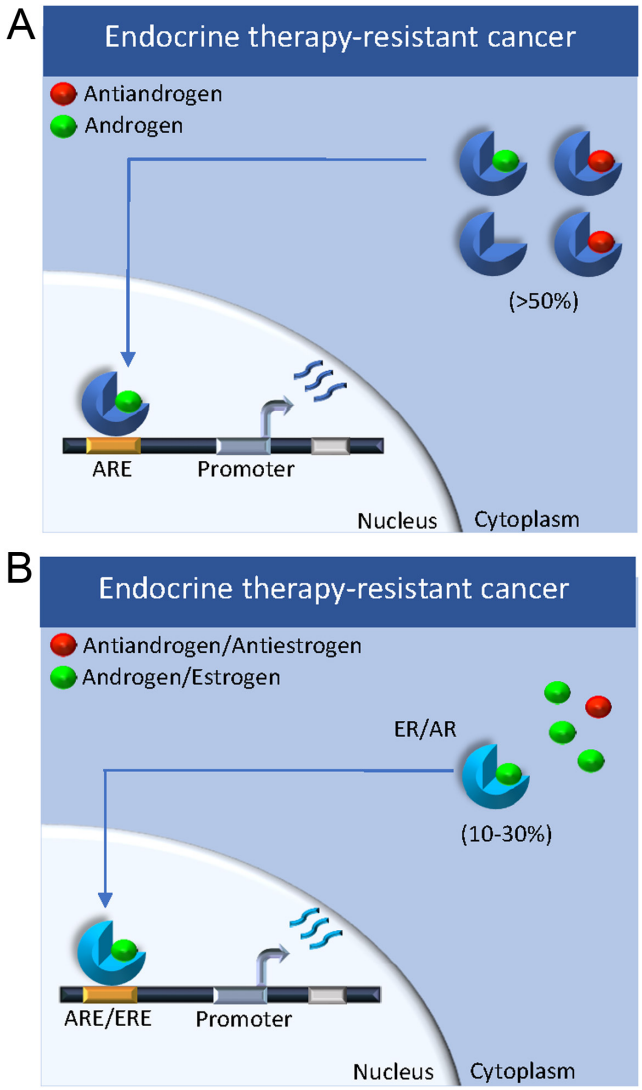

Figure 2
NR activation in endocrine therapy-resistant cancers. (A) NR overexpression (shown for AR): AR overexpression alters the stoichiometric ratio of AR to antagonist, enabling availability of AR to bind to even small amounts of agonists and activate gene expression. NR overexpression is not as commonly seen with ER after tamoxifen therapy. (B) Enhanced NR ligand synthesis: enhanced ligand synthesis can increase the stoichiometric ratio of agonist to antagonists, effectively outcompeting antagonists to bind NR, and increase activation of NR transcriptional programs. This mechanism of resistance has been reported for both AR and ER. Percentages within parentheses after each mechanism reflect their known prevalence. A full color version of this figure is available at https://doi.org/10.1530/ERC-20-0272.

2009, Kawata *et al.* 2010). This mechanism of resistance is more commonly observed in EnzR-PCa than TamR-BCa.

NR overexpression in PCa

A majority (>70%) of EnzR-CRPC patients have AR pathway alterations, resulting in enhanced AR signaling. Increased AR expression is driven either by genomic amplification of AR or the more recently discovered enhancer amplification. Observed in up to 50% of cases, AR gene amplification is one of the most frequent genetic alterations reported for CRPC tumors (Grasso *et al.* 2012, Robinson *et al.* 2015, Abida *et al.* 2019). In contrast to primary tumors, AR amplification is commonly seen in EnzR-CRPC, and is predictive of poorer outcomes (Azad *et al.* 2015). More recent reports show amplification of an enhancer locus ~650 kb upstream of the AR gene in CRPC tumors, but not localized tumors. This enhancer is enriched for H3K27 acetylation, and chromosome capture analysis indicates that this putative enhancer region interacts with the AR promoters, directly contributing to the transcriptional upregulation of the AR gene (Takeda *et al.* 2018, Ramanand *et al.* 2020). Ultimately, the authors showed that the AR gene enhancer was required for EnzR-CRPC cell survival, as AR enhancer knock-in sufficiently conferred enzalutamide resistance to enzalutamide-sensitive cells. This study and confirmatory studies demonstrated the clinical relevance of this AR enhancer, where almost 90% of metastatic CRPC (mCRPC) tumors (compared to <1% of primary tumors) and 70% of mCRPC patients by cell-free DNA analysis contained this duplication (Viswanathan *et al.* 2018, Ramanand *et al.* 2020). Taken together, these endocrine therapy-induced alterations dramatically enhance AR expression in EnzR-CRPC. Thus, developing therapeutic strategies with more potent antiandrogen or degrading activity may be an effective means to overcome enzalutamide resistance driven by AR overexpression. Clinical trials with AR-targeted degraders, such as ARV-110, have already begun to show promising results (NCT03888612).

NR overexpression in BCa

Similarly, ER is the driver in ER+ BCa, which accounts for 70% of breast cancers. While the majority of TamR-BCa still express some ER, loss of ER is a primary mechanism of endocrine therapy resistance in patients, whereas overexpression of ER is much less common (Encarnación *et al.* 1993, Dowsett & Haynes 2003). ER copy-number alterations (gain or amplification) are noted in 15% of metastatic and just 5% of primary ER+ BCa; however, no definitive correlation exists between ER amplification and survival outcomes, as some data suggest that ER amplification may be associated with improved clinical response to antiestrogens (Holst *et al.* 2007, Tomita *et al.* 2009, Basudan *et al.* 2019). Unlike AR amplification in PCa, further investigation is required to determine whether ER

amplification can be considered a proven mechanism of antiestrogen resistance. As a result, therapeutic strategies specifically designed to overcome ER overexpression have not yet been explored.

Point mutations in the NR ligand-binding domain

Since antagonists target the NR ligand-binding domain (LBD), point mutations in the NR LBD can effectively inhibit antagonist activity. Moreover, these point mutations may convert the antagonist to an agonist or represent a gain-of-function (GOF) mutation and contribute to endocrine therapy resistance due to constitutive activation of the receptor (Fig. 3).

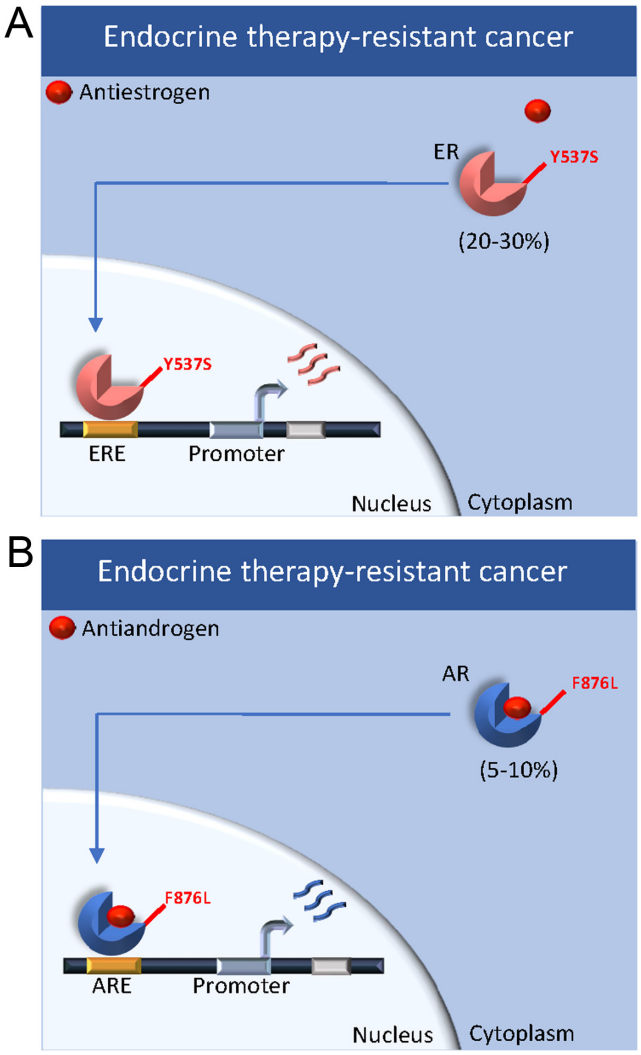

Figure 3
Point mutations in the NR ligand-binding domain (LBD). (A) ER proteins with LBD point mutations, such as the Y537S, are constitutively nuclear, do not bind antiestrogens, and are capable of activating transcription from an ERE in a ligand-independent manner. (B) In contrast, AR proteins with LBD point mutations, such as the F876L, convert their antagonists into agonists and drive transcription from an ARE. Percentages within parentheses after each mechanism reflect their known prevalence. A full color version of this figure is available at https://doi.org/10.1530/ERC-20-0272.

Point mutations in the AR LBD in PCa

The selective pressure of each antiandrogen is associated with specific point mutations in the AR LBD, as evidenced by the AR T877A mutation for flutamide, AR W741C mutation for bicalutamide, and AR F876L for enzalutamide and apalutamide. These point mutations convert the antagonist to an agonist, and independently drive resistance to specific antiandrogens (Veldscholte *et al.* 1990, Hara *et al.* 2003, Chen *et al.* 2004, Balbas *et al.* 2013, Joseph *et al.* 2013) (Fig. 3). Consequently, another antiandrogen may still have activity against the AR LBD point mutants. For example, AR T877A and W741C mutants are sensitive to enzalutamide and apalutamide (Chen *et al.* 2004). Although these mutations to the AR LBD have been identified in patient tumor samples and are recognized as drivers of antiandrogen resistance, their individual observed frequencies are low. As AR mutants develop in response to specific antiandrogens, more potent antiandrogens or AR degraders may also be effective against AR mutants.

Point mutations in the ER LBD in BCa

ER LBD mutations are seen in ~20–30% of endocrine therapy-resistant BCa (Toy *et al.* 2013, Razavi *et al.* 2018). The most common ER LBD mutants are Y537S, Y537N, and D538G. These mutations induce a significant conformational change in the ER LBD that even in the absence of estrogens, effectively lock the ER LBD into a constitutively active state (Zhang *et al.* 1997, Nettles *et al.* 2008, Toy *et al.* 2017) (Fig. 3). For example, the Y537S mutation stabilizes helix 12 in a conformation that allows a coactivator to bind in an estrogen-independent manner by forming a hydrogen bond with another amino acid in the LBD (Nettles *et al.* 2008). Consequently, these ER LBD mutants are always functionally active, are insensitive to tamoxifen, and can drive TamR-BCa *in vitro* (Toy *et al.* 2017, Fanning *et al.* 2018) (Fig. 3). Importantly, E2 can bind to these ER LBD mutants, enhance interaction with coregulators, and further increase transcriptional activity driven by these mutants (Toy *et al.* 2017, Fanning *et al.* 2018). BCa cells with ER LBD mutations are still driven by ER, and thus retain some sensitivity to selective ER degraders, such as fulvestrant. Multiple preclinical compounds that are effective in the ER mutant setting,

including bazedoxifene and ERX-11, are in development, yet their clinical efficacy in therapy-resistant tumors awaits confirmation (Raj *et al.* 2017, Fanning *et al.* 2018, Viswanadhapalli *et al.* 2019).

NR splice variants

The expression of alternatively spliced isoforms of NRs that naturally occur in low frequencies can be enhanced following selection pressure by endocrine therapies. These isoforms can drive the NR transcriptional program in a ligand-independent manner, albeit less effectively than in the ligand-dependent mechanism (Fig. 4).

NR splice variants in PCa

AR-variants (AR-Vs), such as AR-V7 and AR-V9, have been widely detected in CRPC, and their expression is further increased in patients treated with antiandrogens, such as enzalutamide (Guo *et al.* 2009, Hörnberg *et al.* 2011, Antonarakis *et al.* 2014, Kohli *et al.* 2017, Luo *et al.* 2018, Li *et al.* 2020). These AR-Vs typically lack the LBD and remain constitutively active in the absence of androgens (Dehm *et al.* 2008, Guo *et al.* 2009, Li *et al.* 2013*b*, Kohli *et al.* 2017, Luo *et al.* 2018) (Fig. 4). In addition, induction of AR-V expression in antiandrogen-sensitive PCa cells promotes resistance to both bicalutamide and enzalutamide (Li *et al.* 2013*b*). Expression of AR-V7 in metastatic tumors correlates with poor clinical response to AR-targeted therapies, and the loss of AR-V7 in antiandrogen-resistant cells has been shown to restore antiandrogen sensitivity *in vitro* (Li *et al.* 2013*b*, 2020, Antonarakis *et al.* 2014). While AR-Vs are transcriptionally active and can drive resistance, their increased expression is typically concordant with AR amplification, and their role as an independent molecular driver of resistance remains undetermined (Watson *et al.* 2010, Hörnberg *et al.* 2011, Li *et al.* 2013*b*, 2020, Luo *et al.* 2018). Only with the emergence of effective AR-V-targeted therapies, can the role of AR-Vs in EnzR-PCa be clarified. Trials with niclosamide are ongoing (NCT02532114, NCT03123978). Thus, while high AR-V expression is a viable biomarker of insensitivity to AR-targeted therapies, they have not yet been proven as a direct molecular driver of therapy resistance or as a viable therapeutic target.

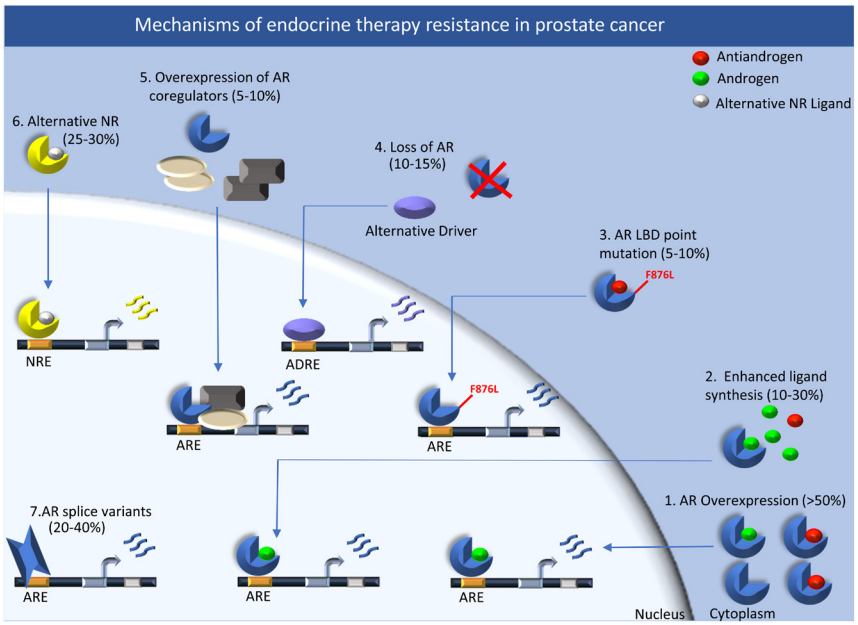

Figure 4
Comparative overview of the most common mechanisms driving endocrine therapy resistance in prostate cancers. 1. AR overexpression alters the stoichiometric ratio of AR to antagonist, enabling AR to bind to even small amounts of agonists and activate gene expression. 2. Enhanced ligand synthesis can increase the stoichiometric ratio of agonist to antagonists, effectively outcompeting antagonists to bind AR, and increase activation of AR transcriptional programs. 3. AR proteins with LBD point mutations, such as the F876L, convert their antagonists into agonists and drive transcription from an ARE. 4. Under the selection pressure of endocrine therapy, AR loss can occur. Alternative non-AR drivers can induce AR-independent transcriptomes to drive the progression from an alternative driver response element (ADRE). 5. Overexpression of AR coregulators effectively increase AR-mediated transcription in the presence of antagonists. 6. Alternative NR drivers may overcome antagonistic blockade of the AR and activate an alternative NR-driven transcriptional program. 7. AR-variants (AR-Vs) form homodimers or heterodimers with full-length AR (AR-FL), are constitutively nuclear, can bind DNA either as a homodimer or heterodimer, and activate AR transcriptional programs. AR-Vs lack the AR-LBD, and are thus not responsive to antagonists. Percentages within parentheses after each mechanism reflect their known prevalence. A full color version of this figure is available at https://doi.org/10.1530/ERC-20-0272.

NR splice variants in BCa

ER splice variants have been detected in TamR-BCa. However, their role as a molecular driver in promoting endocrine therapy resistance is unclear and requires further investigation (Beije *et al.* 2018).

Enhanced NR ligand synthesis

Enhanced synthesis of androgens and estrogens within the tumor microenvironment can effectively alter the stoichiometric ratio of the NR, agonist, and antagonist to overcome the ability of the antagonist to effectively inhibit NR activity (Fig. 3).

Enhanced ligand synthesis in PCa

Intratumoral androgen synthesis is increased in CRPC and overcomes the pituitary suppression of androgen synthesis by the testes. Abiraterone is a second-generation androgen synthesis inhibitor that blocks adrenal and intratumoral androgen synthesis, providing significant clinical benefit to CRPC patients (Locke *et al.* 2008, Cai *et al.* 2011, Mohler *et al.* 2011, Mostaghel *et al.* 2011, Fankhauser *et al.* 2014, Liu *et al.* 2015, 2019). In EnzR cells, upregulation of aldo-keto reductase family 1 member C3 (AKR1C3) has been shown to enhance intracrine androgen synthesis and subsequently enzalutamide resistance. Loss of AKR1C3 by genetic ablation or pharmacological inhibition causes EnzR-PCa cells to become enzalutamide-sensitive *in vitro* and *in vivo* (Liu *et al.* 2015, 2019). Clinical trials in CRPC patients with a combination of enzalutamide and AKR1C3 inhibitors (NCT02935205) are ongoing but are likely to have limited utility, since increased AKR1C3 expression has only been shown in a small percentage of EnzR-PCa patients. Cholesterol is required for steroid synthesis. In PCa, overexpression of 3-hydroxy-2-methyl-glutaryl-CoA reductase (HMGCR), the rate-limiting enzyme for cholesterol synthesis, may mediate enzalutamide resistance (Brown & Goldstein 1980, Kong *et al.* 2018). Additionally, cholesterol synthesis inhibitors suppress the growth of EnzR-PCa *in vitro* and *in vivo* (Kong *et al.* 2018). The clinical utility of this remains to be seen.

Enhanced ligand synthesis in BCa

The most compelling evidence for increased hormone synthesis driving antiestrogen resistance comes from the clinic, where aromatase inhibitors (AI) have repeatedly shown to increase overall survival and duration of response in metastatic TamR-BCa patients (Dombernowsky *et al.* 1998). Aromatase is a cytochrome P450 that promotes ER activation by converting androgens into estrogens (Brodie 1985). Mechanistic studies have further shown that TamR-BCa cells have increased aromatase expression and activity, aromatase precursors increase the growth of TamR cells, and TamR cell growth is inhibited by aromatase inhibitors *in vitro* (Catalano *et al.* 2014). Like in PCa, cholesterol synthesis may play an important role in antiestrogen resistance. Studies have indicated that supplementation with cholesterol precursors promotes tamoxifen resistance, and cholesterol synthesis inhibitors suppress the growth of TamR-BCa *in vivo* (Nelson *et al.* 2013). However, similar to PCa, the clinical relevance of this remains unclear.

NR coregulators and transcriptional machinery

NR coregulators (coactivators and corepressors) are essential for modulating NR-mediated transcription at either the promoter or chromatin level (Figs 4 and 5). Coactivators typically bind the NR when it is bound by the agonist, increasing gene transcription, while corepressors are recruited when the antagonist is bound, decreasing target gene expression (Halachmi *et al.* 1994, Baniahmad *et al.* 1995, Cavaillès *et al.* 1995, Chen & Evans 1995, Oñate *et al.* 1995). A number of NR coregulators are overexpressed in hormone-dependent malignancies and can enhance NR activity. The high expression of some coactivators may enhance NR agonist activity and contribute to endocrine therapy resistance (Figs 4 and 5). Conversely, lower levels of corepressors may reverse the drug's role from antagonist to agonist, ultimately driving therapy resistance.

NR coregulators and transcriptional machinery in PCa

AR coactivators, such as nuclear receptor coactivator 2 (NCOA-2, SRC-2, TIF-2), are upregulated in mCRPC and correlate with worse recurrent-free survival (Agoulnik *et al.* 2006, Robinson *et al.* 2015, Abida *et al.* 2019). Genetic ablation of SRC-2 restores bicalutamide sensitivity in PCa cells (Feng *et al.* 2009). Additionally, knockdown of pioneering factors, such as forkhead box A1 (FOXA1), which are altered in a small subset (10%) of mCRPC patients, overcomes resistance to enzalutamide (Jin *et al.* 2014, Abida *et al.* 2019, Hwang *et al.* 2019). Other coregulators that may be important for antiandrogen resistance and require further investigation include nuclear receptor coactivator 3 (SRC-3) and E1A binding protein p300 (p300/CBP300),

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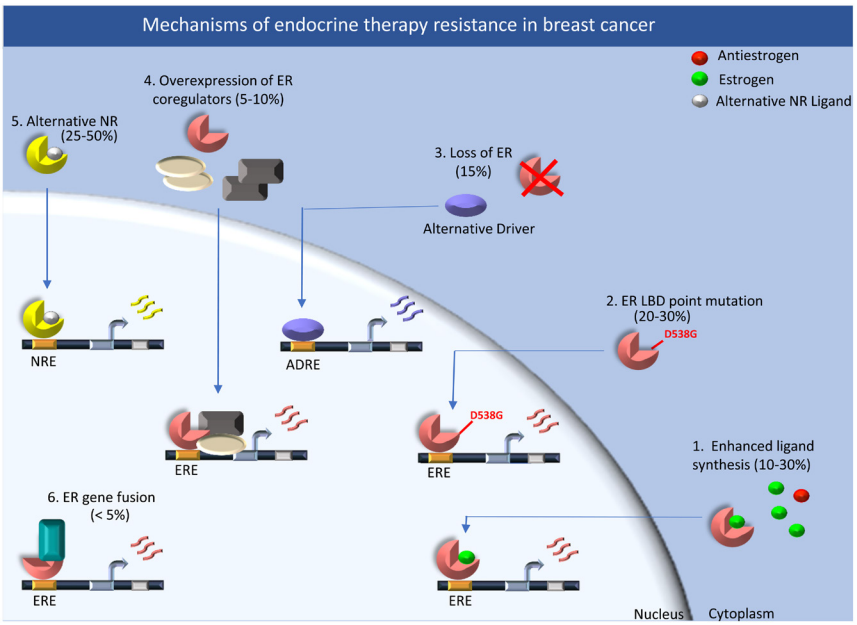

Figure 5
Comparative overview of the most common mechanisms driving endocrine therapy resistance in breast cancers. 1. Enhanced ligand synthesis can increase the stoichiometric ratio of agonist to antagonists, effectively outcompeting antagonists to bind ER, and increase activation of ER transcriptional programs. 2. ER proteins with LBD point mutations, such as the Y537S, are constitutively nuclear, do not bind antiestrogens, and are capable of activating transcription from an ERE in a ligand-independent manner. 3. Under the selection pressure of endocrine therapy, ER loss can occur. Alternative non-NR drivers can drive NR-independent transcriptomes from alternative driver response elements (ADRE) to drive progression. 4. Overexpression of ER coregulators effectively increase ER-mediated transcription in the presence of antagonists. 5. Alternative NR drivers may overcome antagonistic blockade of the ER and activate an alternative NR-driven transcriptional programs. 6. Gene fusions between the ER N-terminus and other genes create hybrid proteins that have the ER N-terminus and a different protein in place of the ER LBD. Consequently, these ER fusion proteins are no longer responsive to either ER agonists or antagonists. Instead, these fusion proteins are able to bind the ERE and activate ER-driven transcriptional programs. Percentages within parentheses after each mechanism reflect their known prevalence. A full color version of this figure is available at https://doi.org/10.1530/ERC-20-0272.

which are associated with worse progression-free survival (PFS) (Debes *et al.* 2003, Zhou *et al.* 2005, Abida *et al.* 2019). Conversely, deletion or loss-of-function (LOF) mutations of either nuclear receptor corepressor 1 or 2 (NCOR1 or NCOR2) are seen in 5–10% of mCRPC patients, and have been shown to promote resistance to enzalutamide in PCa cells *in vitro* (Robinson *et al.* 2015, Lopez *et al.* 2016, Abida *et al.* 2019). Additionally, genetic ablation of NCOR1 has been shown to promote bicalutamide and enzalutamide resistance in sensitive cells *in vitro* (Lopez *et al.* 2016). While these data are suggestive, clear mechanistic evidence for the ability of coregulators to independently drive antiandrogen resistance *in vivo* is lacking.

NR coregulators and transcriptional machinery in BCa

Amplification of multiple ER coregulators has been reported in TamR-BCa. Overexpression of one such ER coactivator, SRC-3, has been shown to increase the agonist activity of tamoxifen *in vitro* and contributes to tamoxifen resistance (Anzick *et al.* 1997, Weiner *et al.* 2013). Small-molecule compounds inhibiting SRC-3 in both *in vitro* and *in vivo* models have been shown to re-sensitize resistant cells to tamoxifen (Wang *et al.* 2014, Song *et al.* 2016). Similar to EnzR-PCa, the pioneering factor FOXA1 is overexpressed in TamR cells and xenografts and drives TamR-BCa *in vitro*, as evidenced by the ability of FOXA1 knockdown to restore tamoxifen sensitivity (Fu *et al.* 2016). As in EnzR-PCa, loss of NCOR expression may play a role in tamoxifen resistance. Low NCOR expression correlates with worse relapse-free survival, and NCOR is decreased in TamR-BCa (Girault *et al.* 2003, Lu *et al.* 2016). *In vitro* and *in vivo* studies have shown that inhibiting NCOR with an NCOR-blocking antibody leads to tamoxifen resistance by conferring tamoxifen with agonistic properties (Lavinsky *et al.* 1998). Compounds targeting coregulators including SRC-3 inhibitors, such as SI-2, bufalin, and gossypol, are in development (Wang *et al.* 2014, Song *et al.* 2016).

NR gene fusions

Intragenic gene fusions with NRs can create novel NR fusion products that no longer respond to either agonists or antagonists, and thus promote therapy resistance (Fig. 5).

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Table 1 Common mechanisms of antiandrogen and antiestrogen resistance in prostate and breast cancer.

Mechanism	Antiandrogen resistance	Antiestrogen resistance	Therapeutic strategy
NR overexpression	Common (>50%) Known driver of resistance	Rare (<5%) Not proven driver of resistance	PCa: More potent AR antagonists or degraders (ARV-110) BCa: Not yet explored
Point mutations in the NR LBD	T877A, W741C, and F876L are infrequent (5–10%) Known driver of resistance	Y537S, Y537N, and D538G are well-described (20–30%) Known driver of resistance	PCa: More potent AR antagonists BCa: SERMs or SERDs (bazedoxifene, ERX-11)
NR splice variants	Commonly seen (20–40%) Not proven driver of resistance	Rare (<5%) Not proven driver of resistance	PCa: Niclosamide BCa: N/A
Enhanced NR ligand synthesis	Well-described (10–30%) Proven mechanism of resistance	Well-described (10–30%) Proven mechanism of resistance	PCa: Abiraterone, AKR1C3 inhibitors BCa: Aromatase inhibitors (AI)
NR coregulators and transrptional machinery	NCOR, SRC family, and FOXA1 alterations are infrequent (5–10%) Not proven driver of resistance	NCOR, SRC family, and FOXA1 alterations are infrequent (5–10%) Not proven driver of resistance	PCa: SRC-3 inhibitors BCa: SRC-3 inhibitors
NR gene fusions	Not detected Not associated with resistance	Rare (<5%) Not proven driver of resistance	N/A
Alternative NR drivers	Increased GR described (25–30%) Likely drivers of resistance	ER-β, ERRα, PR alterations seen (25–30%) Not proven driver of resistance	PCa: GR antagonists BCa: ERRα antagonists or PR modulators + antiestrogens
Alternative non-NR drivers	AR-independent tumors with TP53/RB1 loss have been described (10–15%) Likely drivers of resistance	Enhanced MAPK and Cyclin D-CDK4/6 signaling well-described (15%) Known driver of resistance	PCa: SOX2/BRN2/ATM inhibitors BCa: EGFR and ERK/MEK inhibitors, CDK4/6 inhibitors (palbociclib) + AI

Table displaying eight mechanisms of therapy resistance in prostate and breast cancer, their relative frequencies, and potential therapeutic strategies currently under investigation.
AKR1C3, aldo-keto reductase family 1 member C3; AR, androgen receptor; ATM, ataxia telangiectasia mutated; BRN2, POU-domain transcription factor BRN2; CDK, cyclin-dependent kinase; EGFR, EGF receptor; ERK, extracellular signal-regulated kinase; ERRα, estrogen-related receptor α; ER-β, estrogen receptor-β; forkhead box A1; FOXA1; GR, glucocorticoid receptor; LBD, ligand-binding domain; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase (MEK) ; NCOR, nuclear receptor corepressor; PR, progesterone receptor; RB1, retinoblastoma 1; SERDs, selective estrogen receptor degraders; SERMs, selective estrogen receptor modulators; SOX2, SRY-box 2; SRC, nuclear receptor coactivator; TP53, tumor protein p53.

NR gene fusions in PCa

NR gene fusions have primarily been reported in BCa, not in PCa, and have no known role in antiandrogen resistance (Table 1).

NR gene fusions in BCa

Transcriptomic analysis of ER+ BCa has identified an enrichment in ER C-terminal fused proteins: ESR1-e6>YAP1 (ESR1-YAP1) and ESR1-e6>PCH11X (ESR1-PCDH11X) in late-stage, therapy-resistant BCa (Lei *et al.* 2018). These rearrangements result in an ER fusion protein that lacks the LBD, but can drive estrogen-independent growth, constituently activate ER transcriptional programs, and remain resistant to ER degraders, like fulvestrant (Lei *et al.* 2018) (Fig. 5). These fusions are difficult to therapeutically target but fortunately have only been identified in a small subset of patients with endocrine therapy-resistant BCa (Li *et al.* 2013*a*, Hartmaier *et al.* 2018, Lei *et al.* 2018). Further efforts are needed to prove whether ER gene fusions are a definitive mechanism of endocrine resistance.

Alternative NR drivers

Overlap in the NR ligand affinities, NR-DNA binding elements, and NR transcriptional programs contributes to significant redundancy, NR cross-talk, and NR substitution in hormone-dependent cancers. Thus, activation of other NRs can effectively overcome antagonistic blockade of the oncogenic NR (Figs 4 and 5).

Alternative NR drivers in PCa

Of the NR3C family members, NR3C1, otherwise known as the glucocorticoid receptor (GR), can effectively substitute for NR3C4 (AR). Upregulation of GR expression is noted in EnzR-PCa cells, xenografts, and patient tumors (Arora *et al.* 2013, Puhr *et al.* 2018, Zhang *et al.* 2020). More than 50% of AR DNA binding sites can also be bound by GR (Arora *et al.* 2013). Additionally, GR agonists can induce enzalutamide resistance in enzalutamide-sensitive cells *in vitro*. Conversely, GR knockdown by RNAi or GR antagonists cause EnzR-PCa cells to regain sensitivity *in vitro* and *in vivo* (Arora *et al.* 2013, Puhr *et al.* 2018). Since GR activation can

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effectively substitute for AR as a molecular driver in PCa, GR antagonists are being evaluated in ongoing clinical trials (NCT03437941).

Alternative NR drivers in BCa

The closely related NR3B family member, NR3B2 (ER-β), is highly homologous to NR3B1 (ER-α) in its DNA-binding domain and its LBD, and binds estradiol with similar affinity to ER-α (Brzozowski *et al.* 1997, Shiau *et al.* 2002). Initial reports indicated that the median ER-β mRNA levels were ~two-fold higher than ER-α levels in TamR-BCa, compared with tamoxifen-sensitive tumors (Speirs *et al.* 1999). However, decreased expression of ER-β protein was observed in higher grade BCa, suggesting a protective role for ER-β against the mitogenic effects of estrogen in advanced cancers (Roger *et al.* 2001). Three additional NRs may modulate ER activity in BCa – the estrogen-related receptor alpha (ERRα), the progesterone receptor (PR), and AR. Increased ERRα expression has been noted in TamR patient tumors and correlates with overall survival on tamoxifen. Furthermore, genetic ablation of ERRα inhibits TamR cell proliferation *in vitro* (Thewes *et al.* 2015). The role of PR in endocrine therapy resistance is controversial. PR expression has been shown to be beneficial: ER+/PR− BCa is more resistant to tamoxifen than ER+/PR+ tumors, and PR loss is associated with progression (Bardou *et al.* 2003). Mechanistic studies indicate that in the presence of agonists, PR associates with ER-α to direct ER-α chromatin binding events in BCa cells, resulting in a unique gene expression program associated with positive clinical outcomes (Mohammed *et al.* 2015). While the cooperative effects of ER and PR argue that PR antagonists, rather than agonists, should be used for treating advanced BCa (Singhal *et al.* 2016), other studies indicate that PR, independent of ligand, enhances the expression of proliferative and invasive estrogen-induced target genes (Daniel *et al.* 2015). Much like PR, the role of AR in both ER+ and ER− BCa is controversial. TamR-BCa have higher AR expression and a higher AR:ER ratio, which correlates with a poorer prognosis (Cao *et al.* 2019). Mechanistically, AR may enable maximal ER chromatin binding, with AR redirected to ER-bound promoter elements in an estrogen-dependent manner (D'Amato *et al.* 2016). Some studies show that AR antagonists inhibit the growth of TamR-BCa *in vitro* and *in vivo* (D'Amato *et al.* 2016, Creevey *et al.* 2019). However, other studies have indicated that AR+ BCa patients may have a better prognosis, and that AR agonists inhibit the growth of ER+ BCa, including those driven by mutant ER (Witzel *et al.* 2013, Aleskandarany *et al.* 2016, Ponnusamy *et al.* 2019). The luminal subtype of triple-negative breast cancer (TNBC) characterized by AR expression may be associated with a better prognosis (Hu *et al.* 2017), but the role of AR as the primary molecular driver in this subset remains to be proven. The utility of AR-targeted agents (like enzalutamide) in this subset of TNBC is being evaluated in clinical trials. Although ERRα antagonists may provide some clinical benefit for TamR-BCa patients, the role PR and AR play in therapy resistance requires further study.

Alternative non-NR drivers

In order to adapt to the selection pressure of NR-targeted therapies, hormone-dependent cancers sometimes lose their dependence on their primary oncogenic NR. Since a significant proportion (>5%) of the entire transcriptional program within these hormone-dependent cancers is driven by the NR, drastic changes within the cancer cell are required (Holzbeierlein *et al.* 2004, Sharma *et al.* 2013). Some hormone-dependent cancers dynamically decrease their NR expression in response to selection pressure and evade the effect of the targeting agent. In others, NR expression level may not be changed, but the canonical NR signaling program is lost (Figs 4 and 5).

Alternative drivers in PCa

A small subset of EnzR-PCa (10–15%) lose AR signaling and undergo lineage plasticity, characterized by epigenetic reprogramming and the acquisition of neuroendocrine characteristics, which allows previously AR-dependent cells to become AR independent (Robinson *et al.* 2015, Abida *et al.* 2019). Concomitant loss of tumor protein p53 (TP53) and retinoblastoma 1 (RB1) mediate this programmatic shift. Loss of TP53 and RB1 in enzalutamide-sensitive cells has been shown to promote resistance *in vitro* and *in vivo* through a number of downstream mediators, including SRY-box 2 (SOX2), POU-domain transcription factor BRN2 (BRN2), N-MYC, enhancer of zeste homolog 2 (EZH2), and ataxia telangiectasia mutated (ATM) (Dardenne *et al.* 2016, Bishop *et al.* 2017, Ku *et al.* 2017, Mu *et al.* 2017, Yin *et al.* 2019). Alterations of these downstream mediators, like ATM upregulation, promote enzalutamide resistance *in vitro*, while genetic ablation or pharmacological inhibition of ATM in EnzR cells confers sensitivity (Yin *et al.* 2019). The clinical translation of these findings remains ongoing.

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Alternative drivers in BCa

Loss of ER expression was initially thought to be the dominant mechanism of *de novo* resistance to tamoxifen (Ingle *et al.* 1991). This ER-negative phenotype may be related to either selection of ER-negative clones or epigenetic alterations. Epigenetic modifiers have been shown to reactivate ER-α expression in ER-negative BCa cells (Li *et al.* 2013*c*). In the absence of ER signaling, amplification of another driver, the human epidermal growth factor receptor 2 (HER2/Neu or ERBB2) is sufficient to confer tamoxifen resistance *in vitro* and *in vivo* (Benz *et al.* 1992, Kurokawa *et al.* 2000). Furthermore, EGFR/HER2 kinase inhibitors have been shown to sensitize TamR-BCa cells (Kurokawa *et al.* 2000). Additional studies have shown the clinical relevance of this therapeutic strategy, where HER2 monoclonal antibody tratuzumab/herceptin improves survival in primary and metastatic BCa patients, independent of HR status (Kaufman *et al.* 2009). In addition, enhanced mitogen-activated protein kinase (MAPK) signaling is noted in ~15% of TamR-BCa (Razavi *et al.* 2018). Alterations in the MAPK pathway (including GOF HER2 mutations, neurofibromin (NF1) loss, and epidermal growth factor receptor (EGFR) amplification) are mutually exclusive with ER mutations in patients, and promote fulvestrant resistance *in vitro*, indicating that MAPK activation may be an important factor in driving therapy resistance (Oh *et al.* 2001, Holloway *et al.* 2004, Razavi *et al.* 2018). In support of this, EGFR kinase and ERK/MEK inhibitors confer sensitivity to fulvestrant in resistant BCa cells (Razavi *et al.* 2018). Clinical trials to assess the efficacy of EGFR and ERK/MEK inhibitors are ongoing.

Cyclin D1 and cyclin-dependent kinase 4/6 are important growth factor cell cycle regulatory components, and enhanced cyclin D1-CDK4/6 signaling is critical for growth factor-independent proliferation (Kato *et al.* 1993, Matsushime *et al.* 1994, Zwijsen *et al.* 1997). Cyclin D1 overexpression is associated with tamoxifen resistance and has been shown to independently activate ER-mediated transcription in the absence of estrogen *in vitro* (Zwijsen *et al.* 1997, Kenny *et al.* 1999). Strong preclinical data has demonstrated that CDK4/6 inhibitor, palbociclib, can overcome acquired tamoxifen resistance and synergize with antiestrogen therapy (Finn *et al.* 2009). Together, these findings have inspired several clinical trials revealing that palbociclib and other CDK 4/6 inhibitors, in combination with fulvestrant, increase PFS in patients with advanced, therapy resistant ER+ BCa, when compared to endocrine therapy alone (Finn *et al.* 2009, Cristofanilli *et al.* 2016). Thus, there is considerable evidence that suggests cyclin D1/CDK 4/6 can act as a driver of tamoxifen resistance *in vitro*, as well as the clinic.

Discussion

Hormone-dependent cancers remain dependent on the NR that drives their primary malignancy, and maintaining NR signaling through NR overexpression, mutation or alternative splicing, increased hormone synthesis, or altering coregulator expression can drive endocrine therapy resistance. However, the frequency with which these alterations occur and drive therapy resistance in BCa and PCa are significantly different. For instance, >50% of EnzR-CRPC patients have AR amplification, and AR overexpression is a proven mechanism of resistance in PCa; in contrast, ~15% of TamR-BCa patients have increased ER expression, and it is unclear whether this enables tamoxifen resistance (Table 1). While increased NR expression enhances responsiveness to low agonist levels, the differences in frequency may be attributed to the relative potency, affinity, and clinical use of the antagonists (enzalutamide and tamoxifen). Tamoxifen has a higher affinity for ER than E2, while enzalutamide has a lower affinity for AR than DHT. The weaker selection pressure of enzalutamide may allow the CRPC cell to overexpress AR and maintain AR signaling. In support of this hypothesis, AR overexpression is much more common (75%) after treatment with less avid antiandrogens, like bicalutamide (Koivisto & Rantala 1999). We speculate that AR amplification may be a less common driver of therapy resistance with a more potent AR antagonist. In addition, enzalutamide is more commonly used at a later clinical stage (after primary hormonal therapy in CRPC rather than in primary PCa) than tamoxifen, which is more commonly given as an adjuvant hormonal therapy to premenopausal, early stage ER+ BCa patients and less frequently to metastatic patients. A better comparison may be to evaluate ER amplification rates after tamoxifen treatment to postmenopausal women whom present with metastatic ER+ BCa. Together, we would expect that earlier use of a more potent AR antagonist or AR degrader may lead to less compensatory AR amplification as a molecular driver of resistance in PCa (Table 1).

The duration of ER-targeted therapies in BCa has been defined by level 1 clinical evidence from randomized clinical trials showing that ten years of tamoxifen treatment is associated with a significantly lower recurrence and mortality rate, compared to five years of treatment (Davies *et al.* 2013). The benefit of prolonging treatment is likely due to tamoxifen's cytostatic effect on dormant micrometastases. While clinical trials with neoadjuvant androgen deprivation therapy did not show a significant benefit in terms of disease recurrence or progression,

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earlier use of AR-targeted agents in non-metastatic PCa have shown clinical utility (Akitake *et al.* 2018, Hussain *et al.* 2018). From a molecular basis, the earlier and more prolonged use of AR-targeted agents in clinically localized PCa may target a limited spectrum of AR alterations more effectively and have the same effect as tamoxifen in BCa. The optimal timing and duration of AR-targeted therapies needs to be delineated from randomized clinical trials for patients with clinically localized PCa.

Interestingly, NR LBD mutations are a much more common mechanism of therapy resistance for ER (20–30%) than AR (5–10%) (Toy *et al.* 2013, Robinson *et al.* 2015, Conteduca *et al.* 2017, Razavi *et al.* 2018, Abida *et al.* 2019) (Table 1). These findings may be attributed to critical differences in the LBD, location of the LBD mutations, and the role of helix 12 in LBD function. Typically, AR LBD mutations occur within the ligand-binding pocket (LBP) of the LBD and alter the binding of the antagonist to the receptor. Individual AR LBD mutants may convert specific antagonists to agonists: AR LBD mutants that arise in response to one antiandrogen (e.g. F876L after enzalutamide) may still respond to a second antiandrogen (bicalutamide). Additionally, AR LBD mutations do not result in ligand-independent activity. In contrast, ER LBD mutations are outside the true LBP (on helix 12) and alter access to the LBD. These mutants have decreased affinity for both agonists and antagonists and have ligand-independent activity. Thus, ER LBD mutations offer a competitive growth advantage to TamR-BCa cells and may explain why ER LBD mutants are more commonly seen and more likely to drive therapy resistance than AR mutations (Table 1). We strongly believe that effective agents targeting ER LBD mutants in BCa represent a significant unmet need.

While AR splice variants have constitutive activity and are more commonly seen in PCa than ER splice variants in BCa, their role as an independent driver of therapy resistance is still not clear. AR splice variants are primarily noted in the context of AR amplification and their ability to function independent of the full-length AR is still controversial (Table 1). Agents that selectively target these variants will be critical for defining whether AR splice variants are proven drivers of antiandrogen resistance.

In both hormone-dependent cancers, alterations in coregulators and transcriptional machinery promote resistance by enhancing transcriptional activity of the cognate NR, and thus have relatively the same prevalence in endocrine therapy-resistant patients. The same is true for increased hormone synthesis, which can enable intratumoral agonists to outcompete antagonists and continue to drive pro-proliferative programs in both PCa and BCa (Table 1). We propose that functional redundancy of coregulators may lead to significant heterogeneity in the coregulator profile and detract from the efficacy of agents targeting individual NR coregulators.

Because NRs have similar NR-DNA binding elements, ligand affinities, and transcriptional programs, NR cross-talk and substitution can effectively enable therapy resistance in both hormone-dependent cancers. Although alternative NRs are commonly seen in therapy-resistant patients and mechanistically shown to drive resistance, therapeutic strategies targeting these alternative NRs are currently being investigated and have not been proven in the clinic (Table 1). We postulate that effective biomarkers may be needed to identify which tumors are driven by alternative NRs, and thus define responsiveness to NR-targeting drugs.

The vast majority of therapy-resistant tumors maintain NR signaling; however, a significant proportion of advanced cancers lose NR signaling and subsequently switch to alternative drivers. Although this is seen in both PCa and BCa, it is a more prevalent mechanism of therapy resistance in BCa. Approximately 10–15% of EnzR-PCa will lose AR, while 15–30% of TamR-BCa lose ER and switch to an alternative driver (Table 1). The reason behind this differential frequency is unclear; however, it may be related to the TP53/RB1 status, where TP53 may act as the primary gate-keeper. Under selective pressure, it is possible that when TP53 is lost by deletion or LOF mutation, it could allow for the acquisition of numerous mutations and aberrant genomic events, including RB1 loss and subsequent NR independence. Approximately 20–30% of BCa have RB1 loss, and RB1 loss is enriched following endocrine therapy in conjunction with ER loss (Pharoah *et al.* 1999, Robinson *et al.* 2017). Loss of RB1 occurs in ~10–15% of mCRPC (in conjunction with TP53) and tends to be concurrent with AR loss (Abida *et al.* 2019). Due to the accumulation of mutations potentially contributed by TP53/RB1 loss, these endocrine therapy-resistant tumors are primarily reliant on alternative molecular drivers. Importantly, significant heterogeneity in the alternative molecular drivers is likely, and further molecular characterization of these tumors will be needed to identify possible therapeutic targets and agents.

Our comparative overview of two hormone-dependent cancers – BCa and PCa – indicates that these cancers have a significant addiction to their primary NR driver and will take extraordinary steps to maintain that oncogenic addiction. While more potent therapies

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targeting/degrading these NRs are in development, their clinical utility must be balanced with both their adverse side effects and their likelihood of selectively targeting a more aggressive, therapy-resistant cancer. The optimal timing and duration of each NR-targeted therapy must be defined. Strategies to target alternative non-NR molecular drivers in these cancers should be explored. With the widespread adoption of somatic sequencing, alternative therapeutic agents, such as poly (ADP-ribose) polymerase (PARP) inhibitors for tumors with DNA damage defects should be evaluated in the upfront setting.

Conclusion

Elucidating the molecular drivers of antiandrogen and antiestrogen resistance has enhanced our understanding of factors that drive endocrine therapy resistance and disease progression. Interestingly, while similar mechanisms are employed in maintaining the addiction of the cancer cell to the primary NR oncogenic driver, their relative contributions to therapy resistance in BCa and PCa are clearly distinct, likely due to differences in the clinical stage, relative affinity and binding of these antagonists to the NR, and intrinsic differences between AR and ER. These data indicate that distinct therapeutic strategies are needed to overcome endocrine therapy resistance in BCa and PCa to prolong patient survival.

Declaration of interest

G V R is a named inventor in a patent for ERX-11.

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