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Review

Targeting Androgen/Estrogen Receptors Crosstalk in Cancer

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The actions of estrogens are mediated by estrogen receptors, ERα and ERβ. Recent genomic landscaping of ERα- and ERβ-binding sites has revealed important distinctions regarding their transcriptional activity. ERβ and its isoforms have been correlated with endocrine treatment responsiveness in breast tumors, while post-translational modifications, receptor dimerization patterns, and subcellular localization are increasingly recognized as crucial modulators in prostate carcinogenesis. Androgen receptor (AR) is essential for the development and progression of prostate cancer as well as of certain breast cancer types. The balance between the activity of these two hormone receptors and their molecular interactions in different clinical settings is influenced by several coregulators. This comprises a dynamic regulatory network enhancing or limiting the activity of AR-directed treatments in breast and prostate tumorigenesis. In this review, we discuss the molecular background regarding the therapeutic targeting of androgen/estrogen receptor crosstalk in breast and prostate cancer.

ERβ and AR Communicate in Breast and Prostate Cancers

Owing to the endocrine nature of breast and prostate carcinogenesis, targeting of hormone receptors (HRs) remains a treatment cornerstone. **Estrogen** (ER; ERα and **ERβ**) and **androgen receptor** (**AR**, see Glossary) are members of the steroid nuclear receptor superfamily [1].

In breast and prostate cancers, activation of ERα and AR, respectively, is responsible for enhanced cell proliferation and cell survival, whereas activated ERβ acts as a tumor suppressor [2]. Hence, androgen deprivation therapy (ADT) remains the basic treatment in patients with **hormone-sensitive prostate cancer (HSPC)**, while in **castration-resistant prostate cancer (CRPC)**, AR targeting with novel drugs has provided significant clinical results [3]. Ongoing research efforts are evaluating the combination of novel AR inhibitors with selective modulators of ERβ for the prevention and treatment of prostate cancer [4]. ERα is an established predictive biomarker of endocrine treatment in breast cancer patients. However, one-third of patients treated with tamoxifen (an estrogen blocker) develop resistance, even though ER status remains unchanged [5]. ERβ has been evaluated as a predictive biomarker of endocrine treatment, with indefinite conclusions so far [6,7]. Although the association of ERβ expression and tamoxifen activity in ERα(+) breast tumors has been described, scarce data exist regarding the association of ERβ expression and other endocrine treatments [8]. An important clinical question is whether ERβ status provides useful information in breast cancer treatment decisions. AR is expressed in many early-stage and metastatic breast carcinomas but its effect varies among patients, depending on ER status [9]. Notably, AR has emerged as a new classification biomarker for **triple-negative breast cancer (TNBC)** [10,11]. In light of the above, clinically important considerations have been aroused regarding the use of AR inhibitors in TNBC and other breast cancer subtypes and the potential role of ERβ as a predictive biomarker in TNBC patient management [9].

Trends

ERα has a well-established role in estrogen-dependent breast tumor growth, whereas ERβ significantly attenuates cell proliferation and invasion in many cancer types, including breast and prostate.

Emerging evidence indicates that AR signaling exerts inhibitory effects on the growth of normal mammary epithelial cells and plays a protective role in breast carcinogenesis.

There are different potential mechanisms by which ERβ isoforms can modulate AR activity during carcinogenesis and this may affect treatment efficacy in prostate and breast cancers. Among them, the most important is the competition for DNA binding that may alter the recruitment of transcription coregulators, depending on the cancer stage.

The importance of subtle differences among ERβ isoforms, AR, and associated coregulators in different clinical settings should be further explored to identify patient subgroups with specific expression patterns and define the optimal application of AR-directed treatments.

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In this review, we discuss the role of ERβ and AR in hormone-dependent carcinogenesis regarding cancer evolution, prognosis, and efficacy of available endocrine treatment options. We provide data showing that ERβ and AR represent a pivotal molecular circuit in breast and prostate cancers that affects treatment outcome, although the exact mechanisms are unclear mainly as a result of inherent limitations of currently used analysis techniques. Finally, we highlight the concept of context-dependent endocrine treatment based on the AR–ERβ equilibrium in certain breast cancer subtypes and prostate cancer, as well as novel assays that may facilitate the optimal use of endocrine treatment and the identification of new targeting strategies.

ERβ: A Partner or a Key Molecule?

Breast Cancer

Five molecular breast cancer subtypes were identified by microarray gene expression: luminal A, luminal B, basal-like, ERBB-2-enriched, and claudin-low [12,13]. TNBC is defined by the absence of ER, progesterone receptor (PR), and ERBB-2. Although the majority of TNBCs have been classified as basal-like, these two categories are not considered synonymous [14]. Further analysis of TNBCs led to the identification of six subgroups: basal-like 1 and basal-like 2 (BL1, BL2), mesenchymal (M), mesenchymal stem-like (MSL), immunomodulatory (IM), and luminal androgen receptor (LAR) [10]. Each subgroup displays a unique biology with distinct clinical outcome and differential sensitivity to chemotherapy and/or targeted agents [15].

Estrogens contribute to hormone-dependent breast carcinogenesis through ERs. Ligand binding to ERα/ERβ induces receptor dimerization and subsequent nuclear translocation and binding to target genes in hormone receptor elements (HREs) and other DNA regulatory elements (Figure 1, insert). Multiple ERβ isoforms are expressed in breast (Figure 2A), but only the full-length receptor (ERβ1) is able to bind 17β-estradiol (E2) and regulate gene expression [16]. Additionally, there is wide fluctuation regarding ERα/ERβ expression ratio during breast carcinogenesis (Figure 2B). Moreover, it has been suggested that ductal and lobular carcinomas, which represent the two most common types of breast cancer, express different ER levels in early and advanced stages [17].

ER isoforms trigger different cellular mechanisms and have been implicated in opposing clinical outcomes. ERβ1 has the capacity to induce apoptosis [5] and inhibit the proliferative response mediated by ERα [18], partly by reducing ERα nuclear translocation [19], while in ERα(–) breast cancers it inhibits growth in a ligand-independent and -dependent manner [20]. It has been also shown that activation of mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) signaling cascades in breast cancer cells can inhibit this growth repressing effect [2], while ERβ1 suppresses breast cancer metastatic potential by affecting the Wnt pathway [20] and upregulating E-cadherin expression [21]. ERβ2 and ERβ5 antagonize wild-type ERα and modulate ERβ1 transcriptional activity through heterodimerization, as well as prevention of ERα-induced transcription [22]. ERβ1 positivity has been associated with better survival, while ERβ2 associates with worse clinical outcome [5,23]. This may be partly attributable to ERβ2-induced proteasome-dependent ERα degradation through the formation of ERβ2/ERα heterodimers [6]. ERβ5 seems to be a marker of worse clinical outcome in certain breast cancers, such as ERBB-2 positive and TNBC [24].

ERα is expressed in 70% of breast carcinomas [25] and is a predictive marker of response to endocrine treatment. Lower expression of ERβ is found in tamoxifen-resistant tumors and high levels of ERβ have been associated with better clinical outcome in ERα(+) tumors [25]. Epigenetic modifications are also implicated in ERβ-related dismal prognosis [26]. It has been shown that in ERα(–) tumors, ERβ presence is related to an aggressive breast cancer phenotype [27]. There is growing evidence for the importance of ERBB-2/ERBB-3 receptor dimerization in breast carcinogenesis [28] and ERBB-2 and ERBB-3 coexpression is associated with endocrine

Glossary

Androgen receptor (AR): a nuclear receptor that is activated upon binding the androgenic hormone (testosterone, dihydrotestosterone) in the cytoplasm and then translocates into the nucleus, stimulating transcription of androgen responsive genes. AR plays a key role in prostate and breast cancer.

Castration-resistant prostate cancer (CRPC): prostate cancer that has undergone enough molecular changes to become resistant to hormone therapy (androgen ablation); however, AR signaling is maintained in CRPC.

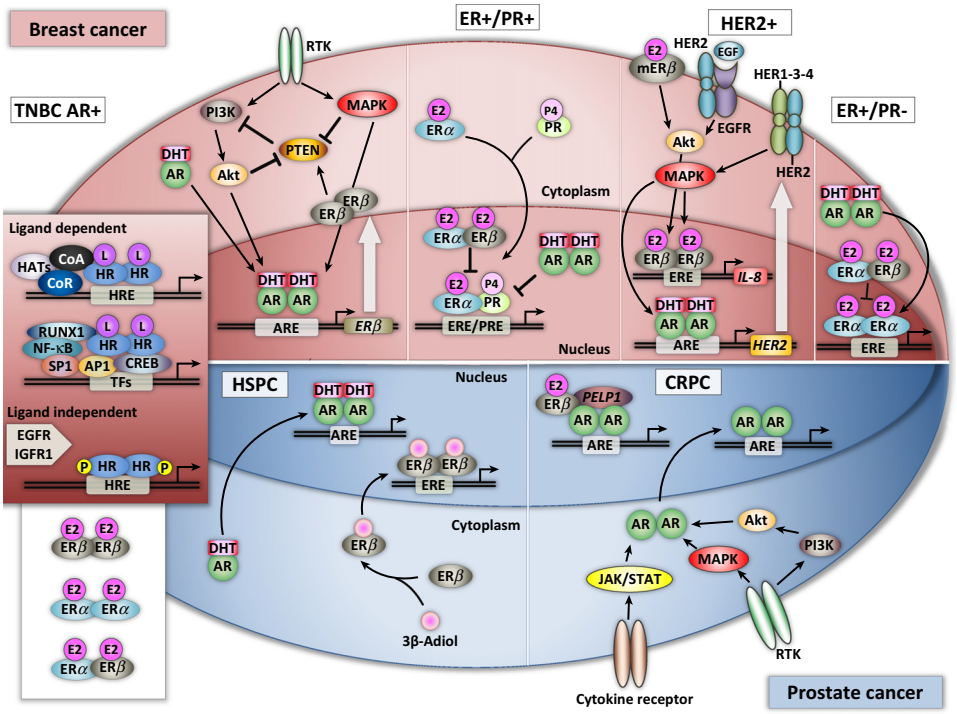
Estrogen receptor β (ERβ): a nuclear receptor that is activated upon binding 17-β-estradiol, estriol, or related ligands in the cytoplasm and then translocates into the nucleus, stimulating transcription of target genes. ERβ plays a key role in breast and prostate cancer.

Hormone-sensitive prostate cancer (HSPC): prostate cancer that depends on testosterone to proliferate and therefore responds to hormone therapy (androgen ablation).

Signaling crosstalk: the molecular mechanisms by which cellular signaling pathways affect each other. This can take place, in part, when components of any two pathways physically interact, or when components of one pathway are enzymatic or transcriptional targets of the other. Multiple other mechanisms of signaling crosstalk exist.

Transcription coregulators (TCRs): nuclear proteins that interact with transcription factors to either repress (corepressors) or activate (coactivators) the transcription of specific genes.

Triple-negative breast cancer (TNBC): breast cancer subtype lacking estrogen receptor α (ERα–), progesterone receptor (PR–), and human epidermal growth factor receptor 2 (HER2–), and, therefore, does not respond to hormonal therapy (tamoxifen, aromatase inhibitors) or anti-HER2 therapies (trastuzumab).



Trends in Cancer

Figure 1. Model of Signaling Crosstalk between AR and ERβ Pathways in Breast and Prostate Cancer. ERβ and AR signaling pathways interact in multiple and complex ways, either directly or indirectly (see Box 1 for further details). Abbreviations: Akt, protein kinase B; AP1, activator protein 1; AR, androgen receptor; ARE, androgen response element; CoA, coactivators; CoR, corepressors; CREB, cAMP response element-binding protein; CRPC, castration-resistant prostate cancer; DHT, dihydrotestosterone; E2, estradiol; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERE, estrogen response element; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; HATs, histone acetyltransferases; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; HRE, hormone response element; HSPC, hormone-sensitive prostate cancer; IGFR1, insulin-like growth factor 1 receptor; IL-8, interleukin 8; JAK, janus kinase; L, ligand; MAPK, mitogen-activated protein kinase; mERβ, membrane estrogen receptor beta; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NRs, nuclear receptors; P4, progesterone; PELP1, proline glutamic leucine rich protein 1; PI3K, phosphoinositide 3-kinase; PR, progesterone receptor; PRE, progesterone response element; PTEN, phosphatase and tensin homolog; RTK, receptor tyrosine kinase; RUNX1, Runt-related transcription factor 1; SP1, specificity protein 1; STAT, signal transducer and activator of transcription; TFs, transcription factors; TNBC, triple-negative breast cancer.

treatment resistance [29]. It has been shown that in ERα(+) breast cancers, ERβ expression can reduce protein kinase B (Akt) activation through downregulation of ERBB-2/ERBB-3 signaling and upregulation of phosphatase and tensin homolog (PTEN), thus increasing tamoxifen sensitivity [30]. ERβ can contribute to aggressiveness of ERBB-2(+) breast carcinomas through its increased expression and binding to interleukin 8 promoter (Figure 1, upper panel) [31]. A novel hypothesis is that higher expression of ERβ isoforms leads to generation of ER(+)/PR(–) breast carcinomas [32], which is in accordance with the suggestion that PR is not just an ERα-induced gene but also an ERα-associated protein that modulates its behavior (Figure 1, upper panel) [33]. Accordingly, there are two groups of breast cancer patients, one in which ERβ is coexpressed with ERα (60%) and the other in which ERβ is expressed alone (15%) [6]. ERβ activity is considered antagonistic to ERα when both receptors are present (Figure 1, upper panel), but in isolation the role of ERβ is not well documented.

Several mechanisms may explain resistance to endocrine treatment. Among them are decreased expression of ERα, loss of PR, and upregulation of ERBB-2 [34]. One way to

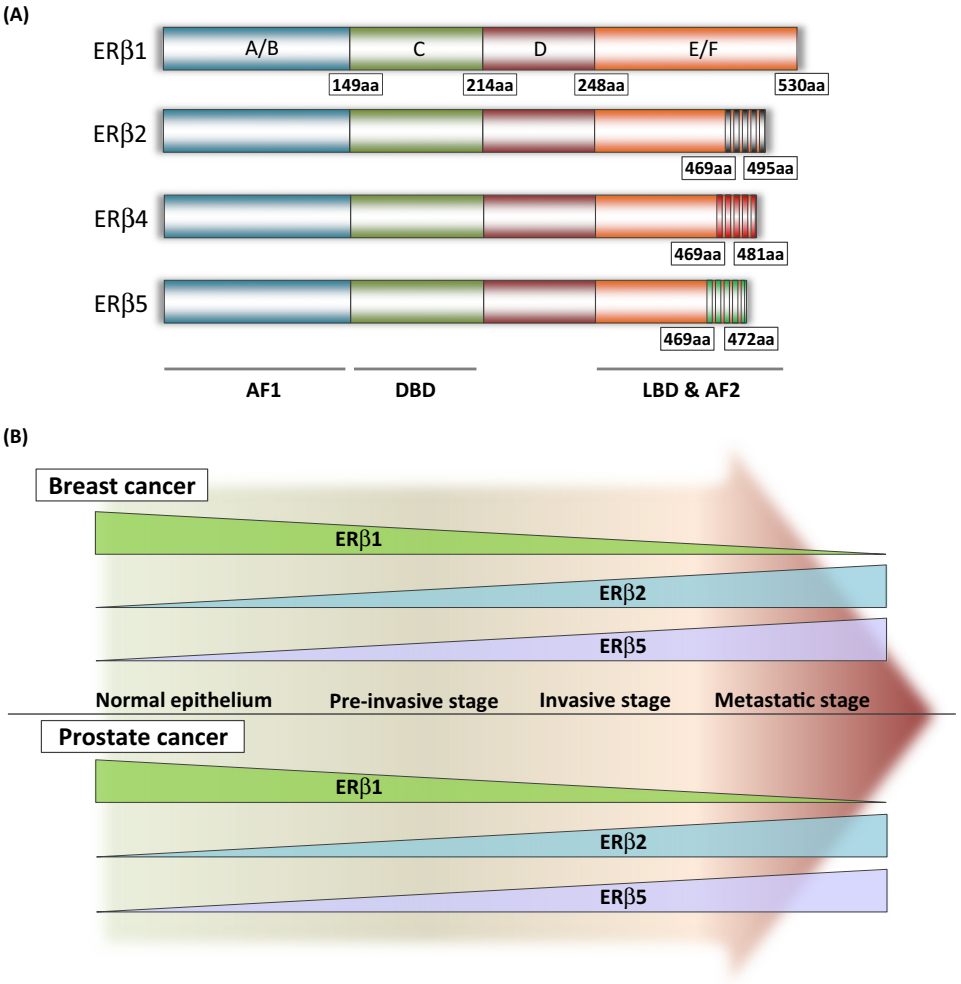


Figure 2. ERβ Isoform Expression during Breast and Prostate Carcinogenesis. (A) Representation of structural and functional protein domains of currently known ERβ isoforms (ERβ1, ERβ2, ERβ4, ERβ5). ERβ3 is not included; its role in cancer is still unknown. A/B is the NTD, which contains a transactivation function (AF1) with ligand-independent action and a coregulatory domain that is responsible for the recruitment of coregulators. C is the DBD, which is necessary for binding to EREs. D contains the hinge region, part of the ligand-dependent activating function and the nuclear localization signal. The C-terminal regions E and F contain the LBD and have a ligand-dependent transactivation function (AF2). This region is also responsible for the binding of coregulators and chaperones, as well as for receptor dimerization and nuclear translocation. ERβ isoforms are generated by alternative splicing of the last coding exon (indicated by the colored striped bars). (B) Illustration of the differential expression of ERβ isoforms during breast and prostate cancer development and progression. Abbreviations: AF1, activation function 1; AF2, activation function 2; aa, amino acids; DBD, DNA-binding domain; ERβ, estrogen receptor beta; LBD, ligand-binding domain; NTD, amino-terminal domain.

bypass this resistance might be the use of ERβ1-selective agonists in certain breast cancer subtypes (Figure 3). Other potential useful strategies might include the combination of ERβ1 agonists with autophagy inhibitors [35] or epigenetic modifiers [36].

Furthermore, targeting ERβ and epidermal growth factor receptor (EGFR) in TNBC may offer new venues for treatment. ERβ1 has been detected in 50–90% of ERα(–) breast cancers [37]. Therefore, it is possible that antiestrogen strategies may act through ERβ1 in TNBC [38]. The combined use of tamoxifen and ERβ-selective agonists has shown additive anticancer effect *in*

Box 1. Proposed AR and ERβ Molecular Interplay in Breast and Prostate Carcinogenesis

In TNBC AR(+), AR is recruited to the androgen response element (ARE)-containing region of the ERβ promoter, leading to an increase in ERβ expression and consequently to an inhibition of tumor growth progression via PTEN, p21, p27, cyclin D1, and cyclin A upregulation, and suppression of *c-myc* [99]. However, ERβ-mediated antiproliferative effects are repressed through MAPK and PI3K signaling [41]. In ER(+)/PR(+) breast cancers, activated PR interacts with ERα causing this complex, along with associated cofactors, to promote the transcription of different genes compared with ERα homodimers, which results in slowing down tumor growth [33]. In addition, ERβ expression levels are associated with low aggressiveness when ERα and PR are also present. Probably, when ERβ forms heterodimers with ERα, there is reduced recruitment of coregulators and thus reduced ERα target gene transcription. ERβ may also inhibit the ERα-mediated binding of other TFs at their cognate motifs. A proposed role for AR in ER(+)/PR(+) breast cancers is that after ligand binding, AR translocates to the nucleus where it competes with ERα and PR for binding to the estrogen response elements (EREs) and thereby inhibits estrogen-dependent signaling. In ER(+)/PR(–) tumors, ERβ probably acts in a dominant negative manner, downregulating transcription of ERα target genes. The role of AR in the absence of PR is probably tumorigenic, enhancing ERα-mediated gene transcription. In HER2-enriched breast cancers, ERβ may contribute to aggressiveness through its increased expression and binding to *interleukin 8* promoter [31]. AR participates in a positive feedback loop with HER-2, inducing the transcription of the *HER-2* gene, which in turn leads to increased HER-2 signaling and AR activation [105]. In the presence of hyperactive receptor tyrosine kinase (RTK) signaling, such as in HER-2 overexpressing tumors, the non-genomic rapid ER action (via mERβ) might become the predominant mode of action of ER signaling and may contribute to the endocrine sensitivity or resistance phenotype of the tumor. Regarding prostate cancer, in HSPC, AR-mediated transcriptional activity is oncogenic [44], while ERβ stimulates the transcription of tumor-suppressive genes [49]. In the setting of CRPC, adaptive mechanisms, such as crosstalk with RTK and cytokine (JAK/STAT) pathways, develop and maintain AR signaling, allowing survival and tumor progression [3]. Furthermore, as the disease progresses to CRPC, ERβ may acquire an oncogenic role and mediate transcription of AR-dependent genes through interaction with proline glutamic leucine rich protein 1 (PELP1) [123] (Figure 1).

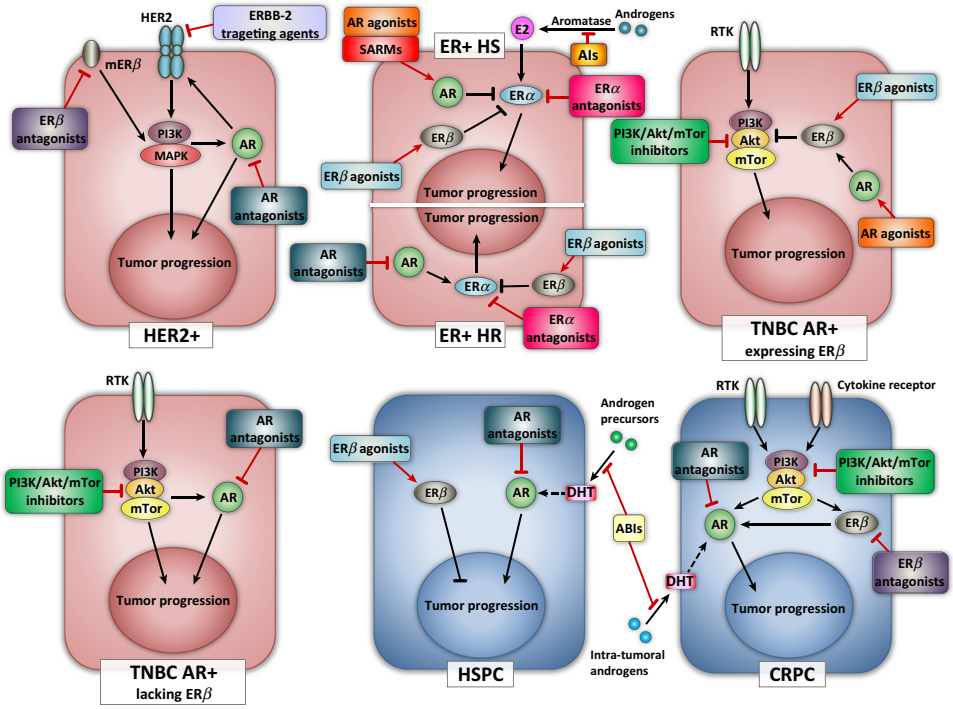
vitro [39], while TNBC patients that are treated with tamoxifen have better survival when the tumors are ERβ1 positive [40]. Under normal conditions, ERβ agonists can induce apoptosis in breast cancer cells. However, EGFR-induced signaling can modulate ERβ growth-inhibitory effects (Figure 1) [41]. In addition, clinical findings have shown an inverse correlation between ERβ1 positivity and EGFR expression [42]. Induction of EGFR signaling in ERβ1-expressing cells can reverse the ERβ1-dependent epithelial phenotype, suggesting that EGFR is a critical mediator in ERβ1-regulated epithelial mesenchymal transition (EMT). It is possible that ERβ1 induces EGFR ubiquitynation and degradation by enhancing EGFR–c-Cbl association [43]. Therefore, the combination of EGFR inhibitors and ERβ-selective agonists seems a rational strategy in TNBC patients expressing ERβ (Figure 3).

Prostate Cancer

Prostate carcinomas are dependent on androgens through AR, which upon ligand binding dissociates from heat shock proteins (Hsp), becomes phosphorylated, and translocates to the nucleus. The active AR homodimers bind HREs and lead to AR target gene activation [44]. Estrogens are also implicated in prostate carcinogenesis [45]. Although both ERs are expressed in prostate tissue, ERα expression is restricted to the stroma, whereas ERβ and its isoforms are present in prostate cells [46] and are differentially expressed during prostate cell cycle [4]. ERβ1 is the only functional isoform, while other isoforms have no intrinsic activity and function as dimerization partners that modulate ERβ1 activity [16].

ERβ is considered as an important modulator of prostate carcinogenesis [4,47] and gene polymorphisms in ERβ1 have been found to associate with a higher risk of prostate cancer [48]. ERβ1 acts as tumor suppressor in prostate and its expression declines during cancer progression [49], whilst ERβ2 and ERβ5 act as oncogenes and are involved in promoting invasion and metastasis (Figure 2B) [50].

ERβ1 expression is primarily regulated at the transcriptional level, while regulation of ERβ2 and ERβ5 is more complex [51]. Although reports have indicated that hypermethylation of the ERβ promoter is associated with loss of receptor expression [52], it has been suggested that loss of



Trends in Cancer

Figure 3. Targeting the Molecular Interplay of AR and ERβ Signaling in Breast and Prostate Cancer. Depending on the molecular profile of breast and prostate cancer patients, selective pharmacological modulation of androgen/estrogen receptors crosstalk could represent a rational therapeutic strategy (see Box 2 for further details). Abbreviations: ABIs, androgen biosynthesis inhibitors; AIs, aromatase inhibitors; Akt, protein kinase B; AR, androgen receptor; CRPC, castration-resistant prostate cancer; DHT, dihydrotestosterone; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; HER2, human epidermal growth factor receptor 2; HS, hormone sensitive; HR, hormone resistant; HSPC, hormone-sensitive prostate cancer; MAPK, mitogen-activated protein kinase; mERβ, membrane estrogen receptor beta; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; RTK, receptor tyrosine kinase; SARMs, selective androgen receptor modulators; TNBC, triple-negative breast cancer.

PTEN results in transcriptional repression of ERβ that involves proto-oncogenes [53] and enables autocrine vascular endothelial growth factor (VEGF) signaling [54]. It has been shown that ERβ triggers the intrinsic apoptotic pathway by increasing the expression of p53-upregulated modulator of apoptosis (PUMA) [55]. ERβ1 also affects multiple cell cycle genes at the mRNA and/or protein levels [49]. Another indication of the antitumor effect of ERβ1 is the upregulation of retinoblastoma (Rb) protein, the loss of which has been correlated to prostate

Box 2. Proposed Model for Rational Combinatorial Hormone Receptor-Directed Therapies in Breast and Prostate Cancer

In HER-2+ breast cancer patients, inhibition of tumor progression may be achieved through combinations of agents such as ERβ antagonists, AR antagonists, HER-2 targeting agents, and androgen biosynthesis inhibitors. In ERα(+) breast cancer patients who are hormone sensitive, suppression of tumor progression may be possible via combinatorial treatment, including ERα antagonists, AR agonists, selective androgen receptor modulators (SARMs), ERβ agonists, and aromatase inhibitors (AIs). By contrast, in ERα(+) breast cancer patients, who are hormone resistant, antitumor responses may be feasible by using combined AR antagonists, ERα antagonists and ERβ agonists, or antagonists. In TNBC AR(+) ERβ(+) patients, blockade of tumor progression could be achieved via multitargeting with agents such as ERβ agonists, AR agonists, and PI3K/Akt/mTOR inhibitors. However, in TNBC AR(+) ERβ(–) patients, antitumor effects may be elicited through the use of AR antagonists, androgen biosynthesis inhibitors, and PI3K/Akt/mTOR inhibitors. HSPC patients may benefit from receiving AR antagonists, ERβ agonists, and androgen biosynthesis inhibitors, while CRPC patients may probably require combined treatment, including AR antagonists, ERβ antagonists, PI3K/Akt/mTOR inhibitors, and androgen biosynthesis inhibitors to stimulate antitumor responses (Figure 3).

Box 3. Coregulatory Network in Breast and Prostate Cancer: The AR/ERβ Paradigm

Nuclear receptors interact with a plethora of transcription coregulators (TCRs) to modulate transcriptional events. Indeed, TCRs that facilitate tumor-promoting activities of AR in prostate cancer cells also facilitate the tumor-promoting activities of ERα in breast cancer cells [119]. Since ERα and ERβ potentially share common TCRs [120], it is possible that AR and ERβ interact with a largely overlapping set of TCRs to subsequently enhance or repress the transcription of target genes. Various studies have implicated TCRs both in carcinogenesis and anticancer treatment efficacy in hormone-sensitive cancers [121,122]. For example, it has been recently reported that both ERβ1 and steroid receptor RNA activator protein (SRAP) are predictive biomarkers of tamoxifen response/benefit in women with ERα-negative breast cancer [122]. The most widely studied group of HR TCRs is the p160 protein family [33], while the repertoire of AR and ERβ TCRs is continuously increasing and becoming better understood [121,122]. Additionally, it has been recognized that TCRs hold a crucial role in both HR ligand-dependent and -independent transcriptional activity. Multiple mechanisms of crosstalk between AR and ERβ exist, and the recruitment of certain TCRs is one of the most important, affecting interactions in normal and malignant states and probably determining the efficacy of HR-directed treatment strategies.

carcinogenesis [56]. ERβ1 has also an inhibitory effect on bone metastases in prostate cancer through downregulation of *RUNX2* by ERβ1-mediated regulation of *Slug*, a gene implicated in bone formation and metastasis [57].

Contrarily, ERβ2 seems to act as an ERβ1 transcriptional repressor and promotes the metastatic potential of prostate cancer through the upregulation of c-Myc, Twist1, and its target gene *DKK1* [4]. Additionally, ERβ2 expression results in upregulation of the EMT genes *TWIST1* and *RUNX2* [49].

There are also data showing that ERβ and its isoforms participate in CRPC conversion [58]. The combination of decreased ERβ1 expression and AR phosphorylation in HSPC correlates with poor clinical outcome and increased risk of subsequent CRPC development [59]. In CRPC, preclinical studies of epigenetic modifiers [60] and selective ERβ agonists showed encouraging results [45]. In the same context, the use of phytoestrogens in ERβ(+) CRPC showed that such agents function as negative regulators of AR [61]. However, so far, clinical results of ERβ-targeted therapy in CRPC are conflicting [62,63]. All these data should be further evaluated, taking also into account the complex interplay of the AR–ERβ–**transcription coregulator (TCR)** network (Box 3).

Targeting AR in Prostate Carcinogenesis

AR-targeted therapy has evolved since the discovery of prostate cancer dependence on androgen [64] and luteinizing hormone-releasing hormone (LHRH) agonists [65]. The 'flare' of testosterone associated with LHRH agonists subsequently led to the development of LHRH antagonists [66], but a paradigm shift in CRPC treatment was abiraterone acetate, an inhibitor of androgen biosynthesis (Table 1) [3]. Likewise, first-generation antiandrogens are initially effective but eventually develop agonist activity [67,68] as a result of mutations in the AR ligand-binding domain (LBD) and AR overexpression (Table 1). Enzalutamide, a second-generation antiandrogen, binds AR with greater affinity than first-generation antiandrogens, reduces AR nuclear translocation, and impairs DNA binding to HREs and recruitment of TCRs [69]. It has provided significant clinical results, although this agent eventually acts as an agonist [3].

Multiple mechanisms of maintained AR activity have been reported. The elucidation of CRPC biology has facilitated the development of novel agents (Table 1). After the clinical success of abiraterone acetate, many androgen biosynthesis inhibitors were developed, such as orteronel (TAK-700) [70], galeterone (TOK-001) [71], and VT-464 [72]. Drugs targeting other components of the androgen biosynthesis pathway have also been developed, such as dutasteride, which inhibits 5-α-reductase (SRD5A1), and ASP9521, which is a potent selective oral inhibitor of the type 5 17-β-hydroxysteroid dehydrogenase AKR1C3 [44]. Dutasteride is currently being tested in combination with abiraterone acetate, while ASP9521 has not demonstrated clinical activity as

Table 1. AR-Directed Strategies in Breast and Prostate Cancer Therapy.

Agent	Target[a]
Prostate Cancer	
Bicalutamide, Nilutamide, Flutamide (*First-generation AR inhibitor*)	Bind to LBD of AR, potential of antagonist-to-agonist reversal of action
Enzalutamide, ARN-509 (*Second-generation AR inhibitor*)	Bind to LBD of AR with higher affinity, reduces nuclear translocation of AR, impairs AR DNA binding to AREs and TCRs, lower potential of antagonist-to-agonist reversal of action
ODM-201 (*Third-generation AR inhibitor*)	Bind to LBD of AR with higher affinity and reduces nuclear translocation of AR, no potential of antagonist-to-agonist reversal of action
EPI-001	Inhibitor of AR NTD
Abiraterone acetate	Selective inhibitor of 17∝-hydroxylase and 17,20-lyase
Orteronel, VT-464	Selective inhibitor of 17,20-lyase
Galeterone	CYP17 lyase inhibitor, AR antagonist, AR degradation
Ganetespib, AT13387	Hsp90 inhibitor
Apatorsen	Hsp27 inhibitor
BKM120, GDC-0068, GDC-0980	Targeting PI3K/Akt/mTOR pathway
Breast Cancer	
Bicalutamide	Bind to LBD of AR in AR(+) TNBC
Enzalutamide, ARN-509	Bind to LBD of AR with higher affinity, reduces nuclear translocation of AR, impairs AR DNA binding to AREs and TCRs in AR(+) TNBC
Enzalutamide with or without aromatase inhibitors (AIs) or fulvestrant	Bypass resistance to antiestrogens, needs higher dose of AIs due to enzalutamide-induced CYP3A4, evaluated in ER(+) breast cancer
Enzalutamide with ERBB-2 targeting agents	Blocks AR-mediated feedback loop with ERBB-2, evaluated in ER(–)/ERBB-2(+) breast cancer
Ganetespib, AT13387	Hsp90 inhibitor evaluated in AR(+) TNBC, ER+, ERBB-2(+) breast cancers
Apatorsen	Hsp27 inhibitor evaluated in AR(+) TNBC, ER+, ERBB-2(+) breast cancers
Enobosarm	Selective AR modulator (SARM), evaluated in AR(+) breast cancer
BKM120, GDC-0941, GDC-0980, NVP-BEZ235	Targeting of PI3K/Akt/mTOR pathway, evaluated in AR(+) TNBC

[a]Abbreviations: Akt, protein kinase B; AR, androgen receptor; AREs, androgen response elements; ER, estrogen receptor; LBD, ligand-binding domain; Hsp, heat-shock protein; mTOR, mammalian target of rapamycin; NTD, amino-terminal domain; PI3K, phosphoinositide 3-kinase; TCRs, transcription coregulators; TNBC, triple-negative breast cancer.

a single agent [73]. ARN-509 has a chemical structure similar to that of enzalutamide and displayed potent AR antagonism [74] and promising clinical activity in CRPC patients [75]. Other novel antiandrogens under development include ODM-201, which inhibits AR nuclear translocation, and AZD3514, which inhibits AR nuclear translocation and downregulates AR levels [76]. Another strategy involves targeting the intrinsically disordered amino-terminal domain (NTD). EPI-001 is an AR NTD antagonist, which blocks protein–protein interactions necessary for AR transcriptional activity [77]. Targeting AR stability is also being evaluated with Hsp inhibitors, such as Hsp27 inhibitor apatorsen [78] and Hsp90 inhibitor AT13387 [79]. These agents have shown additive preclinical activity. Since targeting AR in CRPC can activate compensatory signaling networks, including kinase-dependent pathways, clinical trials are assessing PI3K/Akt [80] and tyrosine kinase inhibitors in combination with AR drugs [81,82].

Targeting AR in Breast Carcinogenesis

AR is expressed in approximately 80% and 60% of primary and metastatic breast carcinomas, respectively [83] and its expression varies across different subtypes [10,84]. An oncogenic role for AR was first described in molecular apocrine breast cancer, an ERα(–)/AR(+) subtype that has a steroid response signature similar to that of ERα(+) breast tumors [85]. Pharmacological modulation of AR was tested in breast cancer with contradictory results. Testosterone was used in nonselected breast cancer patients with a 20–25% response rate [86]. Nevertheless, it was replaced by ER-targeted therapies. Androgens showed clinical efficacy when combined with ER-targeted agents [87] or as single agent in metastatic ERα(+) breast cancer patients after failure of ER-directed therapies [88]. Clinical trials with first-generation antiandrogens in unselected patients failed [89], although their application in AR(+) TNBC gave promising results [9]. Currently, many AR-targeted therapies are being evaluated in breast cancer (Table 1) [90–96].

TNBC with an AR molecular signature is termed luminal androgen receptor (LAR) subtype due to the resemblance of its gene expression profile to that of ER(+) breast cancer [10]. Data of AR in TNBC [9] seems to suggest that no single anti-AR strategy can produce significant results in unselected patient cohorts beyond what chemotherapy has already achieved. Moreover, various pathways interact with AR and participate in TNBC initiation and progression. For example, LAR breast cancer cells are sensitive to AR antagonists and Hsp90 inhibitors, whilst an association between activating *PI3KCA* mutations and AR expression has been reported [94]. Interestingly, it was shown that AR(+) TNBCs lacking an LAR expression profile were also sensitive to AR inhibition [97]. Thus, the combined targeting of AR and PI3K/Akt could be of clinical importance for AR(+) TNBC patients (Figure 3). The identification of patient subgroups for targeted agents is a prerequisite for individualized treatment. So far, clinical trials evaluating AR inhibitors have enrolled unselected AR(+) TNBC patients. However, the presence of BRCA mutations has not yet been reported, while the crosstalk between AR and ERβ remains to be addressed (Figure 1, upper panel).

ERα(+) breast cancer represents the predominant AR-expressing subtype [84]. ER and AR signaling share similarities, including TCRs and DNA-binding sites (Box 3). AR positivity is associated with improved outcome in ERα(+) breast cancer, but AR becomes oncogenic in a tamoxifen-resistant setting [98]. Preclinical studies demonstrated that AR antagonizes ER growth stimulatory effect [99,100]. Clinical data also link AR expression to improved clinical outcome [100,101] and better response to ER-directed therapies [83]. An indirect mechanism of ER inhibition through AR-mediated upregulation of ERβ has been demonstrated [101]. Finally, AR contributes to the antitumor effect of aromatase inhibitors [102], supporting clinical observations that high circulating androgen and tumoral AR levels in postmenopausal women are associated with better response to aromatase inhibitors [103]. It remains to be clarified how ER and AR signal transduction cascades interact and to what extent during breast carcinogenesis. For example, there are data showing that enzalutamide can inhibit E2-induced proliferation [104]. Given this, clarifying the role of ERα/ERβ expression ratio and dimerization profile is of paramount importance.

AR expression is strongly associated with ERBB-2 overexpression in breast carcinomas. One of the mechanisms underlying this functional **signaling crosstalk** is the positive feedback loop leading to direct ERBB-2 upregulation by AR, which in turn activates AR transcription through downstream mediators (Figure 1, upper panel) [105]. AR signaling interacts with ERBB-2 signaling through the formation of ERBB-2/ERBB-3 heterodimers, leading to c-myc-mediated amplification of AR signaling [106]. Therefore, AR antagonists could be evaluated in this breast cancer subtype (Figure 3). In fact, since ERBB-2 signaling is a dominant oncogenic driver in ERBB-2 overexpressing breast tumors and ERBB-2-directed therapies are already in use, current clinical trials are now assessing the combined AR/ERBB-2 therapy (Table 1, Figure 3).

Novel Technologies for Hormone Receptor-Directed Cancer Therapeutics

Modulation of HR activity is quantitatively analyzed by assaying target gene transcription or downstream cascades with various techniques. However, these parameters are indirect and are the result of HR interaction with several TCRs. To study these interactions, methods such as intermolecular free length, yeast two-hybrid, phage display, and colocalization studies in fluorescence microscopy have been employed. One major drawback of these methods is the restriction to study a single receptor–coregulator pair at a time. New technologies, such as the MARCoNI-based coregulator binding assay can provide valuable information in AR- and ER-dependent responses induced by various drug compounds and their interactions with multiple TCRs [107]. In this technique, compounds are profiled for their ability to modulate HR activity (e.g., receptor–coregulator binding). The main advantage of this technique is that it can directly evaluate these interactions and it can be applied in minimal tissue volumes. These data can then be used to create computational models that may predict the evolution of HR–TCR association in various contexts, facilitating the optimal application of transcription factor (TF)-directed antitumor treatments [108].

Despite the fact that ERα/ERβ heterodimers in association with AR have important biological roles [4,9], there is a lack of reliable techniques to evaluate receptor dimerization in each clinical setting [e.g., early- versus late-stage cancer, breast versus prostate cancer, HR(+) versus HR(–) breast cancer]. Immunohistochemistry is insufficient to investigate ER subtypes and AR coexpression in tumor samples [109], while other techniques such as AQUA based on tissue microarray technology [110] or proximity ligation assay based on a sophisticated fluorescent *in situ* hybridization [27] offer new venues for identifying tumors coexpressing AR and ER subtypes.

Further levels of complexity of the AR–ERβ circuit that should be considered are the presence of both HRs at the plasma membrane, cytoplasm, and other organelles [7], as well as post-translational modifications that affect their genomic and non-genomic action during carcinogenesis [111]. Up to now, available methods failed to provide reliable measurements of these enzymatic activities throughout cancer development and progression [112]. New-generation techniques are being developed to illuminate these dynamic processes and understand the interactions between parallel signaling cascades as well as the feedback loops that are built over time and affect the activity of anticancer agents [113].

Breast cancer subtypes were identified using gene expression microarrays. Subsequent advances, such as next-generation sequencing and pathway signaling profiling, facilitated the integration of these data, resulting in the definition of more clinical relevant subtypes [14,114]. Typically, studies are designed to assess correlations between alterations that enhance or repress pathways that modulate one or more 'cancer hallmarks' [115,116]. However, cancer evolution is complex and tissue- and context-dependent. For example, protein function and signaling cascades are affected by many factors, such as expression, localization, affinity, dimerization, interactions, and post-translational modifications [117]. Data acquired with novel techniques are entered into bioinformatics databases and used for identification of new targets and synthetic 'molecular targeted' compounds [118]. However, the spatial and temporal plasticity of signaling events represents a major drawback, and could benefit from the creation of mathematical models that could simulate these multiple cellular events. Mathematical simulation might offer better understanding of biological processes and contribute significantly to the successful targeting of dynamic and complex cellular networks.

Concluding Remarks

In cancer development and progression, ERα has a well-established role in promoting estrogen-dependent breast tumor growth, whereas ERβ significantly attenuates cell proliferation and

Outstanding Questions

Are ERα- and AR-mediated enhanced proliferation and apoptosis inhibition, and the presence of ERβ as a tumor suppressor in hormone-dependent tissues clinically relevant?

Are the currently used techniques reliable in terms of evaluating hormone receptors and their associated networks during cancer evolution?

What is the exact role of ERβ isoforms in hormone-dependent carcinogenesis and endocrine treatment response?

In which breast cancer subtypes does the ERβ–AR molecular interplay have clinical importance?

Can the combination of ERβ modulation and AR targeting offer any clinically important results in prostate cancer patients? At which stage of the disease? Is it effective for patients with certain molecular characteristics?

Can AR targeting in breast cancer patient subgroups based only on AR expression be considered as a promising treatment strategy? What is the optimal molecular profile of a breast cancer patient for successful AR targeting?

Is the concurrent or sequential modulation of the ERβ–AR network better regarding treatment efficacy? Could breast and prostate cancers be considered the same?

What is the role of transcription coregulator complexes and post-translational modifications as far as ERβ–AR transcriptional activity is concerned?

Which is the most reliable and easily reproducible technique to study the ERβ–AR molecular interplay in real time? Is this actually feasible with tissue and/or blood samples?

Could mathematical models help to elucidate complex transcriptional networks? Is it safe to base therapeutic decisions on theoretical assumptions?

progression in a number of cancer types, including breast and prostate. The identification of ERβ isoforms generated new data regarding the role of this receptor in tumor pathogenesis, evolution, and treatment. There are also various potential mechanisms by which ERβ isoforms can modulate AR activity during carcinogenesis and this may improve treatment of prostate and breast cancers (see Outstanding Questions).

Several reports have shown that the biological functions of ERβ-specific genes are very diverse and include DNA replication, cell cycle, apoptosis and autophagy, DNA transcription, intracellular trafficking, mRNA maturation, or cell signaling. Although the clinical significance and predictive value of ERβ isoforms is still conflicting, it seems that the expression, dimerization pattern with other isoforms or with HRs, or the interaction with transcription coregulatory factors is cell- and context-dependent, making the development of compounds specific for ERβ subtype a major challenge. AR implication in prostate carcinogenesis is well characterized, while the high expression rates in breast cancer subtypes and its molecular role in breast carcinogenesis has created expectations for novel treatment options. Indeed, agents targeting AR are currently being evaluated in breast cancer patients. However, the elucidation of the AR–ERβ signaling network has provided important clues about optimal combinations of AR-directed treatment with ERβ modulators and other therapies. The rationale of targeting more than one signaling cascade in breast and prostate carcinogenesis seems promising as long as the right targets can be identified in real time and the most effective and less toxic combinations of agents are used.

With the use of new biological techniques and computer-based analytical methods, we have begun to unravel the crucial role of the androgen/estrogen receptor crosstalk in breast and prostate cancers. Furthermore, we are now beginning to assess this 'molecular communication' as a therapeutic target. Undoubtedly, future technical advances will assist in identifying the major players that participate in the complex interaction between AR- and ERβ-signaling pathways. Based on this knowledge, we may make progress in the therapeutic targeting of breast and prostate cancer patients.

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