Genome-wide crosstalk between steroid receptors in breast and prostate cancers

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ABSTRACT

Steroid receptors (SRs) constitute an important class of signal-dependent transcription factors (TFs). They regulate a variety of key biological processes and are crucial drug targets in many disease states. In particular, estrogen (ER) and androgen receptors (AR) drive the development and progression of breast and prostate cancer, respectively. Thus, they represent the main specific drug targets in these diseases. Recent evidence has suggested that the crosstalk between signal-dependent TFs is an important step in the reprogramming of chromatin sites; a signal-activated TF can expand or restrict the chromatin binding of another TF. This crosstalk can rewire gene programs and thus alter biological processes and influence the progression of disease. Lately, it has been postulated that there may be an important crosstalk between the AR and the ER with other SRs. Especially, progesterone (PR) and glucocorticoid receptor (GR) can reprogram chromatin binding of ER and gene programs in breast cancer cells. Furthermore, GR can take the place of AR in antiandrogen-resistant prostate cancer cells. Here, we review the current knowledge of the crosstalk between SRs in breast and prostate cancers. We emphasize how the activity of ER and AR on chromatin can be modulated by other SRs on a genome-wide scale. We also highlight the knowledge gaps in the
interplay of SRs and their complex interactions with other signaling pathways and suggest how to
eperimentally fill in these gaps.

INTRODUCTION

The nuclear receptor (NR) superfamily consists of 48 transcription factors (TFs), most, if not all, are
key regulators of essential biological functions, such as development, metabolism, and reproduction.
Notably, many of the NRs are associated with multiple disease states and serious illnesses
(Mangelsdorf et al. 1995, Lazar 2017, Achermann et al. 2017). The majority of the NRs have the
same domain structure with a variable N-terminal domain (NTD), a DNA-binding domain (DBD), a
hinge region, and a C-terminal ligand/hormone-binding domain (LBD). Transcriptional coregulators
often interact selectively with the LBD, depending on the conformational change which has been
induced by different ligands (Perissi & Rosenfeld 2005, Arnal et al. 2017). The DBD is the best
conserved domain of the NRs which distinguishes these receptors from other TFs (Lambert et al.
2018). NRs are associated with several human cancers. Depending on NRs’ cellular context and
composition, they can function either as oncogenes or tumor suppressors (Dhiman et al. 2018,
Holbeck et al. 2010). A subfamily of the NRs, steroid receptors (SRs) are particularly associated with
breast cancer (BCa) and prostate cancer (PCa); these are cancers whose development and growth are
initially steroid hormone-dependent (Dhiman et al. 2018, Metcalfe et al. 2017).

The family of SRs consists of estrogen receptor (ER), and 3-ketosteroid receptors (NR3Cs);
glucocorticoid (GR, NR3C1), mineralocorticoid (MR, NR3C2), progesterone (PR, NR3C3), and
androgen (AR, NR3C4) receptor (Carson-Jurica et al. 1990). ERs are encoded by two different
genes, ERα (NR3A1) and ERβ (NR3A2) (Arnal et al. 2017). Unless specifically indicated, we will
use the abbreviation ER to represent ERα from now on. In the absence of a hormonal stimulus, the
SRs usually, but not invariably, reside in the cytoplasm associated with a chaperone complex which
maintains the SR in an inactive form but still capable of binding a hormonal ligand (Arnal et al.
2017, Timmermans et al. 2019). Subsequently, after ligand binding, a conformational change occurs
in the SR, resulting in the dissociation of the chaperone complex, its translocation to the nucleus,
oligomerization, and binding to regulatory elements at enhancers on chromatin. The SRs have been
widely assumed to form homodimers, but recent evidence suggests that the SRs can form higher
oligomerization states, such as tetramers (Presman et al. 2016, Fuentes-Prior et al. 2018). On
chromatin, SRs bind often, but not always, to hormone response elements (HREs) that usually consist of an imperfect palindrome sequence of two 6 bp half-sites separated by three nucleotides. The response element for ER (ERE) is commonly 5’-AGGTCAnnnTGACCT-3’, while the corresponding response element for the 3-ketosteroid receptors (GRE, MRE, PRE, ARE) is 5’-GGTACAnnnTGTTC-3’ (Coons et al. 2017). The binding sites of SRs contain a variable number of response element sequences; these sites mostly occur distal to gene promoters (Everett & Lazar 2013). At these sites, SRs recruit a variety of other TFs and transcriptional coregulators, coactivators and corepressors, to chromatin, ultimately influencing the transcription and expression of their target genes (Perissi & Rosenfeld 2005, Lempiäinen et al. 2017, Papachristou et al. 2018). Most of the coregulators recruited by the SRs harbor histone-modifying and chromatin-remodeling activities and they are often shared between the SRs (Lempiäinen et al. 2017). Since corepressors are expressed at a large excess over coactivators (Gillespie et al. 2020), competition between coactivators, e.g. through squelching, could occur when multiple SRs are activated. Finally, since in most cases, the SR-binding sites are located outside of the target gene promoters, the SRs are thought to enable the formation of chromatin loops with the promoters (Fullwood et al. 2009, D’Ippolito et al. 2018, Zhang et al. 2019).

In hormone-dependent cancers such as BCa and PCa, ER’s growth-promoting transcriptional programs as well as those of AR are considered as the key drivers of cancer development and progression (Swinstead et al. 2018, Feng & He 2019). Thus, in the therapy of these cancers, the ER and the AR are targeted by antagonist compounds (Metcalfe et al. 2017). In addition, coregulators recruited by the ER and AR have recently emerged as potential targets for cancer therapies (Groner & Brown 2017, Wimalasena et al. 2020). The druggable coregulators include EP300 and various proteins of the bromodomain and extra-terminal (BET) family (Lasko et al. 2017, Murakami et al. 2019, Gilan et al. 2020). The concomitant targeting of the ER or the AR with a coregulator could help to resolve drug resistance occurring with single drug treatments (Metcalfe et al. 2017, Boumahdi & de Sauvage 2019, Carceles-Cordon et al. 2020). Interestingly, also other SRs have emerged as important “coregulators” for the ER and AR in cancer cells (Kach et al. 2015). Since recent work has shown that TFs can modulate chromatin binding and the activity of other TFs through multiple mechanisms, including cooperative binding, tethering and assisted loading (Long et al. 2016, Morgunova & Taipale 2017, Swinstead et al. 2018), here we reviewed the crosstalk and interplay between SRs from the view of steroid-dependency in BCa and PCa. We will focus on how signaling via ER and AR in BCa and PCa, respectively, can be altered by other SRs on a genome-wide scale.
**BREAST CANCER**

BCa is the most common cancer in women and among the leading causes of cancer deaths in both the United States and Finland (Centers for Disease Control and Prevention 2017, Finnish Cancer Registry 2018). Estrogens, principally estradiol (E2), and ER are considered as the main drivers of BCa development and progression (Swinstead et al. 2018). BCa is primarily classified by the expression of ER, PR and human epidermal growth factor 2 receptor (HER2/ERBB2), and divided into three main subtypes ER+/PR+/HER2+, ER-/PR-/HER2+ and ER-/PR-/HER2- (Waks & Winer 2019). The latter subtype is commonly indicated as triple negative BCa (TNBC) i.e. the cancer cells do not express any of the three proteins. ER+ BCa is usually treated with ER antagonists, such as tamoxifen or fulvestrant that compete with E2 for binding to the LBD (Arnal et al. 2017, Waks & Winer 2019). In addition, aromatase inhibitors (AI) can be used to block the synthesis of E2. However, resistance to AI or ER antagonist treatment can occur. The mechanisms underlying the resistance vary, including ER mutations generating ligand-independent receptor forms (Hanker et al. 2020). The TNBC is usually treated with chemotherapy, however, as highlighted in the next sections, other SRs in addition to ER and PR could be considered as potential and alternative targets of therapy. Interestingly, in ER+ BCa cells, the genome-wide binding of GR to chromatin is similar to that of ER, while AR binds in a similar manner as PR (Kittler et al. 2013). Furthermore, in male and female BCa patients, AR, PR, and GR have been shown to occupy ER-binding chromatin sites (Severson et al. 2018). Thus, it is important to understand how these SRs interact on chromatin and regulate transcription and consequently how they influence the development and progression of BCa. We will focus on the data derived from genome-wide experiments.

**Genome-wide crosstalk between the ER and the PR in BCa**

Historically, PR expression in BCa has been used as a proxy for the function of ER in the disease. *PGR* encoding PR is a well-known E2-regulated gene and its expression is thought to reflect the transcriptional activity of ER (Creighton et al. 2009, Siersbæk et al. 2018). Thus, both receptors are expressed at a similar frequency of ~50-80% in all BCa cases, although the PR is not expressed in all ER+BCa patients, the actual percentage is around 75% (McGuire et al. 1978, Swinstead et al. 2018). In addition as acting as TFs, both ER and PR have been shown to function as local and global genome organizers in their unliganded state in BCa cells (Le Dilly et al. 2018). This suggests that the expression status of the ER and PR could also influence BCa survival through the regulation of
chromosome organization. Indeed, the organization often changes during the development and progression of cancer (See et al. 2019).

Recently, PR has been suggested to play a more prominent role in BCa rather than being a mere diagnostic marker (Carroll et al. 2017). In 2015, the Carroll laboratory reported that the activation of PR by progesterone could reprogram the chromatin occupancy of ER in a BCa cell line (Mohammed et al. 2015). This reprogramming resulted in thousands of new ER-binding sites not observed in BCa cells stimulated by E2 alone. Proteomic and motif analyses suggested that this had occurred through tethering of ER to chromatin-bound PR (Figure 1A). Interestingly, the activation of PR decreased ER-driven proliferation and blocked tumor growth. This suggests that instead of being a mere marker of functional ER in BCa, the PR is a major determinant of ER-driven gene programs in BCa.

Similar, but not entirely complementary, results have been reported by the Greene laboratory (Singhal et al. 2016). They revealed that the PR can reprogram chromatin-binding of ER and act not only as a genomic estrogen agonist, but also as a phenotypic estrogen antagonist in ER+ PR+ BCa. However, the tethering of ER to the PR was not suggested as the main mechanism of interaction between the SRs on chromatin (Figure 1A). Sequential (re)ChIP-seq experiments demonstrated that only some, not all, SR-bound chromatin regions harbor both ER and PR concomitantly. Even though the ER and the PR could be found on the same chromatin fragment, this does not necessarily indicate a direct interaction (i.e. tethering) between the SRs. These results suggest that the ER-PR interplay on chromatin most likely occurs through several different modes of interaction (Swinstead et al. 2018).

Forkhead box protein A1 (FOXA1) was speculated to act as a pioneer TF, influencing the crosstalk between ER and PR (Mohammed et al. 2015). However, only ~50% of the PR-induced ER chromatin-binding sites contain a FOXA1 motif, and a knockdown of FOXA1 had only a minor impact on the PR-induced expression of the ER target genes (Mohammed et al. 2015, Singhal et al. 2016). It remained to be investigated whether activation of PR could alter the chromatin binding of FOXA1. Since ER has been reported to influence the chromatin occupancy of FOXA1 (Swinstead et al. 2016, Paakinaho et al. 2019a), it is likely that PR could act in a similar manner. As a variable number of ER-binding sites are lost after the activation of PR (Mohammed et al. 2015, Singhal et al. 2016), it is tempting to speculate that the PR could sequester FOXA1 from the ER-bound sites, thereby influencing the proliferation of BCa cells and tumor growth.

Other layers of complexity in the ER-PR interplay derive from the different isoforms of PR. The function of two alternate promoters of PGR give rise to two different receptors, PR-A and PR-B. The
PR-A lacks 164 N-terminal amino acids of the larger PR-B isoform (Cenciarini & Proietti 2019). In other respects, the PR isoforms share the same amino acid sequence. Despite the overall similarity, while PR-A and PR-B have shared functions, they also have certain unique properties. Interestingly, in the genome-wide ER-PR crosstalk, the PR-A mainly inhibited, while the PR-B reprogrammed the chromatin-binding of ER (Singhal et al. 2017). This was reflected at the level of transcription, e.g. estrogen-driven proliferation was attenuated by PR-A but augmented by PR-B (Figure 1B). In theory, this kind of isoform-specific modulation of PR activity could lead to a favorable outcome of BCa. Indeed, the PR-A- and PR-B-induced gene signatures were associated with poorer and better patient survival, respectively (Singhal et al. 2017). Although, the above investigations strongly point to tumor suppressor capabilities of PR, more recent work has suggested that both PR-A and PR-B can exert tumor promoting effects in ER+BCa (Truong et al. 2019). PR-B was revealed as a driver of BCa cell proliferation (Truong et al. 2019), which is complementary to the results reported by Singhal and coworkers (Singhal et al. 2017). Moreover, the Lange laboratory demonstrated that PR-A was a driver of cancer stem cell (CSC) expansion in BCa cells and that phosphorylation of PR-A was required for the expression of CSC-associated genes (Truong et al. 2019). Thus, the poor patient survival linked with the PR-A gene signature (Singhal et al. 2017), could derive from the expansion of BCa CSC. However, more investigations will be needed to fully appreciate the impact PR and its isoforms on BCa development and progression as well as the PR’s role as a potential drug target in BCa. Furthermore, it is largely unknown if and how ERβ and PR can influence each other’s transcriptional activity.

In addition to influencing the progression of BCa in cell and animal models, the status of PR correlates with patient survival (Table 1). The loss of PR expression in ER+ BCa decreases patient survival, whereas patients who have ER+ and PR+ BCa show increased survival compared to PR+ and ER- patients. Due to the evident importance of PR activity in BCa survival, clinical trials are being conducted to assess the influence of a PR agonist in the treatment of ER+ BCa (NCT03306472, NCT03024580).

**Genome-wide crosstalk between the ER and the AR in BCa**

Compared to our knowledge of the cross-regulatory role of PR with ER, the genome-wide interplay between AR and ER in BCa is a relatively recently recognized phenomenon. The AR is expressed in ~80% of BCa cases (McNamara et al. 2014), with a high expression of AR in ER+ BCa patients correlating with a better survival (Table 2). However, this is not the case in ER- BCa patients (Peters...
et al. 2009). AR interestingly regulates the same transcriptional programs in molecular apocrine BCa (AR+TNBC) cells as ER in luminal BCa cells (McNamara et al. 2014, Robinson et al. 2011). Thus, AR has been recognized as a valuable drug target in TNBC, as its inhibition could offer an alternative form of therapy (McNamara et al. 2014, Gerratana et al. 2018). Moreover, the AR’s value as a drug target is strengthened by the concept that the molecular apocrine BCa and castration resistant PCa (CRPC) cells share a core AR cistrome and target gene signature linked to cancer cell growth (Malinen et al. 2015). However, a recent study indicated that AR+ TNBC displayed heterogeneity in AR levels, which influenced AR-targeted therapy in combination with cell cycle inhibitors (Christenson et al. 2021). These results suggest that in the presence of the ER, the AR could suppress BCa cell growth, whereas the AR promoted it in the absence of ER.

Although a high expression level of AR has been associated with increased survival of ER+ BCa patients, the Richer group found that inhibition of AR by enzalutamide (ENZ) (a 2nd generation antiandrogen) decreased BCa cell proliferation and tumor size (D’Amato et al. 2016). Interestingly, these investigators showed that ER and AR could bind to the same genomic sites and that ENZ inhibited chromatin binding not only of the AR but also the ER (Figure 2A). These results suggest that the AR supports the chromatin-binding of ER, influencing BCa cell proliferation and tumor growth. In clinical trials, a combination of bicalutamide (1st generation antiandrogen) with AI did not however confer any clinical benefit (NCT02910050) in ER+ and AR+ BCa patients, but a clinical trial combining fulvestrant (ER degrader) and ENZ is underway (NCT02953860).

A later study suggested that a selective AR modulator (SARM)/agonist, rather than ENZ, would be capable of inhibiting ER+ BCa tumor growth (Ponnusamy et al. 2019). Intriguingly, while the SARM-bound AR reduced ER occupancy on chromatin at a subset of sites, it also redistributed ER to new genomic sites (Figure 2B). Furthermore, some of these effects were not restricted to wild-type ER, since AR was also able to inhibit the growth of BCa tumors expressing an estrogen-independent ER-Y537S mutant. In confirmation, a more recent investigation indicated a redistribution of ER occupancy and the inhibition of ER-induced BCa proliferation upon AR activation with an agonist (Hickey et al. 2021). Mechanistically, the redistribution of ER by AR was suggested to be tightly linked to a squelching of EP300 from ER- to AR-binding sites (Figure 2C). Thus, the ER and the EP300 are redistributed from loci associated with a poor patient survival to AR-regulated loci associated with a good patient survival outcome. These results suggest that in ER+ BCa, it is activation rather than inhibition of AR that should be pursued. The differences observed between
ENZ (D’Amato et al. 2016) and SARM/agonist (Ponnusamy et al. 2019, Hickey et al. 2021) could be attributed to the different models used in the studies. Furthermore, another study utilizing endocrine therapy resistant BCa models revealed that the resistance (to ER inhibition) could be reversed by knockdown of AR, but not by ENZ (Chia et al. 2019). The apparent differences in the effect of ENZ could derive from the different concentrations of ENZ used in these studies. Since not all of the above experiments have been performed in the same cellular milieu, other TFs or coregulators (with different expression levels in the models) that influence the ER-AR crosstalk could also explain the observed differences. Since the squelching of EP300 by AR from ER-binding sites in ER+ BCa appears to be the prevalent crosstalk mechanism between the SRs (Hickey et al. 2021), the EP300 might act as an important regulator of AR’s action in BCa. Indeed, the activity of AR in TNBC is sensitive to the inhibition of EP300 (Garcia-Carpizo et al. 2019) (Figure 2D). Thus, there are other coregulators, such as BET family proteins that are known to exert distinct effects on ER-regulated gene expression programs (Murakami et al. 2019), their presence could influence the crosstalk between AR and ER in ER+ BCa cells.

Genome-wide crosstalk between the ER and the GR in BCa

Corticosteroids, such as dexamethasone, are widely used in the treatment of breast cancer to alleviate the side effects of chemotherapy and to treat symptoms related to advanced cancer. The genomic crosstalk in BCa cells between the ER and the GR has been the most extensively studied of the different SR pairs. Originally, the Hager laboratory demonstrated that chromatin binding of ERpBox, a DBD ER mutant that binds to GRE instead of ERE, to a subset of chromatin sites was enabled by the GR (Voss et al. 2011). This mechanism was termed assisted loading (Figure 3A) and in this process, an initiator TF (such as GR) could bind to a closed chromatin site; this induced a remodeling of chromatin thereby assisting the binding of a second TF (such as ER). The second TF was incapable of binding to the closed site without the action of the first TF. This occurred in a symmetric and enhancer-specific manner such that the dependency of TF could be reversed, i.e. the ER could assist the binding of GR at some sites. It is notable that even though the TFs can bind to the same site, they do not compete for the binding-site due to their rapid binding kinetics on chromatin (Paakinaho et al. 2017). Since the initiator TF induced remodeling of chromatin at assisted sites, chromatin remodeler complexes were postulated to play a key role in mediating assisted loading (Swinstead et al. 2018). The assisted loading between ER and GR has been demonstrated at a genome-wide level in mouse mammary cells (Miranda et al. 2013), highlighting the importance of the crosstalk between ER and GR in human BCa.
In human BCa patients, high levels of GR are associated with an increased survival in ER+ BCa individuals, while in ER- BCa patients, the high levels are associated with a worse survival (Pan et al. 2011). This relationship is seen both in pre- and post-therapy treated patients, and the better survival of ER+ BCa patients with high GR levels occurs irrespective of PR expression level (Table 3). In ER+ BCa cells, concomitant activation of ER and GR leads to alterations in transcriptional programs that promote differentiation and decrease epithelial-mesenchymal transition (West et al. 2016), which is reflected as a large overlap between ER and GR on chromatin (Figure 3B). This is reminiscent of the assisted loading reported by the Hager laboratory (Miranda et al. 2013). There are also other similarities, as activator protein 1 (AP-1) was shown to be an important mediator of assisted loading in mouse mammary cells (Miranda et al. 2013) and AP-2 motifs are enriched at many sites showing co-occupancy of ER and GR (West et al. 2016). Thus, it seems likely that other TFs and coregulators also participate in the crosstalk between the SRs. Whether the ER and the GR physically interact on chromatin, tethering to each other, remains an open question, although the tethering is implied by the lack of a response element for one of the receptors at the co-occupying sites (West et al. 2016). In support of the tethering concept and the role of post-translational modification, small ubiquitin-related modifier (SUMO)-modified GR has been reported to specifically tether to ER, resulting in the recruitment of corepressor complexes and repression of ER-driven transcription (Figure 3C) (Yang et al. 2017). However, this mechanism was shown to operate only on a few selected loci, whereas at the genome- and proteome-wide level, SUMO modification of GR fine-tunes the chromatin occupancy and interactome of the receptor, impacting on gene expression in a target gene selective manner (Paakinaho et al. 2014, Paakinaho et al. 2021). A recent study revealed that in addition to pure agonists, selective GR modulators (SGRMs), possess the ability to antagonize transcription of canonical GR target genes and thus could inhibit estrogen-induced proliferation in ER+ BCa models (Tonsing-Carter et al. 2019). SGRMs decreased the occupancy of the ER at several enhancers and that the displacement of ER from chromatin by the liganded GR was associated with decreased expression of key genes mediating E2-driven proliferation (Tonsing-Carter et al. 2019) (Figure 3D). The SGRM-bound GR was also able to inhibit the actions of the estrogen-independent ER-Y537S mutant. These results support the concept of developing selective SR modulators for the treatment for endocrine therapy-resistant ER+ BCa.

As indicated earlier, high levels of GR in ER- BCa are associated with a poor patient survival (Table 3). Further analyses of patient data indicated that a similar association was evident with different subtypes of TNBC; basal-like 1, mesenchymal, and luminal AR (West et al. 2018). In TNBC cells,
GR induces the genes related to cell survival and suppresses those related to cell death. Interestingly, this GR-mediated regulation can be reversed by using RU486 (mifepristone), a steroidal antagonist that also influences chromatin binding of the GR (Figure 3F); in fact, RU486 is currently being tested in clinical trials for GR+ TNBC (NCT02788981). In addition to RU486, a non-steroidal SGRM, Compound A (CpdA), was similarly demonstrated to reduce GR-mediated regulation of pro-tumorigenic genes (Chen et al. 2015).

Complications of the ER-GR crosstalk with other signaling pathways in BCa

The above investigations highlight the apparent overall benefit of the GR-ER crosstalk in the suppression of BCa cell growth. However, new cautionary and complicating results are emerging, not from the direct crosstalk between the SRs, but from the effects of SRs on other signaling pathways and TFs. For example, in conjunction with the ER, the proinflammatory TF nuclear factor-κB (NF-κB) regulates a subset of cell growth-related genes, resulting in a restriction and reduction of BCa cell proliferation (Franco et al. 2015). The GR is a well-known suppressor of NF-κB activity in immune cells (Syed et al. 2020). More recently, the GR has been demonstrated to suppress the actions of NF-κB also in long-term estrogen-deprived BCa cells (Fan et al. 2019), leading to an inhibition of TNF-α production and a complete blockade of E2-induced apoptosis and the consequent survival of cancer cells (Figure 3E). Thus, glucocorticoids should be used cautiously in patients who have been extensive treated with AI. This caution is supported by recent observations showing that the activation of GR enhances the ability of TNBC to metastasize (Obradović et al. 2019). GR and ER also undergo a crosstalk with a TF signal transducer and activator of transcription 3 (STAT3) that becomes activated after phosphorylation in response to interferons, epidermal growth factor (EGF), interleukin (IL-)5 and IL-6. For example, in basal-like TNBC cells, GR operates with STAT3 in a genome-wide manner to drive BCa growth (Conway et al. 2020). Since the activation of STAT3 reprograms binding of ER on enhancers and induces metastasis of ER+ BCa (Siersbæk et al. 2020), it could act as a central TF, defining the tumor inducing or repressing role of the SR. The GR can also have an important ligand-independent activity in TNBC. The Lange laboratory has recently shown that transforming growth factor β1 (TGFβ1) could increase the phosphorylation of GR at S134 (Perez Kerkvliet et al. 2020), which induced the transcriptional activation of the GR in a ligand-independent manner, driving migration and anchorage-independent growth of TNBC cells. Interestingly, at least the latter effect can be inhibited by RU486.
Taken together, these results indicate that GR plays different roles in BCa cells depending on the presence of the ER as well as the activity of other signaling pathway-regulated TFs. The ER-GR crosstalk operates through several different modes in different BCa cell types and disease stages. Finally, the ER-GR crosstalk and the importance of SR expression levels for cancer survival are not restricted to BCa, as similar, though not identical, mechanisms of crosstalk have been observed between the ER and the GR in endometrial cancer (Vahrenkamp et al. 2018).

PROSTATE CANCER

PCa is the most common cancer in men and among the leading causes of cancer deaths in United States and Finland (Centers for Disease Control and Prevention 2017, Finnish Cancer Registry 2018). The primary cancer is almost always dependent on the AR signaling (Wang et al. 2018a). Nonetheless, PCa patients with localized tumor are usually initially treated with radiotherapy and/or surgery or mere active surveillance, depending on the evaluation of the risk level of the disease. For advanced disease, androgen deprivation therapy (ADT) has for a long time been the golden-standard treatment (Wang et al. 2018a, Swami et al. 2020). Although, a recent clinical trial has indicated that chemotherapy with ADT is more efficient primary treatment than ADT alone, when there is a high metastatic burden (Sweeney et al. 2015). After the relapse of the primary ADT, second-line androgen deprivation or chemotherapy is typically administrated. ADT is based on either surgical or chemical castration or antiandrogens to prevent the production or action of androgens and thereby the growth of PCa cells. In antiandrogen therapy, AR action is blocked with an antagonist, such as bicalutamide, ENZ, apalutamide or darolutamide; these are compounds that compete with androgens for binding to the AR (Wang et al. 2018a, Feng & He 2019, Swami et al. 2020). After the initial ADT, CRPC can occur such that AR signaling is restored through variable mechanisms. This state can be treated with additional ADT. The synthesis of androgens can be blocked with abiraterone, a drug that inhibits the CYP17A1 enzyme, which catalyzes a critical step in the synthesis of androgen. Coadministration of a glucocorticoid is required to compensate for the abiraterone-induced reduction in serum cortisol and to block the compensatory increase in adrenocorticotropic hormone (ACTH). However, further resistance, such as the development of neuroendocrine PCa, which is unresponsive to further ADT, can take place (Ku et al. 2019, Carceles-Cordon et al. 2020). In addition, other SRs, such as GR, can contribute to the therapy resistance (Narayanan et al. 2015), emphasizing the importance of investigating how SRs interact on chromatin and together influence the development and progression of PCa. Surprisingly, there is a real scarcity of information on how ER and PR can influence AR
signaling on a genome-wide scale in PCa. In the case of ER, the focus of the investigations has been on the differential role of ERα and ERβ in PCa. While early analyses suggested that ERα, but not ERβ, was expressed at various stages of PCa (Bonkhoff et al. 1999), more recently, both ER forms have been implicated in PCa development and tumor progression (Bonkhoff & Berges 2009). Furthermore, many PCa datasets display an increased level of ERα in more advanced cancers in comparison to less advanced cancers or benign prostate tissue (Chakravarty et al. 2014). On the other hand, variable levels of PR transcripts have been measured in hormone-refractory tumors (Latil et al. 2001). PR-B, but not PR-A, has been reported to be an independent predictor of PCa recurrence (Grindstad et al. 2018). Since genome-wide level information of the crosstalk between ER and PR with AR in PCa is lacking, we will focus on the interplay between AR and GR, which has been examined in PCa cells in an unbiased genome-wide fashion.

**Genome-wide crosstalk between the AR and the GR in PCa**

The potential role of glucocorticoids, but not that of GR, PCa was discovered in the early 2000s, when an AR mutant from a patient was shown to be activated by glucocorticoids (Zhao et al. 2000, Chang et al. 2001). Similar promiscuous LBD mutations, L702H and T878A (L701H and T877A in early release of human genome builds), were found in PCa patients and commonly used PCa cell lines (Veldscholte et al. 1990, Robinson et al. 2015). In addition to natural and synthetic glucocorticoids (Chang et al. 2001), an AR with the T878A mutation was found to be activated by E2 and progesterone (Zhao et al. 2000), and PCa cells expressing one of the mutant ARs could obtain a growth advantage after cortisol exposure (Krishnan et al. 2002).

After the discovery of glucocorticoid-activated AR mutations, the GR itself was found to possess tumor suppressor activity in PCa cells (Yemelyanov et al. 2007). Glucocorticoids inhibited the growth of PCa cells expressing both GR and wild type AR. Interestingly, the expression levels of GR were either decreased or absent in 70-85% of PCa patients compared to those with a benign form of the disease. In a follow-up study, CpdA was found to inhibit the growth of both AR- and GR-expressing PCa cells (Yemelyanov et al. 2008). In both studies, the restriction of cell growth was attributed to GR-mediated inhibition of MAPK signaling and decreased expression of AP-1 and NF-κB. It has been suggested that GR can repress the activity of these TFs, both indirectly by inhibiting the MAPK signaling and directly through CpdA-induced transrepression. Interestingly, GRβ, an alternative splicing isoform of GR that does not bind glucocorticoids (Timmermans et al. 2019) possessed the ability to modify the growth of PCa cells, as its depletion decreased PCa cell...
proliferation (Ligr et al. 2012), suggesting that the GRβ could modulate the tumor suppressor capability of the full-length GR in PCa.

The initial crosstalk between AR and GR in PCa was observed in their similar response to chromatin binding after FOXA1 depletion from PCa cells (Sahu et al. 2011). Depletion of FOXA1 resulted in a reprogramming of the AR and GR chromatin occupancy; some binding sites were unchanged or lost and more sites were gained. These gained and lost chromatin-binding sites were also reflected in the capability of the SRs to regulate transcription, indicating that the AR and the GR behave similarly with the FOXA1 on chromatin in PCa cells. Indeed, there is a large overlap between the SR-binding sites in PCa cells, and FOXA1 has been postulated to specify unique binding sites of AR and GR, depending on the PCa cell line (Sahu et al. 2013). Conversely, activation of AR can redistribute FOXA1 on chromatin (Paakinaho et al. 2019a). The importance of FOXA1 for PCa biology is also supported by the findings that the expression level and mutations of FOXA1 substantially influence PCa progression (Sahu et al. 2011, Adams et al. 2019, Parolia et al. 2019).

A major breakthrough in clarifying the crosstalk between the AR and the GR came from the Sawyers group (Arora et al. 2013). These investigators discovered that resistance to antiandrogens in PCa can occur through the replacement of AR by the GR. They postulated that the AR represses the GR gene (NR3C1) (Figure 4A), which is alleviated upon long-term ENZ treatment, resulting in an enhanced expression of GR and a substitution of the AR by the GR in transcriptional regulation (Figure 4B). Interestingly this replacement occurs at only around half, not all, of the AR-bound chromatin sites. Furthermore, the FOXA1 motif is enriched at the GR-replaced AR-binding sites, suggesting that it plays a role in the GR-mediated resistance to ENZ in PCa cells. In support of this concept, inhibition of GR was found to rescue ENZ sensitivity to prevent tumor growth (Arora et al. 2013), and furthermore a depletion of GR significantly decreased the initiation and progression of resistant PCa tumors (Ishikbay et al. 2014). One particular GR target gene, serum and glucocorticoid-regulated kinase 1 (SGK1), has been shown to have a prominent role in ENZ resistance (Ishikbay et al. 2014); inhibition of SGK1 decreased PCa cell viability, while its overexpression increased tumor initiation. Thus, GR and its target gene products play an important role in antiandrogen resistance.

Subsequently, the importance of GR has been confirmed in PCa patient material. While the levels of GR initially rise in PCa during ADT, the levels can decrease to pre-castration levels due to restored AR signaling in castration resistant PCa (Xie et al. 2015). In primary PCa, the expression of GR is reduced, but it is restored in PCa metastases, with ENZ-treated patients showing a higher GR
expression than therapy naïve patients (Shah et al. 2017, Puhr et al. 2018). These results are thus complementary to data from preclinical models, showing significantly increased levels of GR upon long-term abiraterone or ENZ treatment (Arora et al. 2013, Puhr et al. 2018). As an outcome of GR expression, patients with a high expression of GR have a poor outcome to ENZ treatment, and high levels of GR are associated with a poor survival (Arora et al. 2013, Puhr et al. 2018). Regardless of the expression levels, the GR is postulated to be a crucial player in both antiandrogen resistant and therapy naïve PCa (Puhr et al. 2018). In addition to the GR, the Sharifi laboratory has indicated that glucocorticoid metabolism plays an additional key role in the maintenance of ENZ resistance (Li et al. 2017). The formation of the bioactive glucocorticoid, cortisol, is regulated by the 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) and 2 (11β-HSD2) (Timmermans et al. 2019). The 11β-HSD2 enzyme converts cortisol into the biologically inactive cortisone that can be converted back to cortisol by the 11β-HSD1 (Figure 4C). Interestingly, PCa cell models and patients treated with ENZ display decreased levels of 11β-HSD2 through autocrine motility factor receptor (AMFR) ubiquitin E3 ligase-mediated degradation (Li et al. 2017). The decrease in 11β-HSD2 elevates the level of cortisol (Figure 4D), which together with the increased amount of GR, leads to a systemic activation of glucocorticoid signaling and antiandrogen resistance. Furthermore, this is not restricted to tumor tissue, as patients treated with ENZ show a systemic rise of cortisol levels (Alyamani et al. 2020).

As indicated above, the AR signaling is an important regulator of GR expression in the prostate. Indeed, most PCa patients and PCa cell lines display high levels of either AR or GR. This suggests that there is an inverse correlation in the expression between the two SRs (Xie et al. 2015). This inverse correlation can be explained by a direct repression of the GR gene (NR3C1) by the AR, via an intronic prostate-specific enhancer in the NR3C1 (Arora et al. 2013). Subsequent studies have revealed that the enhancer is repressed through an AR-induced and EZH2-mediated mechanism that is lost in ENZ resistant PCa cells (Shah et al. 2017). The repression can be restored by a BET family inhibitor, JQ1. In addition, GATA-binding factor 2 (GATA2), mediator complex (Yuan et al. 2019) and corepressor transducin-like enhancer protein 3 (TLE3) (Palit et al. 2019) have been shown influence the AR-mediated repression of NR3C1. The functionality of the enhancer was proven by its CRISPR-Cas9-mediated mutation (Shah et al. 2017, Yuan et al. 2019).

The “intronic enhancer” (Arora et al. 2013) that lacks AREs and only weakly binds AR is surprisingly located in the promoter region of NR3C1, very close to the initiation codon ATG (Figure
In contrast, another study suggested that the enhancer-mediating the repressive effect of AR on the expression of \textit{NR3C1} would be located far more upstream from the gene promoter (Xie \textit{et al.} 2015). This upstream enhancer contains an ARE sequence and shows prominent binding of AR (Figure 5A). Thus, the decrease of GR levels after CRISPR-Cas9-facilitated deletion of the putative “intrinsic enhancer” (Shah \textit{et al.} 2017, Yuan \textit{et al.} 2019); this may be due to the disruption of the promoter function rather than that of its interaction with the AR with it. However, it remains to be investigated how disruption of the upstream enhancer would influence the expression of \textit{NR3C1}. Since both putative enhancers reside within the same topological associating domain (Figure 5B) (ENCODE Project Consortium 2012, Wang \textit{et al.} 2018b), the upstream enhancer could in fact loop to the promoter.

In addition to GR, AR splice variants are well known drivers of antiandrogen resistance in PCa (Blatt \textit{et al.} 2021). The splice variants, such as AR-V7, lack an LBD; these receptors are constitutively active and do respond to ENZ treatment. Interestingly, ENZ- and abiraterone-resistant PCa patients show a negative correlation between AR-V7 and GR expression (Shah \textit{et al.} 2017). Thus, AR-V7 and the replacement of the AR by the GR might represent a mutually exclusive mechanism of antiandrogen resistance. However, both AR-V7 and GR display high expression levels in some individual patients (Shah \textit{et al.} 2017). This suggests that antiandrogen resistance could be derived simultaneously or sequentially from both AR-V7 and GR. Nevertheless, larger patient cohorts will need to be analyzed to estimate the relative contribution of AR-V7 vs. that of GR to the antiandrogen resistance. Compared to PCa, the occurrence and the role of ER splice variants are relatively unknown in therapy naïve or endocrine resistant BCa (Blatt \textit{et al.} 2021). However, ER gene fusion with other proteins, such as YAP1, has been observed to occur in BCa. These types of SR fusions have not been demonstrated for the AR in PCa. However, the ER gene fusions do not seem to be involved in the crosstalk between SRs in BCa.

Overall, the above data indicate that glucocorticoids should be used with caution, especially in patients undergoing ENZ therapy. Synthetic glucocorticoids, such as prednisolone, are widely used to alleviate therapy-related side-effects, to reduce inflammation and to counteract the decrease in cortisol levels due to abiraterone treatment (Narayanan \textit{et al.} 2015). Thus, alternative ways of modulating GR activity in PCa have been under investigation. Interestingly, an SGRM has been reported to decrease the GR-mediated PCa cell proliferation and CRPC tumor growth and viability without inhibiting the activity of the AR (Kach \textit{et al.} 2017). Therefore, two different SGRMs
combination with ENZ are currently being evaluated in clinical trials to treat metastatic PCa (NCT03437941, NCT03674814). Even though RU486 can bind to AR and PR in addition to GR (Kach et al. 2017), a combination therapy with RU486 and ENZ is under evaluation in clinical trials in hormone resistant PCa (NCT02012296). Based on the results from the preclinical models, targeting of the DNA binding half-site sequence of ARE/GRE by a pyrrole-imidazole polyamide instead of the GR, may emerge as an alternative approach to block the actions of both the AR and GR (Kurmis et al. 2017). Taken together, these results support the importance of augmented GR signaling as a key player in antiandrogen resistance of PCa, warranting further research on developing novel approaches to target the signaling in PCa tissue without compromising the beneficial effects of glucocorticoids at a system-wide level.

DIFFERENTIAL ROLE OF THE GR IN THE STEROID RECEPTOR CROSSTALK IN BREAST AND PROSTATE CANCERS

The AR and the ER play analogous roles in PCa and BCa, respectively. Both SRs are frequently mutated or alternatively spliced in response to endocrine therapy (suppression of hormone availability or receptor activity), resulting in a resistant disease (Metcalfe et al. 2017). Moreover, the activity of both receptors can be similarly reprogrammed during the progression of these cancers. For example, the chromatin-binding of the AR and ER can be pioneered by FOXA1 (Sahu et al. 2011, Hurtado et al. 2011), mutations of FOXA1 impact on the activity of both the AR and the ER (Arruabarrena-Aristorena et al. 2020, Adams et al. 2019, Parolia et al. 2019), and both SRs also affect the chromatin binding of FOXA1 (Paakinaho et al. 2019a). However, the characteristics of the GR-ER crosstalk in BCa and those of the GR-AR crosstalk in PCa differ from each other. In BCa cell models, it is well established that the activation of GR can expand the binding of ER to chromatin and vice versa (Miranda et al. 2013, West et al. 2016), mainly through an assisted loading mechanism (Swinstead et al. 2018). This has not been explicitly demonstrated for the GR and the AR in PCa. The AR and the GR share binding sites in PCa cells, and at some chromatin sites, GR binding is enhanced upon activation of both SRs, which is reminiscent of assisted loading (Sahu et al. 2013). However, AR binding seemed unchanged upon GR activation. Thus, while the crosstalk between the GR and the ER in BCa is symmetric, that between the GR and the AR in PCa appears to be asymmetric. Even though coactivation of ER and GR in BCa leads mainly to a synergistic transcription and expression of ER target genes (West et al. 2016), the expression of some ER target genes was restricted upon activation of GR. In contrast, PCa cell models showed no clear synergy...
between the GR and the AR in target gene regulation (Sahu et al. 2013). At some selected target
genes, it seemed that the expression of AR-regulated genes was restricted by GR, while that of GR-
regulated genes was conversely enhanced by AR. Some of the differences between the GR-ER and
GR-AR crosstalk may however derive from cell models: MCF-7 cells that endogenously express
both ER and GR were used as the model for BCa experiments (West et al. 2016), but most PCa
experiments were performed in LNCaP-1F5 cells expressing endogenous AR and rat GR from an
engineered, integrated gene (Sahu et al. 2013). Thus, it is currently unclear how the crosstalk
between GR and AR in PCa occurs in a more natural setting of PCa cells. Since the expression of AR
and that of GR are usually inversely correlated, there are only a few native PCa cells, such as VCaP
and CWR22RV1 cells that endogenously express both GR and AR (Puhr et al. 2018). For example,
VCaP cells were used to show that GR and AR chromatin-binding sites overlap (Sahu et al. 2013),
but it remains to be determined how the SRs influence each other’s chromatin binding.

Furthermore, in the case of the crosstalk linked to gene repression, the AR and the ER operate
differentially with the GR. In BCa, the activation of both GR and ER can lead to a redistribution of
ER from some sites to new sites that cannot be occupied by ER without GR activation (Tonsing-
Carter et al. 2019). Nonetheless, it is not known how or even if ER represses GR activity in BCa.
However, the repression of GR-regulated genes by ER has been observed (Miranda et al. 2013, West
et al. 2016, Yang et al. 2017). Thus, it does seem that at least GR can actively repress the activity of
ER. As indicated earlier, AR does not repress the activity of GR, but the expression of NR3C1 was
blocked in PCa models (Shah et al. 2017). Moreover the expression of GR is enhanced upon
inhibition of AR by ENZ, leading to replacement of the AR in transcriptional regulation (Arora et al.
2013). A similar mechanism of therapy resistance where the GR substitutes for the ER in
transcriptional regulation has not been observed in endocrine-resistant BCa. As indicated earlier, no
mechanism has been elucidated for GR-mediated suppression of AR target genes, (Sahu et al. 2013).
The suppression could be due to GR-mediated repression of TFs, such as AP-1 and ETS factors
(Yemelyanov et al. 2007), especially since the latter factors are known collaborating TFs of the AR
in PCa (Zhang et al. 2019, Yu et al. 2010). Thus, the GR could indirectly restrict the action of AR.

Finally and interestingly, the activity of GR is rather similar in TNBC models and ENZ-resistant PCa
models where the GR drives the progression of the disease (West et al. 2018, Arora et al. 2013).
Since the GR acts similarly in BCa and PCa in the absence of ER or AR, respectively, this is
convincing evidence that the differential crosstalk of GR with the AR and the ER stems from the
inherent activity of the ER and AR within the cells. Thus, while the activity of GR is similar in BCa and PCa cells, the crosstalk of GR with ER and AR differs as a result of their differential chromatin binding and recruitment of interaction partners (TFs and coregulators). Indeed, even though FOXA1 plays a similar role with the SRs, AR has a more pronounced effect on the chromatin binding of FOXA1 in PCa cells than ER in BCa cells (Paakinaho et al. 2019a). Moreover, depletion of FOXA1 generally decreases the binding of ER to chromatin in BCa cells (Hurtado et al. 2011), but in PCa cells, the occupancy of the majority of AR binding sites remains unchanged and new binding sites are generated (Sahu et al. 2011). This highlights the importance of investigating how other TFs and coregulators impact on the crosstalk between different SRs.

As indicated earlier, glucocorticoids are widely used in the treatment of PCa and BCa patients e.g. to alleviate the unwanted effects of chemotherapy and to reduce inflammation. Even though GR has tumor promoting effects in TNBC and in ENZ-resistant PCa, the overall clinical benefit of glucocorticoids outweighs their disadvantages. Thus, a more viable option to treat TNBC or GR-induced ENZ-resistant PCa would be to inhibit the action of the GR through other signaling pathways, while retaining the beneficial systemic effect of glucocorticoids. In ENZ-resistant PCa, these kinds of pathways could be BET (Shah et al. 2017) or PI3K/AKT (Adelaiye-Ogala et al. 2020), whereas in TNBC, STAT3 (Conway et al. 2020) or p38 MAPK (Perez Kerkvliet et al. 2020) could be targeted. Future investigations will determine if sufficient attenuation of GR signaling can be obtained through these pathways or if other yet to be discovered pathways will be more effective.

FUTURE PERSPECTIVES

SRs are important transcriptional regulators in many different cancers (Dhiman et al. 2018), and the revelation of the crosstalk between the SRs opens a new avenue of targeting these diseases (De Bosscher et al. 2020). Overall, we propose that the physiological effects of steroid hormones in tissues are dictated not by cognate hormone-SR pairs but instead by the crosstalk between SRs and their interaction with different transcriptional complexes. All transcriptional programs in steroid target tissues and steroid-dependent diseases are likely to be governed by the crosstalk between the SRs. This crosstalk is naturally dependent on the concentrations and types of ligands present at any given time. The development of novel, more targeted therapies with fewer side effects will have to be based on a better understanding of the molecular mechanisms of this crosstalk. The crosstalk between different pairs of SRs is thus an important aspect that should be considered in the treatment of steroid hormone-regulated cancers. In particular, the crosstalk of the GR with the ER in BCa, and that with
the AR in PCa will be particularly critical for the development of therapies in the future (Kach et al. 2015). Since these SRs can reprogram each other’s chromatin binding (Miranda et al. 2013) as well as binding of other cancer relevant TFs, such as FOXA1 (Swinstead et al. 2016, Paakinaho et al. 2019a), the regulation of the chromatin landscape of hormone-dependent cancers warrants a thorough scrutiny in future investigations. Many of the chromatin remodeling complexes have become mutated, deleted, or dysregulated in cancers (Valencia & Kadoch 2019), including BCa (Nagarajan et al. 2020) and PCa (Sandoval et al. 2018), potentially influencing the chromatin binding of SRs and the capability of SRs to reprogram the chromatin occupancy of other TFs. Indeed, the loss of the SWI/SNF subunit ARID1A influences ER activity in BCa by altering the BET family activity at the receptor bound enhancers (Nagarajan et al. 2020). Furthermore, the loss of CHD1 contributes to the antiandrogen resistance in PCa, including GR-driven processes (Zhang et al. 2020). Thus, improved knowledge of the remodelers and the impact of their dysregulation on SR crosstalk should yield important information on how to improve targeting of the SRs in BCa and PCa.

One aspect that has been rather neglected in the SR crosstalk is the effect of steroid hormone abundance and the contribution of precursor hormones to the crosstalk. Many, if not all, investigations into SR crosstalk have been performed with saturating hormone concentrations. It is not known how physiological levels of steroid hormones and overall steroid hormone exposure influence the SR crosstalk. Studies addressing these questions are crucially important, since also the metabolism of steroid hormones is altered in BCa and PCa (Capper et al. 2016). Treatment of PCa tumors with ADT or abiraterone leads to reduced levels of androgens (Knuutila et al. 2019), although in CRPC, the malignancy can maintain its growth by intratumoral production of androgens (Montgomery et al. 2008, Narayanan et al. 2015). The inhibition of steroid hormone metabolism can also lead to elevated levels of adrenal androgen precursors which in turn may activate promiscuously mutated AR (Chang et al. 2001). Furthermore, many components of the steroidogenesis pathway are dysregulated in PCa, leading to a sustained production of androgens despite ADT (Narayanan et al. 2015). As well as the tumor, the PCa stroma can increase its androgen metabolism through TGFβ1 produced in the tumor’s microenvironment (Piao et al. 2013). Interestingly, while the expression of GR is reduced in PCa as compared to benign tissue, the PCa stroma appears to maintain its GR expression levels (Mohler et al. 1996). Thus, the GR could have wider effects on PCa through the stroma. The changes in steroid hormone metabolism in BCa are less well-known. The occurrence of different steroids in breast cancer has been reviewed (Africander and Storbeck 2018), but little is known about the changes occurring in steroid hormone metabolism after treatment with ER
antagonists or AI. The abundance of steroid hormones is likely to affect SR crosstalk (Reddy et al. 2012). Furthermore, many steroid hormones are secreted in cycles, e.g. an ultradian rhythm for cortisol and the menstrual cycle for estrogen and progesterone. Thus, the hormone concentrations may have a varying effect, depending on the moment of time and therapies used for cancer treatments.

In addition to chromatin, the interactomes of SRs could help to find novel mechanisms and subsequent drug targets influencing the crosstalk. Several chromatin proteomics methods have been developed, including ChIP-SICAP (Rafiee et al. 2016) and qPLEX-RIME (Papachristou et al. 2018). These methods have not yet been utilized in studies addressing the SR crosstalk, although the GR and the AR have been shown to possess a highly overlapping set of interacting chromatin proteins (Lempiäinen et al. 2017). Furthermore an agonist-induced post-translational modification has been shown to regulate the interaction of GR with coregulators on chromatin (Paakinaho et al. 2021).

Finally, oligomerization of the receptor should also be considered as a potential step in the regulation of the SR crosstalk. Many SRs form higher structures than dimer oligomers upon DNA binding (Presman et al. 2016). In the case of GR, it has been demonstrated the receptor’s transcriptional activity is increased if the receptor is in a higher oligomerization state prior to chromatin binding (Paakinaho et al. 2019b). It was also postulated that SRs could form atypical oligomers with each other (Jiménez-Panizo et al. 2019, De Bosscher et al. 2020), which would indeed influence the SR crosstalk. Since oligomerization of TFs has been thought to activate EP300 (Ortega et al. 2018) and inhibition of EP300 can restrain the crosstalk and assisted loading between non-SR TFs (Goldstein et al. 2017), receptor oligomerization to an atypical or higher state, via EP300 could potentially influence the crosstalk of SRs. The role of coregulators, such as EP300, could be investigated with the proteomic approaches described above.

The genome-wide crosstalk between non-SR TFs is thought to operate like the crosstalk between SRs. For example, the crosstalk between STAT3 and NF-κB during the acute phase response in hepatocytes (Goldstein et al. 2017) resembles that between ER and GR in BCa cells (Miranda et al. 2013), i.e. the crosstalk occurs at some but not all TF-binding sites. The activity of SR in BCa and PCa can be modulated via other signal-activated TFs, such as STAT3 and NF-κB. The activation of STAT3 in BCa cells expands the chromatin-binding of ER, driving the metastasis of the disease (Siersbæk et al. 2020). Similarly, the crosstalk between GR and STAT3 can impact on cell growth of basal-like TNBC (Conway et al. 2020). In the case of NF-κB, the chromatin-binding of ER in BCa
(Franco et al. 2015) and that of AR in PCa cells (Malinen et al. 2016) are expanded by TNF-α. In both cancers, NF-κB most likely influences the outcome of the disease. Since in addition to coregulators, ChIP-SICAP (Rafiiee et al. 2016) and qPLEX-RIME (Papachristou et al. 2018) can capture the TFs interacting with the SRs, currently unknown partners mediating the crosstalk could be discovered in the future by exploiting proteomic techniques.

As indicated earlier, for some of the SRs, there is little to no genome-wide information on how they influence cancer progression and development or how they influence each other’s chromatin binding. Whether estrogen and progesterone signaling can influence PCa cells and how the ER and PRs interact with the AR are also rather underexplored areas. The MR has been the least extensively studied SR in BCa and PCa. However, some investigators have explored the MR’s potential role in both cancers. Both glucocorticoids and mineralocorticoids, together with PR, can decrease BCa cell proliferation (Leo et al. 2004), and a high cytoplasmic expression of MR has been associated with a poor survival of ER+/PR+/HER2- BCa patients (Jääskeläinen et al. 2019). In PCa, abiraterone treatment can result in an excess of aldosterone and its precursors, which increases the risk for hypertension and cardiovascular diseases (Pia et al. 2013, Narayanan et al. 2015). This can be bypassed by administering MR antagonists or suppressing ACTH production with glucocorticoids. Interestingly, the MR has been claimed to have a potential role in ENZ resistance in PCa (Shiota et al. 2018).

In conclusion, there are many aspects of SR crosstalk that still need to be resolved. For example, only the influence of the relatively unknown SRs, but also how oligomerization, the chromatin landscape, and other TFs and coregulators influence the crosstalk between the receptors are relatively unexplored areas. In many cases, a combined therapy to target multiple pathways and factors could well be beneficial in overcoming therapeutic resistance in these hormonal cancers (Boumahdi & de Sauvage 2019, Carceles-Cordon et al. 2020). It should be emphasized that the mechanical aspects of SR crosstalk indicated above need to be supported by data from clinical samples. Indeed, there are several ongoing clinical trials testing the importance of the SR crosstalk in patients (NCT03306472, NCT03024580, NCT02953860, NCT02788981, NCT03437941, NCT03674814, NCT02012296), and TF interactomes have been analyzed from patient samples (Siersbæk et al. 2020). Moreover, single cell transcriptomics (scRNA-seq), chromatin accessibility (scATAC-seq) and spatial transcriptomic analyses will soon be able to reveal the level of cellular heterogeneity in the crosstalk, initially in pre-clinical models and subsequently in clinical cancer samples.
Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

V.P. and J.J.P. wrote the paper.

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References

Achermann JC, Schwabe J, Fairall L, Chatterjee K 2017 Genetic disorders of nuclear receptors. *J Clin Invest* 127 1181-1192.

Adams EJ, Karthaus WR, Hoover E, Liu D, Gruet A, Zhang Z, Cho H, DiLoreto R, Chhangawala S, Liu Y, *et al.* 2019 FOXA1 mutations alter pioneering activity, differentiation and prostate cancer phenotypes. *Nature* 571 408-412.

Africander D, Storbeck KH 2018 Steroid metabolism in breast cancer: Where are we and what are we missing? *Mol Cell Endocrinol* 466 86-97.

Alyamani M, Li J, Patel M, Taylor S, Nakamura F, Berk M, Przybycin C, Posadas EM, Madan RA, Gulley JL, *et al.* 2020 Deep androgen receptor suppression in prostate cancer exploits sexually dimorphic renal expression for systemic glucocorticoid exposure. *Ann Oncol* 31 369-376.

Arnal JF, Lenfant F, Metivier R, Flouriot G, Henrion D, Adlanmerini M, Fontaine C, Gourdy P, Chambon P, Katzenellenbogen B, *et al.* 2017 Membrane and Nuclear Estrogen Receptor Alpha Actions: From Tissue Specificity to Medical Implications. *Physiol Rev* 97 1045-1087.

Arora VK, Schenkein E, Murali R, Subudhi SK, Wongvipat J, Balbas MD, Shah N, Cai L, Efstatiou E, Logothetis C, *et al.* 2013 Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. *Cell* 155 1309-1322.
Arruabarrena-Aristorena A, Maag JLV, Kittane S, Cai Y, Karthaus WR, Ladewig E, Park J, Kannan S, Ferrando L, Cocco E, et al. 2020 FOXA1 Mutations Reveal Distinct Chromatin Profiles and Influence Therapeutic Response in Breast Cancer. Cancer Cell 38 534-550.e9.

Blatt EB, Kopplin N, Kumar S, Mu P, Conzen SD, Raj GV 2021 Overcoming oncogene addiction in breast and prostate cancers: a comparative mechanistic overview. Endocr Relat Cancer 28 R31-R46.

Bonkhoff H, Fixemer T, Hunsicker I, Remberger K 1999 Estrogen receptor expression in prostate cancer and premalignant prostatic lesions. Am J Pathol 155 641-647.

Bonkhoff H, Berges R 2009 The evolving role of oestrogens and their receptors in the development and progression of prostate cancer. Eur Urol 55 533-542

Boumahdi S, de Sauvage FJ 2020 The great escape: tumour cell plasticity in resistance to targeted therapy. Nat Rev Drug Discov 19 39-56.

Capper CP, Rae JM, Auchus RJ 2016 The Metabolism, Analysis, and Targeting of Steroid Hormones in Breast and Prostate Cancer. Horm Cancer 7 149-64.

Carceles-Cordon M, Kelly WK, Gomella L, Knudsen KE, Rodriguez-Bravo V, Domingo-Domenech J 2020 Cellular rewiring in lethal prostate cancer: the architect of drug resistance. Nat Rev Urol 17 292-307.

Carroll JS, Hickey TE, Tarulli GA, Williams M, Tilley WD 2017 Deciphering the divergent roles of progestogens in breast cancer. Nat Rev Cancer 17 54-64.

Carson-Jurica MA, Schrader WT, O'Malley BW 1990 Steroid receptor family: structure and functions. Endocr Rev 11 201-220.

Cenciarini ME, Proietti CJ 2019 Molecular mechanisms underlying progesterone receptor action in breast cancer: Insights into cell proliferation and stem cell regulation. Steroids 152 108503.

Centers for Disease Control and Prevention 2017 https://gis.cdc.gov/Cancer/USCS/DataViz.html [Accessed April 26, 2021].
Chakravarty D, Sboner A, Nair SS, Giannopoulou E, Li R, Hennig S, Mosquera JM, Pauwels J, Park K, Kossai M, et al. 2014 The oestrogen receptor alpha-regulated lncRNA NEAT1 is a critical modulator of prostate cancer. *Nat Commun* 5 5383.

Chang CY, Walther PJ, McDonnell DP 2001 Glucocorticoids manifest androgenic activity in a cell line derived from a metastatic prostate cancer. *Cancer Res* 61 8712-8717.

Chen Z, Lan X, Wu D, Sunkel B, Ye Z, Huang J, Liu Z, Clinton SK, Jin VX, Wang Q 2015 Ligand-dependent genomic function of glucocorticoid receptor in triple-negative breast cancer. *Nat Commun* 6 8323.

Chia K, Milioli H, Portman N, Laven-Law G, Coulson R, Yong A, Segara D, Parker A, Caldon CE, Deng N, et al. 2019 Non-canonical AR activity facilitates endocrine resistance in breast cancer. *Endocr Relat Cancer* 26 251-264.

Christenson JL, O'Neill KI, Williams MM, Spoelstra NS, Jones KL, Trahan GD, Reese J, Van Patten ET, Elias A, Eisner JR, et al. 2021 Activity of combined androgen receptor antagonism and cell cycle inhibition in androgen receptor-positive triple-negative breast cancer. *Mol Cancer Ther* molcanther.0807.2020.

Conway ME, McDaniel JM, Graham JM, Guillen KP, Oliver PG, Parker SL, Yue P, Turkson J, Buchsbaum DJ, Welm BE, et al. 2020 STAT3 and GR Cooperate to Drive Gene Expression and Growth of Basal-Like Triple-Negative Breast Cancer. *Cancer Res* 80 4355-4370.

Coons LA, Hewitt SC, Burkholder AB, McDonnell DP, Korach KS 2017 DNA Sequence Constraints Define Functionally Active Steroid Nuclear Receptor Binding Sites in Chromatin. *Endocrinology* 158 3212-3234.

Creighton CJ, Kent Osborne C, van de Vijver MJ, Foekens JA, Klijn JG, Horlings HM, Nuyten D, Wang Y, Zhang Y, Chamness GC, et al. 2009 Molecular profiles of progesterone receptor loss in human breast tumors. *Breast Cancer Res Treat* 114 287-299.

D'Amato NC, Gordon MA, Babbs B, Spoelstra NS, Carson Butterfield KT, Torkko KC, Phan VT, Barton VN, Rogers TJ, Sartorius CA, et al. 2016 Cooperative Dynamics of AR and ER Activity in Breast Cancer. *Mol Cancer Res* 14 1054-1067.
D'Ippolito AM, McDowell IC, Barrera A, Hong LK, Leichter SM, Bartelt LC, Vockley CM, Majoros WH, Safi A, Song L, et al. 2018 Pre-established Chromatin Interactions Mediate the Genomic Response to Glucocorticoids. *Cell Syst* 7 146-160.e7.

De Bosscher K, Desmet SJ, Clarisse D, Estébanez-Perpiña E, Brunsveld L 2020 Nuclear receptor crosstalk - defining the mechanisms for therapeutic innovation. *Nat Rev Endocrinol* 16 363-377.

Dhiman VK, Bolt MJ, White KP 2018 Nuclear receptors in cancer - uncovering new and evolving roles through genomic analysis. *Nat Rev Genet* 19 160-174.

ENCODE Project Consortium 2012 An integrated encyclopedia of DNA elements in the human genome. *Nature* 489 57-74.

Everett LJ, Lazar MA 2013 Cell-specific integration of nuclear receptor function at the genome. *Wiley Interdiscip Rev Syst Biol Med* 5 615-629.

Fan P, Siwak DR, Abderrahman B, Agboke FA, Yerrum S, Jordan VC 2019 Suppression of Nuclear Factor-κB by Glucocorticoid Receptor Blocks Estrogen-Induced Apoptosis in Estrogen-Deprived Breast Cancer Cells. *Mol Cancer Ther* 18 1684-1695.

Feng Q, He B 2019 Androgen Receptor Signaling in the Development of Castration-Resistant Prostate Cancer. *Front Oncol* 9 858.

Finnish Cancer Registry 2018 https://cancerregistry.fi/statistics/cancer-in-finland/ [Accessed April 26, 2021].

Franco HL, Nagari A, Kraus WL 2015 TNFα signaling exposes latent estrogen receptor binding sites to alter the breast cancer cell transcriptome. *Mol Cell* 58 21-34.

Fuentes-Prior P, Rojas A, Hagler AT, Estébanez-Perpiñá E 2019 Diversity of Quaternary Structures Regulates Nuclear Receptor Activities. *Trends Biochem Sci* 44 2-6.

Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, Orlov YL, Velkov S, Ho A, Mei PH, et al. 2009 An oestrogen-receptor-alpha-bound human chromatin interactome. *Nature* 462 58-64.
Garcia-Carpizo V, Ruiz-Llorente S, Sarmentero J, González-Corpas A, Barrero MJ 2019 CREBBP/EP300 Bromodomain Inhibition Affects the Proliferation of AR-Positive Breast Cancer Cell Lines. *Mol Cancer Res* 17 720-730.

Gerratana L, Basile D, Buono G, De Placido S, Giuliano M, Minichillo S, Coinu A, Martorana F, De Santo I, Del Mastro L, et al. 2018 Androgen receptor in triple negative breast cancer: A potential target for the targetless subtype. *Cancer Treat Rev* 68 102-110.

Gilan O, Rioja I, Knezevic K, Bell MJ, Yeung MM, Harker NR, Lam EYN, Chung CW, Bamborough P, Petretich M, et al. 2020 Selective targeting of BD1 and BD2 of the BET proteins in cancer and immunoinflammation. *Science* 368 387-394.

Gillespie MA, Palii CG, Sanchez-Taltavull D, Shannon P, Longabaugh WJR, Downes DJ, Sivaraman K, Espinoza HM, Hughes JR, Price ND, et al. 2020 Absolute Quantification of Transcription Factors Reveals Principles of Gene Regulation in Erythropoiesis. *Mol Cell* 78 960-974.e11.

Goldstein I, Paakinaho V, Baek S, Sung MH, Hager GL 2017 Synergistic gene expression during the acute phase response is characterized by transcription factor assisted loading. *Nat Commun* 8 1849.

Grindstad T, Richardsen E, Andersen S, Skjefstad K, Rakaee Khanekenari M, Donnem T, Ness N, Nordby Y, Bremnes RM, Al-Saad S, et al. 2018 Progesterone Receptors in Prostate Cancer: Progesterone receptor B is the isoform associated with disease progression. *Sci Rep* 8 11358.

Groner AC, Brown M 2017 Role of steroid receptor and coregulator mutations in hormone-dependent cancers. *J Clin Invest* 127 1126-1135.

Hanker AB, Sudhan DR, Arteaga CL 2020 Overcoming Endocrine Resistance in Breast Cancer. *Cancer Cell* 37 496-513.

Hickey TE, Selth LA, Chia KM, Laven-Law G, Milioli HH, Roden D, Jindal S, Hui M, Finlay-Schultz J, Ebrahimie E, et al. 2021 The androgen receptor is a tumor suppressor in estrogen receptor-positive breast cancer. *Nat Med* 27 310-320.

Holbeck S, Chang J, Best AM, Bookout AL, Mangelsdorf DJ, Martinez ED 2010 Expression profiling of nuclear receptors in the NCI60 cancer cell panel reveals receptor-drug and receptor-gene interactions. *Mol Endocrinol* 24 1287-1296.
Hurtado A, Holmes KA, Ross-Innes CS, Schmidt D, Carroll JS 2011 FOXA1 is a key determinant of estrogen receptor function and endocrine response. *Nat Genet* 43 27-33.

Isikbay M, Otto K, Kregel S, Kach J, Cai Y, Vander Griend DJ, Conzen SD, Szmulewitz RZ 2014 Glucocorticoid receptor activity contributes to resistance to androgen-targeted therapy in prostate cancer. *Horm Cancer* 5 72-89.

Jiménez-Panizo A, Pérez P, Rojas AM, Fuentes-Prior P, Estébanez-Perpiñá E 2019 Non-canonical dimerization of the androgen receptor and other nuclear receptors: implications for human disease. *Endocr Relat Cancer* 26 R479-R497.

Jääskeläinen A, Jukkola A, Haapasaari KM, Auvinen P, Soini Y, Karihtala P 2019 Cytoplasmic Mineralocorticoid Receptor Expression Predicts Dismal Local Relapse-free Survival in Non-triple-negative Breast Cancer. *Anticancer Res* 39 5879-5890.

Kach J, Conzen SD, Szmulewitz RZ 2015 Targeting the glucocorticoid receptor in breast and prostate cancers. *Sci Transl Med* 7 305ps19.

Kach J, Long TM, Selman P, Tonsing-Carter EY, Bacalao MA, Lastra RR, de Wet L, Comiskey S, Gillard M, VanOpstall C, *et al.* 2017 Selective Glucocorticoid Receptor Modulators (SGRMs) Delay Castrate-Resistant Prostate Cancer Growth. *Mol Cancer Ther* 16 1680-1692.

Kittler R, Zhou J, Hua S, Ma L, Liu Y, Pendleton E, Cheng C, Gerstein M, White KP 2013 A comprehensive nuclear receptor network for breast cancer cells. *Cell Rep* 3 538-551.

Knuuttila M, Hämäläinen E, Poutanen M 2019 Applying mass spectrometric methods to study androgen biosynthesis and metabolism in prostate cancer. *J Mol Endocrinol* 62 R255-R267.

Krishnan AV, Zhao XY, Swami S, Brive L, Peehl DM, Ely KR, Feldman D 2002 A glucocorticoid-responsive mutant androgen receptor exhibits unique ligand specificity: therapeutic implications for androgen-independent prostate cancer. *Endocrinology* 143 1889-1900.

Ku SY, Gleave ME, Beltran H 2019 Towards precision oncology in advanced prostate cancer. *Nat Rev Urol* 16 645-654.

Kurmis AA, Yang F, Welch TR, Nickols NG, Dervan PB 2017 A Pyrrole-Imidazole Polyamide Is Active against Enzalutamide-Resistant Prostate Cancer. *Cancer Res* 77 2207-2212.
Lambert SA, Jolma A, Campitelli LF, Das PK, Yin Y, Albu M, Chen X, Taipale J, Hughes TR,
Weirauch MT 2018 The Human Transcription Factors. Cell 172 650-665.

Lasko LM, Jakob CG, Edalji RP, Qiu W, Montgomery D, Digiammarino EL, Hansen TM, Risi RM,
Frey R, Manaves V, et al. 2017 Discovery of a selective catalytic p300/CBP inhibitor that targets
lineage-specific tumours. Nature 550 128-132.

Latil A, Bièche I, Vidaud D, Lidereau R, Berthon P, Cussenot O, Vidaud M 2001 Evaluation of
androgen, estrogen (ER alpha and ER beta), and progesterone receptor expression in human prostate
cancer by real-time quantitative reverse transcription-polymerase chain reaction assays. Cancer Res
61 1919-1926.

Lazar MA 2017 Maturing of the nuclear receptor family. J Clin Invest 127 1123-1125.

Le Dily F, Vidal E, Cuartero Y, Quilez J, Nacht AS, Vicent GP, Carbonell-Caballero J, Sharma P,
Villanueva-Cañas JL, Ferrari R, et al. 2019 Hormone-control regions mediate steroid receptor-
dependent genome organization. Genome Res 29 29-39.

Lempiäinen JK, Niskanen EA, Vuoti KM, Lampinen RE, Göös H, Varjosalo M, Palvimo JJ 2017
Agonist-specific Protein Interactomes of Glucocorticoid and Androgen Receptor as Revealed by
Proximity Mapping. Mol Cell Proteomics 16 1462-1474.

Leo JC, Guo C, Woon CT, Aw SE, Lin VC 2004 Glucocorticoid and mineralocorticoid cross-talk
with progesterone receptor to induce focal adhesion and growth inhibition in breast cancer cells.
Endocrinology 145 1314-1321.

Li J, Alyamani M, Zhang A, Chang KH, Berk M, Li Z, Zhu Z, Petro M, Magi-Galluzzi C, Taplin
ME, et al. 2017 Aberrant corticosteroid metabolism in tumor cells enables GR takeover in
enzalutamide resistant prostate cancer. Elife 6 e20183.

Ligr M, Li Y, Logan SK, Taneja S, Melamed J, Lepor H, Garabedian MJ, Lee P 2012 Mifepristone
inhibits GRβ coupled prostate cancer cell proliferation. J Urol 188 981-988.

Long HK, Prescott SL, Wysocka J 2016 Ever-Changing Landscapes: Transcriptional Enhancers in
Development and Evolution. Cell 167 1170-1187.
Malinen M, Toropainen S, Jääskeläinen T, Sahu B, Jänne OA, Palvimo JJ 2015 Androgen receptor- and PIAS1-regulated gene programs in molecular apocrine breast cancer cells. *Mol Cell Endocrinol* 414 91-98.

Malinen M, Niskanen EA, Kaikkonen MU, Palvimo JJ 2017 Crosstalk between androgen and pro-inflammatory signaling remodels androgen receptor and NF-κB cistrome to reprogram the prostate cancer cell transcriptome. *Nucleic Acids Res* 45 619-630.

Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schütz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, *et al.* 1995 The nuclear receptor superfamily: the second decade. *Cell* 83 835-839.

McGuire WL, Horwitz KB, Zava DT, Garola RE, Chamness GC 1978 Hormones in breast cancer: update 1978. *Metabolism* 27 487-501.

McNamara KM, Moore NL, Hickey TE, Sasano H, Tilley WD 2014 Complexities of androgen receptor signalling in breast cancer. *Endocr Relat Cancer* 21 T161-T181.

Metcalfe C, Fiedman LS, Hager JH 2018 Hormone-Targeted Therapy and Resistance. *Annu Rev Cancer Biol* 2 291-312.

Miranda TB, Voss TC, Sung MH, Baek S, John S, Hawkins M, Grøntved L, Schiltz RL, Hager GL 2013 Reprogramming the chromatin landscape: interplay of the estrogen and glucocorticoid receptors at the genomic level. *Cancer Res* 73 5130-5139.

Mohammed H, Russell IA, Stark R, Rueda OM, Hickey TE, Tarulli GA, Serandour AA, Birrell SN, Bruna A, Saadi A, *et al.* 2015 Progesterone receptor modulates ERα action in breast cancer. *Nature* 523 313-317.

Mohler JL, Chen Y, Hamil K, Hall SH, Cidlowski JA, Wilson EM, French FS, Sar M 1996 Androgen and glucocorticoid receptors in the stroma and epithelium of prostatic hyperplasia and carcinoma. *Clin Cancer Res* 2 889-895.

Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalhorn TF, Higano CS, True LD, Nelson PS 2008 Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. *Cancer Res* 68 4447-4454.
Morgunova E, Taipale J 2017 Structural perspective of cooperative transcription factor binding. *Curr Opin Struct Biol* 47 1-8.

Murakami S, Li R, Nagari A, Chae M, Camacho CV, Kraus WL 2019 Distinct Roles for BET Family Members in Estrogen Receptor α Enhancer Function and Gene Regulation in Breast Cancer Cells. *Mol Cancer Res* 17 2356-2368.

Nagarajan S, Rao SV, Sutton J, Cheeseman D, Dunn S, Papachristou EK, Prada JG, Couturier DL, Kumar S, Kishore K, *et al.* 2020 ARID1A influences HDAC1/BRD4 activity, intrinsic proliferative capacity and breast cancer treatment response. *Nat Genet* 52 187-197.

Narayanan S, Srinivas S, Feldman D 2016 Androgen-glucocorticoid interactions in the era of novel prostate cancer therapy. *Nat Rev Urol* 13 47-60.

Obradović MMS, Hamelin B, Manevski N, Couto JP, Sethi A, Coissieux MM, Münst S, Okamoto R, Kohler H, Schmidt A, *et al.* 2019 Glucocorticoids promote breast cancer metastasis. *Nature* 567 540-544.

Ortega E, Rengachari S, Ibrahim Z, Hoghoughi N, Gaucher J, Holehouse AS, Khochbin S, Panne D 2018 Transcription factor dimerization activates the p300 acetyltransferase. *Nature* 562 538-544.

Paakinaho V, Kaikkonen S, Makkonen H, Benes V, Palvimo JJ 2014 SUMOylation regulates the chromatin occupancy and anti-proliferative gene programs of glucocorticoid receptor. *Nucleic Acids Res* 42 1575-1592.

Paakinaho V, Presman DM, Ball DA, Johnson TA, Schiltz RL, Levitt P, Mazza D, Morisaki T, Karpova TS, Hager GL 2017 Single-molecule analysis of steroid receptor and cofactor action in living cells. *Nat Commun* 8 15896.

Paakinaho V, Swinstead EE, Presman DM, Grøntved L, Hager GL 2019a Meta-analysis of Chromatin Programming by Steroid Receptors. *Cell Rep* 28 3523-3534.e2.

Paakinaho V, Johnson TA, Presman DM, Hager GL 2019b Glucocorticoid receptor quaternary structure drives chromatin occupancy and transcriptional outcome. *Genome Res* 29 1223-1234.
Paakinaho V, Lempiäinen JK, Sigismondo G, Niskanen EA, Malinen M, Jääskeläinen T, Varjosalu M, Krijgsveld J, Palvimo JJ 2021 SUMOylation regulates the protein network and chromatin accessibility at glucocorticoid receptor-binding sites. *Nucleic Acids Res* 49 1951-1971.

Palit SA, Vis D, Stelloo S, Lieftink C, Prekovic S, Bekers E, Hofland I, Šuštić T, Wolters L, Beijersbergen R, *et al.* 2019 TLE3 loss confers AR inhibitor resistance by facilitating GR-mediated human prostate cancer cell growth. *Elife* 8 e47430.

Pan D, Kocherginsky M, Conzen SD 2011 Activation of the glucocorticoid receptor is associated with poor prognosis in estrogen receptor-negative breast cancer. *Cancer Res* 71 6360-6370.

Papachristou EK, Kishore K, Holding AN, Harvey K, Roumeliotis TI, Chilamakuri CSR, Omarjee S, Chia KM, Swarbrick A, Lim E, *et al.* 2018 A quantitative mass spectrometry-based approach to monitor the dynamics of endogenous chromatin-associated protein complexes. *Nat Commun* 9 2311.

Parolia A, Cieslik M, Chu SC, Xiao L, Ouchi T, Zhang Y, Wang X, Vats P, Cao X, Pitchiaya S, *et al.* 2019 Distinct structural classes of activating FOXA1 alterations in advanced prostate cancer. *Nature* 571 413-418.

Perez Kerkvliet C, Dwyer AR, Diep CH, Oakley RH, Liddle C, Cidlowski JA, Lange CA 2020 Glucocorticoid receptors are required effectors of TGFβ1-induced p38 MAPK signaling to advanced cancer phenotypes in triple-negative breast cancer. *Breast Cancer Res* 22 39.

Perissi V, Rosenfeld MG 2005 Controlling nuclear receptors: the circular logic of cofactor cycles. *Nat Rev Mol Cell Biol* 6 542-554.

Peters AA, Buchanan G, Ricciardelli C, Bianco-Miotto T, Centenera MM, Harris JM, Jindal S, Segara D, Jia L, Moore NL, *et al.* 2009 Androgen receptor inhibits estrogen receptor-alpha activity and is prognostic in breast cancer. *Cancer Res* 69 6131-6140.

Pia A, Vignani F, Attard G, Tucci M, Bironzo P, Scagliotti G, Arlt W, Terzolo M, Berruti A 2013 Strategies for managing ACTH dependent mineralocorticoid excess induced by abiraterone. *Cancer Treat Rev* 39 966-973.

Piao YS, Wiesenfeld P, Sprando R, Arnold JT 2013 TGFβ1 alters androgenic metabolites and hydroxysteroid dehydrogenase enzyme expression in human prostate reactive stromal primary cells:
Is steroid metabolism altered by prostate reactive stromal microenvironment? *J Steroid Biochem Mol Biol* 138 206-213.

Ponnusamy S, Asemota S, Schwartzberg LS, Guestini F, McNamara KM, Pierobon M, Font-Tello A, Qiu X, Xie Y, Rao PK, *et al.* 2019 Androgen Receptor Is a Non-canonical Inhibitor of Wild-Type and Mutant Estrogen Receptors in Hormone Receptor-Positive Breast Cancers. *iScience* 21 341-358.

Presman DM, Ganguly S, Schiltz RL, Johnson TA, Karpova TS, Hager GL 2016 DNA binding triggers tetramerization of the glucocorticoid receptor in live cells. *Proc Natl Acad Sci U S A* 113 8236-8241.

Puhr M, Hoefer J, Eigentler A, Ploner C, Handle F, Schaefer G, Kroon J, Leo A, Heidegger I, Eder I, *et al.* 2018 The Glucocorticoid Receptor Is a Key Player for Prostate Cancer Cell Survival and a Target for Improved Antiandrogen Therapy. *Clin Cancer Res* 24 927-938.

Rafiee MR, Girardot C, Sigismondo G, Krijgsfeld J 2016 Expanding the Circuitry of Pluripotency by Selective Isolation of Chromatin-Associated Proteins. *Mol Cell* 64 624-635.

Reddy TE, Gertz J, Crawford GE, Garabedian MJ, Myers RM 2012 The hypersensitive glucocorticoid response specifically regulates period 1 and expression of circadian genes. *Mol Cell Biol* 32 3756-3767.

Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, Montgomery B, Taplin ME, Pritchard CC, Attard G, *et al.* 2015 Integrative clinical genomics of advanced prostate cancer. *Cell* 161 1215-1228.

Robinson JL, Macarthur S, Ross-Innes CS, Tilley WD, Neal DE, Mills IG, Carroll JS 2011 Androgen receptor driven transcription in molecular apocrine breast cancer is mediated by FoxA1. *EMBO J* 30 3019-3027.

Sahu B, Laakso M, Ovaska K, Mirtti T, Lundin J, Rannikko A, Sankila A, Turunen JP, Lundin M, Konst J, *et al.* 2011 Dual role of FoxA1 in androgen receptor binding to chromatin, androgen signalling and prostate cancer. *EMBO J* 30 3962-3976.
Sahu B, Laakso M, Pihlajamaa P, Ovaska K, Sinielnikov I, Hautaniemi S, Jänne OA 2013 FoxA1 specifies unique androgen and glucocorticoid receptor binding events in prostate cancer cells. *Cancer Res* 73 1570-1580.

Sandoval GJ, Pulice JL, Pakula H, Schenone M, Takeda DY, Pop M, Boulay G, Williamson KE, McBride MJ, Pan J, *et al.* 2018 Binding of TMPRSS2-ERG to BAF Chromatin Remodeling Complexes Mediates Prostate Oncogenesis. *Mol Cell* 71 554-566.e7.

See YX, Wang BZ, Fullwood MJ 2019 Chromatin Interactions and Regulatory Elements in Cancer: From Bench to Bedside. *Trends Genet* 35 145-158.

Severson TM, Kim Y, Joosten SEP, Schuurman K, van der Groep P, Moelans CB, Ter Hoeve ND, Manson QF, Martens JW, van Deurzen CHM, *et al.* 2018 Characterizing steroid hormone receptor chromatin binding landscapes in male and female breast cancer. *Nat Commun* 9 482.

Shah N, Wang P, Wongvipat J, Karthaus WR, Abida W, Armenia J, Rockowitz S, Drier Y, Bernstein BE, Long HW, *et al.* 2017 Regulation of the glucocorticoid receptor via a BET-dependent enhancer drives antiandrogen resistance in prostate cancer. *Elife* 6 e27861.

Shiota M, Fujimoto N, Higashijima K, Imada K, Kashiwagi E, Takeuchi A, Inokuchi J, Tatsugami K, Kajioka S, Uchiumi T, *et al.* 2018 Mineralocorticoid receptor signaling affects therapeutic effect of enzalutamide. *Prostate* 10.1002/pros.23661.

Singhal H, Greene ME, Tarulli G, Zarnke AL, Bourgo RJ, Laine M, Chang YF, Ma S, Dembo AG, Raj GV, *et al.* 2016 Genomic agonism and phenotypic antagonism between estrogen and progesterone receptors in breast cancer. *Sci Adv* 2 e1501924.

Singhal H, Greene ME, Zarnke AL, Laine M, Al Abosy R, Chang YF, Dembo AG, Schoenfelt K, Vadhi R, Qiu X, *et al.* 2017 Progesterone receptor isoforms, agonists and antagonists differentially reprogram estrogen signaling. *Oncotarget* 9 4282-4300.

Siersbæk R, Kumar S, Carroll JS 2018 Signaling pathways and steroid receptors modulating estrogen receptor α function in breast cancer. *Genes Dev* 32 1141-1154.

Siersbæk R, Scabia V, Nagarajan S, Chernukhin I, Papachristou EK, Broome R, Johnston SJ, Joosten SEP, Green AR, Kumar S, *et al.* 2020 IL6/STAT3 Signaling Hijacks Estrogen Receptor α Enhancers to Drive Breast Cancer Metastasis. *Cancer Cell* 38 412-423.e9.
Swami U, McFarland TR, Nussenzveig R, Agarwal N 2020 Advanced Prostate Cancer: Treatment Advances and Future Directions. *Trends Cancer* 6 702-715.

Sweeney CJ, Chen YH, Carducci M, Liu G, Jarrard DF, Eisenberger M, Wong YN, Hahn N, Kohli M, Cooney MM, *et al.* 2015 Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer. *N Engl J Med* 373 737-746.

Swinstead EE, Miranda TB, Paakinaho V, Baek S, Goldstein I, Hawkins M, Karpova TS, Ball D, Mazza D, Lavis LD, *et al.* 2016 Steroid Receptors Reprogram FoxA1 Occupancy through Dynamic Chromatin Transitions. *Cell* 165 593-605.

Swinstead EE, Paakinaho V, Hager GL 2018 Chromatin reprogramming in breast cancer. *Endocr Relat Cancer* 25 R385-R404.

Syed AP, Greulich F, Ansari SA, Uhlenhaut NH 2020 Anti-inflammatory glucocorticoid action: genomic insights and emerging concepts. *Curr Opin Pharmacol* 53 35-44.

Timmermans S, Souffriau J, Libert C 2019 A General Introduction to Glucocorticoid Biology. *Front Immunol* 10 1545.

Tonsing-Carter E, Hernandez KM, Kim CR, Harkless RV, Oh A, Bowie KR, West-Szymanski DC, Betancourt-Ponce MA, Green BD, Lastra RR, *et al.* 2019 Glucocorticoid receptor modulation decreases ER-positive breast cancer cell proliferation and suppresses wild-type and mutant ER chromatin association. *Breast Cancer Res* 21 82.

Truong TH, Dwyer AR, Diep CH, Hu H, Hagen KM, Lange CA 2019 Phosphorylated Progesterone Receptor Isoforms Mediate Opposing Stem Cell and Proliferative Breast Cancer Cell Fates. *Endocrinology* 160 430-446.

Vahrenkamp JM, Yang CH, Rodriguez AC, Almomen A, Berrett KC, Trujillo AN, Guillen KP, Welm BE, Jarboe EA, Janat-Amsbury MM, *et al.* 2018 Clinical and Genomic Crosstalk between Glucocorticoid Receptor and Estrogen Receptor α In Endometrial Cancer. *Cell Rep* 22 2995-3005.

Valencia AM, Kadoch C 2019 Chromatin regulatory mechanisms and therapeutic opportunities in cancer. *Nat Cell Biol* 21 152-161.
Veldscholte J, Ris-Stalpers C, Kuiper GG, Jenster G, Berrevoets C, Claassen E, van Rooij HC, Trapman J, Brinkmann AO, Mulder E 1990 A mutation in the ligand binding domain of the androgen receptor of human LNCaP cells affects steroid binding characteristics and response to anti-androgens. *Biochem Biophys Res Commun* 173 534-540.

Voss TC, Schiltz RL, Sung MH, Yen PM, Stamatoyannopoulos JA, Biddie SC, Johnson TA, Miranda TB, John S, Hager GL 2011 Dynamic exchange at regulatory elements during chromatin remodeling underlies assisted loading mechanism. *Cell* 146 544-554.

Waks AG, Winer EP 2019 Breast Cancer Treatment: A Review. *JAMA* 321 288-300.

Wang G, Zhao D, Spring DJ, DePinho RA 2018a Genetics and biology of prostate cancer. *Genes Dev* 32 1105-1140.

Wang Y, Song F, Zhang B, Zhang L, Xu J, Kuang D, Li D, Choudhary MNK, Li Y, Hu M, *et al.* 2018b The 3D Genome Browser: a web-based browser for visualizing 3D genome organization and long-range chromatin interactions. *Genome Biol* 19 151.

West DC, Pan D, Tonsing-Carter EY, Hernandez KM, Pierce CF, Styke SC, Bowie KR, Garcia TI, Kocherginsky M, Conzen SD 2016 GR and ER Coactivation Alters the Expression of Differentiation Genes and Associates with Improved ER+ Breast Cancer Outcome. *Mol Cancer Res* 14 707-719.

West DC, Kocherginsky M, Tonsing-Carter EY, Dolcen DN, Hosfield DJ, Lastra RR, Sinnwell JP, Thompson KJ, Bowie KR, Harkless RV, *et al.* 2018 Discovery of a Glucocorticoid Receptor (GR) Activity Signature Using Selective GR Antagonism in ER-Negative Breast Cancer. *Clin Cancer Res* 24 3433-3446.

Wimalasena VK, Wang T, Sigua LH, Durbin AD, Qi J 2020 Using Chemical Epigenetics to Target Cancer. *Mol Cell* 78 1086-1095.

Xie N, Cheng H, Lin D, Liu L, Yang O, Jia L, Fazli L, Gleave ME, Wang Y, Rennie P, *et al.* 2015 The expression of glucocorticoid receptor is negatively regulated by active androgen receptor signaling in prostate tumors. *Int J Cancer* 136 E27-E38.
Yang F, Ma Q, Liu Z, Li W, Tan Y, Jin C, Ma W, Hu Y, Shen J, Ohgi KA, et al. 2017
Glucocorticoid Receptor: MegaTrans Switching Mediates the Repression of an ERα-Regulated
Transcriptional Program. Mol Cell 66 321-331.e6.
Yemelyanov A, Czwornog J, Chebotaev D, Karseladze A, Kulevitch E, Yang X, Budunova I 2007
Tumor suppressor activity of glucocorticoid receptor in the prostate. Oncogene 26 1885-1896.
Yemelyanov A, Czwornog J, Gera L, Joshi S, Chatterton RT Jr, Budunova I 2008 Novel steroid
receptor phyto-modulator compound a inhibits growth and survival of prostate cancer cells. Cancer
Res 68 4763-4773.
Yu J, Yu J, Mani RS, Cao Q, Brenner CJ, Cao X, Wang X, Wu L, Li J, Hu M, et al. 2010 An
integrated network of androgen receptor, polycomb, and TMPRSS2-ERG gene fusions in prostate
cancer progression. Cancer Cell 17 443-454.
Yuan F, Hankey W, Wu D, Wang H, Somarelli J, Armstrong AJ, Huang J, Chen Z, Wang Q 2019
Molecular determinants for enzalutamide-induced transcription in prostate cancer. Nucleic Acids Res
47 10104-10114.
Zhang Z, Chng KR, Lingadahalli S, Chen Z, Liu MH, Do HH, Cai S, Rinaldi N, Poh HM, Li G, et al.
2019 An AR-ERG transcriptional signature defined by long-range chromatin interactomes in prostate
cancer cells. Genome Res 29 223-235.
Zhang Z, Zhou C, Li X, Barnes SD, Deng S, Hoover E, Chen CC, Lee YS, Zhang Y, Wang C, et al.
2020 Loss of CHD1 Promotes Heterogeneous Mechanisms of Resistance to AR-Targeted Therapy
via Chromatin Dysregulation. Cancer Cell 37 584-598.e11.
Zhao XY, Malloy PJ, Krishnan AV, Swami S, Navone NM, Peehl DM, Feldman D 2000
Glucocorticoids can promote androgen-independent growth of prostate cancer cells through a
mutated androgen receptor. Nat Med 6 703-706.

FIGURE LEGENDS

Figure 1. Crosstalk between PR and ER modulates BCa cell proliferation. ER regulates pro-proliferation
(Proprolif.) pathways in BCa. (A) Upon activation of the PR, the ER tethers to the PR (upper) or binds near or
next to the PR (lower), inhibiting BCa pro-proliferation and cell survival pathways. (B) PR-A inhibits binding
of the ER to chromatin and E2-induced proliferation (prolif.) (upper). PR-B induces binding of the ER, enhancing E2-induced proliferation (lower).

**Figure 2. Different AR ligands modulate binding of ER to chromatin in BCa cells.** (A) In ER+ BCa cells, a subset of ER binding sites (left) can be occupied by activated AR (middle), regulating cell proliferation (prolif.). ENZ inhibits the binding of AR and ER, repressing cell proliferation (right). (B) ER binding and its regulation of tumor growth pathways (left) can be inhibited by SARM-bound AR (middle). Binding of ligand-independent ER-Y537S mutant is also inhibited by AR. SARM-bound AR redistributes ER to other chromatin sites (right). (C) Agonist activated AR redistributes (squelches) EP300 from ER- to AR-binding sites. The ER is redistributed to a subset of these sites. (D) In apocrine BCa (ER-/AR+) cells, the AR can regulate the same targets as the ER in ER+ BCa cells (upper). Inhibition of EP300 by specific acetyltransferase inhibitor (i) can repress the AR-regulated transcription (lower).

**Figure 3. GR has multiple different mechanisms to modulate ER action in BCa cells.** (A) Assisted loading model was initially shown with ER and GR. The initiating factor (ER in left, GR in right) binds to a closed chromatin region, and upon recruitment of chromatin remodeling factors, the secondary factor (GR on the left, ER on the right) can bind to the site. (B) In ER+ BCa cells, upon coactivation, GR and ER bind to the same sites, and induce pro-differentiation (Prodiffer.) and repress epithelial-mesenchymal transition (EMT). (C) SUMO-modified GR can tether to the ER and repress ER’s target genes. (D) GR can actively inhibit the chromatin-binding of ER and ligand-independent ER-Y537S mutant at regulatory sites of genes involved in E2-driven pro-proliferation. (E) In long-term E2 derived BCa cells, the GR can inhibit the action of NF-κB thereby reducing the levels on TNF-α and E2-induced apoptosis. (F) In ER- BCa cells, GR can induce cell survival and repress cell death pathways (left). Inhibition of the GR with antagonist RU486 (RU), induces cell death and represses cell survival pathways (right).

**Figure 4. GR can replace ENZ-inhibited AR in PCa.** (A) In therapy naïve PCa cells, the AR actively represses the transcription of the GR gene (NR3CI). (B) In ENZ treated PCa cells, the activity of the AR is inhibited, leading to a de-repression of NR3CI. GR can substitute for the ENZ-inhibited AR at some but not at all of the AR’s binding sites. (C) Active glucocorticoid, cortisol, is converted by 11β-HSD2 to its inactive metabolite cortisone, and cortisone is converted back to cortisol by 11β-HSD1. (D) In ENZ-treated PCa cells, the protein levels of 11β-HSD2 are decreased, leading to elevated levels of cortisol and reduced levels of cortisone.

**Figure 5. Localization of the AR-regulated genomic region that mediates the repression of NR3CI.** (A) Genome browser tracks of AR ChIP-seq from 22Rv1 (GSE94013), C4-2B (GSE40050), LNCaP (GSE40050), LREX (GSE51497), and VCaP (GSE56086) cells (red color). Genome browser tracks of H3K27ac ChIP-seq from 22Rv1 (ENCSR391NPE), C4-2B (ENCSR279KIX), LNCaP (GSE118514), LREX (GSE103449), and...
VCaP (ENCSR597ULV) cells (black color). Data are mapped to human hg38 genome. Location of ARE motifs (based on HOMER are.motif sequence) shown as blue bars below the tracks. The location of \( \text{NR3C1} \) promoter region is depicted as well as the initiation codon ATG (red arrow). Enhancer A: AR-regulated enhancer region as suggested in Shah et al. 2017 and Yuan et al. 2019. Enhancer B: AR-regulated enhancer region as suggested in Xie et al. 2015. (B) \( \text{NR3C1} \) and both suggested AR-regulated enhancers reside within the same TAD (topologically associating domain). The AR-regulated enhancer region proposed by Shah et al. 2017 and Yuan et al. 2019 (enhancer A) and suggested by Xie et al. 2015 (enhancer B) (red arrows) are within the same TAD based on Hi-C data from LNCaP cells (ENCSR346DCU). Data were obtained using 3D Genome Browser (Wang et al. 2018b). The interaction matrix shown above with a scale from 0-5. Genes on the positive strand are shown in black, and genes on the negative strand are shown in light blue. TADs are distinguished with different colors.
| Type    | Comparison          | Increased survival | P-value | HR    | Reference                  |
|---------|---------------------|--------------------|---------|-------|----------------------------|
| ESR1+   | PGR loss vs neutral/gain | PGR neutral/gain   | 0.001   | 1.46  | Mohammed et al. 2015       |
| PGR-    | ESR1- vs ESR1+      | no difference      | 0.27    | NA    | Singhal et al. 2016        |
| PGR+    | ESR1- vs ESR1+      | ESR1+              | 3.50E-02| NA    | Singhal et al. 2016        |

ESR1, estrogen receptor α; PGR, progesterone receptor; HR, hazard ratio; NA, not applicable or indicated
| Type        | Comparison          | Increased survival | P-value | HR   | Reference       |
|-------------|---------------------|--------------------|---------|------|-----------------|
| ESR1+       | AR low vs high      | AR-high            | 0.002   | 0.22 | Peters et al. 2009 |
| ESR1-       | AR low vs high      | no difference      | 0.32    | NA   | Peters et al. 2009 |
| TCGA-BRCA   | AR low vs high      | AR-high            | 1.10E-16| 0.52 | Ponnusamy et al. 2019 |

ESR1, estrogen receptor α; PGR, progesterone receptor; AR, androgen receptor; TCGA-BRCA, The Cancer Genome Atlas Breast Invasive Carcinoma; HR, hazard ratio; NA, not applicable or indicated.
| Type                  | Comparison                  | Increased survival | P-value | HR   | Reference          |
|----------------------|-----------------------------|--------------------|---------|------|--------------------|
| ESR1+ untreated      | NR3C1 low vs high           | NR3C1-high         | 0.03    | 0.6  | Pan et. al 2011    |
| ESR1+ tamoxifen      | NR3C1 low vs high           | NR3C1-high         | 7.70E-08| 0.25 | Pan et. al 2011    |
| ESR1- untreated      | NR3C1 low vs high           | NR3C1-low          | 0.001   | 2.23 | Pan et. al 2011    |
| ESR1- chemotherapy    | NR3C1 low vs high           | NR3C1-low          | 5.80E-07| 6.83 | Pan et. al 2011    |
| ESR1+                | NR3C1 low vs high           | NR3C1-high         | 7.80E-14| 0.35 | West et al. 2016   |
| ESR1+ PGR-high       | NR3C1 low vs high           | NR3C1-high         | 2.30E-07| 0.35 | West et al. 2016   |
| ESR1+ PGR-low        | NR3C1 low vs high           | NR3C1-high         | 4.10E-06| 0.4  | West et al. 2016   |
| TNBC Basal-like 1    | NR3C1 low vs high           | NR3C1-low          | 0.013   | 1.87 | West et al. 2018   |
| TNBC Basal-like 2    | NR3C1 low vs high           | no difference      | 0.64    | NA   | West et al. 2018   |
| TNBC Mesenchymal     | NR3C1 low vs high           | NR3C1-low          | 0.04    | 1.65 | West et al. 2018   |
| TNBC Luminal AR      | NR3C1 low vs high           | NR3C1-low          | 0.015   | 1.68 | West et al. 2018   |

ESR1, estrogen receptor α; PGR, progesterone receptor; AR, androgen receptor; NR3C1, glucocorticoid receptor; TNBC, triple negative breast cancer; HR, hazard ratio; NA, not applicable or indicated.
Figure 1 (in color for the web version of the journal)

174x53mm (600 x 600 DPI)
Figure 2 (in color for the web version of the journal)

143x145mm (600 x 600 DPI)
Figure 3 (in color for the web version of the journal)

203x122mm (600 x 600 DPI)
Figure 4 (in color for the web version of the journal)
Figure 5 (in color for the web version of the journal)