Published in final edited form as:

J Steroid Biochem Mol Biol. 2014 September ; 0: 160–173. doi:10.1016/j.jsbmb.2014.02.010.

Estrogen signaling crosstalk: implications for endocrine resistance in ovarian cancer

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Abstract

Resistance to anti-estrogen therapies is a prominent challenge in the treatment of ovarian cancer. Tumors develop endocrine resistance by acquiring adaptations that help them rely on alternative oncogenic signaling cascades, which crosstalk with estrogen signaling pathways. An understanding of estrogen signaling crosstalk with these growth promoting cascades is essential in order to maximize efficacy of anti-estrogen treatments in ovarian cancer. Herein, we provide an overview of estrogen signaling in ovarian cancer and discuss the major challenges associated with anti-estrogen therapies. We also review what is currently known about how genomic and non-genomic estrogen signaling pathways crosstalk with several major oncogenic signaling cascades. The insights provided here illustrate existing strategies for targeting endocrine resistant ovarian tumors and may help identify new strategies to improve the treatment of this disease.

Keywords

Estrogen signaling; estrogen receptor; estradiol; endocrine resistance; ovarian cancer

1.Introduction

Ovarian cancer is the most deadly gynecological cancer [1], which frequently presents at an advanced stage [2] and has a high rate of recurrence after chemotherapy [3]. Efforts have been made to exploit the estrogen production and dependence of the ovary to treat malignant ovarian tumors of both epithelial and granulosa cell origin, although we will focus primarily on epithelial ovarian cancer (EOC) in this review. In contrast to breast cancer, anti-estrogen therapies have only proven effective in a small subset of patients and have largely failed to produce increases in overall survival (OS) of ovarian cancer patients [4-14]. There are a

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number of cellular scenarios that may render ovarian tumors resistant to endocrine therapy. Similarly to chemoresistance, which develops as tumors acquire adaptive mechanisms to evade apoptosis, tumors also acquire adaptations that help them rely on growth promoting pathways other than estrogen signaling. The complexity of estrogen signaling crosstalk with alternative oncogenic signaling pathways provides ample opportunities for ovarian tumors to develop productive adaptive mechanisms. Although many of these pathways are well studied in the context of breast cancer, their contribution to the development of endocrine resistance in ovarian cancer is less thoroughly explored. In this review, we will begin with an overview of estrogen signaling and a discussion of clinical benefits and challenges of anti-estrogen therapy; we will then discuss how genomic and non-genomic estrogen signaling pathways interact with a variety of oncogenic signaling cascades in ovarian cancer. Relevant studies from other estrogen-responsive cancers, primarily breast cancer, will be included where research in ovarian cancer is lacking. These studies have profound implications for understanding the development of endocrine resistance in ovarian cancer and highlight the need for combinatorial strategies to increase the therapeutic efficacy of anti-estrogenic therapies.

1.1 Estrogen Signaling

The paradigm of how we approach ovarian cancer research is changing as we gain a greater understanding of the cell types of origin and molecular signatures associated with various subtypes of ovarian tumors. For example, high-grade and low-grade serous epithelial ovarian tumors (HGSC, LGSC), and the rarer epithelial subtypes including mucinous, clear cell and endometrioid tumors have unique cell types of origin and may be more effectively treated if approached as discrete diseases [15-18]. However, one common denominator in all of these subtypes is that they preferentially arise or grow in the ovarian milieu. Many HGSC are now known to originate from the fimbrial epithelium of the fallopian tube [19], in addition to the ovarian surface epithelium [20]. By focusing on commonalities between subtypes and between HGSC of differing origins, we may be able to gain insight into why the ovary is an optimal site for tumor growth.

One long-standing hypothesis is that normal endocrine signaling within the pre-menopausal ovary contributes to establishment of a unique and pro-tumorigenic microenvironment. Perturbation of the delicate balance of hormonal signaling factors may thus increase the risk of ovarian tumorigenesis later in life [21]. Moreover, epidemiological studies, which reveal that long-term use of estrogen-only hormone replacement therapy increases a woman's risk of ovarian cancer, support the hypothesis that estrogen-signaling contributes to the etiology of the disease [22-25]. In addition, experimental studies demonstrate the growth promoting effects of estrogen on ovarian tumors in mice [26-28] and in human ovarian cancer cell lines [29-31]. Thus, while anti-estrogen therapies alone may be ineffective, they should not be ruled out as part of future combinatorial therapeutic approaches to better treat ovarian cancer.

We are only beginning to appreciate the complexity of estrogen signaling pathways, which can occur by way of genomic, non-genomic or converging mechanisms. Classical genomic estrogen signaling occurs via the classical estrogen receptors (ERs), ER α and ER β .

Homodimerization or heterodimerization of these receptors occurs in response to binding of a ligand, such as 17 β -estradiol (estradiol), prompting transport to the nucleus, where the dimer binds to canonical estrogen response elements (EREs) in target gene promoters [32, 33]. Non-classical, or tethered, genomic signaling occurs when ligand-bound ERs interact with AP1 or SP1 transcription factors, which are bound to their own DNA recognition sites [33]. An intermediary mechanism has been described whereby ERs bind to one or two ERE-half sites, and this interaction is stabilized by transcription factor binding at a downstream AP1 or SP1 site [34, 35]. Likewise, ERs bound at EREs can stabilize interactions of other transcription factors to imperfect response elements, as occurs with Nuclear Factor Kappa B (NF κ B) at a latent NF κ B response element upstream of an ERE [36]. Thus, activation of ERs and direct modulation of downstream transcriptional programs is a well-characterized function of estrogen.

Non-genomic estrogen signaling pathways are typically mediated by activation of a membrane bound ER, which transactivates protein kinase cascades, leading to rapid cell signaling and transcriptional activation of downstream targets of these kinase cascades [37-39]. The G protein-coupled receptor GPR30, also named G protein-coupled estrogen receptor 1 (GPER1), has been extensively studied as a membrane ER that activates non-genomic estrogen signaling pathways in various tissues [40]. Filardo *et al.* show that GPER1 transactivates epidermal growth factor receptor (EGFR) signaling in breast cancer cells, which in turn activates the mitogen activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling cascade [41]. However, controversy still exists as to the role it plays in mediating reproductive estrogenic responses, since GPER1-deficient mice have normal reproductive functions and mammary development [42].

Ligand-independent events represent an alternate mechanism for estrogenic action as well, whereby classical ERs are phosphorylated by activated MAPK/ERK [43, 44] or phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) [45, 46]. Alternative isoforms of ER α have recently been shown to act at the cell membrane and participate in non-genomic estrogen signaling pathways as well [38, 47, 48]. Many of these pathways act in tandem; therefore, inhibition of one mechanism of estrogen signaling may not completely abolish estrogenic or ER regulatory actions in a cell.

Adding another layer of complexity to estrogen signaling, ERs are differentially regulated in tissue- and cell type-specific manners. Although some target genes may overlap between tissues, many genes may only be regulated in a particular cell type or in a specific context, as their regulation is dependent upon specific combinations of transcriptional cofactors and the ratio of various ERs present [32, 49]. In this manner, the relative levels of ER corepressors and coactivators can also affect tumor progression and response to hormone therapy [32, 49]. The differential functions of the various ERs play an important role in ovarian cancer as well; ER α [29, 50-53] and GPER1 [52, 54-56] are primarily thought to be protumorigenic, whereas studies report ER β to be primarily anti-proliferative [57-60].

These differing ER roles can affect tumor phenotypes depending upon the relative ratios of each ER; this point is illustrated in a study that found varying levels of ER α , ER β and GPER1 in three different human ovarian cancer cell lines, and also observed varying effects

of estrogen on each of these cell lines [61]. The variability in expression of ERs is reflected in a clinical setting as well, where approximately 67% of ovarian cancer patients are ER-positive [62], and ovarian tumors display an increased ER α :ER β mRNA ratio compared to normal ovarian tissue or benign cysts [63]. Moreover, levels of ER α and ER β have not been found to correlate with levels of GPER1 [64]. These results make individualized approaches toward therapeutic strategies all the more important, including definitive identification of specific ER status in ovarian tumors.

1.2 Clinical Response to Anti-Estrogens

While clinical benefit of anti-estrogens ranging from 13-51% is reported in EOC (marked by complete response [CR], partial response [PR], or stable disease [SD]), anti-estrogen therapy has elicited no improvement in OS [4-14]. These studies largely suggest that inhibition of estrogen signaling alone is not adequate to cause significant tumor regression once tumorigenesis has already been initiated. However, there are several considerations of these studies to take into account. First, only one study selected for patients with ER-positivity [9], which has been correlated with increased clinical benefit to anti-estrogens in ovarian cancer patients [5, 11, 65]. In addition to ER α , Argenta *et al.* also significantly correlate expression of the estrogen response genes TFF1 and vimentin with clinical benefit and/or PFS in response to the anti-estrogen fulvestrant [65]. Smyth *et al.* confirm higher TFF1 expression and lower vimentin expression as predictors of endocrine sensitivity [9], supporting the idea that further research is merited to identify subsets of patients who might benefit most from anti-estrogen treatments. Perhaps by measuring a panel of endocrine-related factors, including multiple ER isoforms, TFF1 and other downstream ER target genes, a subset of EOC patients that may respond to anti-estrogen therapies can be identified.

Another consideration of clinical studies that involve anti-estrogen therapy is that they typically involve recurrent ovarian cancer cases, which are resistant to platinum chemotherapy. The use of anti-estrogens in chemo-naïve patients, alone or in combination with standard chemotherapeutics or novel small molecule inhibitors has not been thoroughly investigated. The need for these types of studies is apparent when examining the retrospective study performed by Gershenson *et al.*, which shows a clinical benefit of various anti-estrogen regimens in 82.7% of patients with platinum-sensitive, recurrent LGSC versus a clinical benefit in 54.1% of platinum-resistant cases [13]. Moreover, although the Gershenson *et al.* study focused only on LGSC, most trials have failed to distinguish ovarian cancer subtypes, potentially further diluting the significance of their results. However, a major challenge to performing studies on rare subtypes lies in obtaining adequate numbers of patients.

The type of anti-estrogen therapy needs to be considered in designing a productive clinical trial as well. Forms of anti-estrogen therapy include: selective estrogen receptor modulators (SERMs), such as raloxifene; aromatase inhibitors (AIs), which block the conversion of androgens to estrogens by aromatase, including letrozole and anastrozole; and selective estrogen receptor degraders (SERDs), such as fulvestrant (also known as ICI 182,780) [66, 67].

The anti-estrogen tamoxifen has frequently been studied in ovarian cancer, however, it exhibits ER α agonist activity at AP1 sites [33], which may be increased in tamoxifen resistant breast cancer [68]. Moreover, Hachisuga *et al.* report an anti-estrogenic effect of tamoxifen on normal endometrium in competition with endogenous estradiol, but an agonist effect in the absence of endogenous estradiol [69]. These results emphasize the limitations in our understanding of how this compound functions in the normal ovary and in the treatment of ovarian cancer.

A study by Badia *et al.* highlights some differences between tamoxifen and fulvestrant. The authors show that long-term treatment of ovarian cancer cells with fulvestrant durably inhibits ovarian cancer cells from transactivating a synthetic ERE and results in slower proliferation. Conversely, after long-term treatment with tamoxifen, the cells remain estrogen-responsive and actually grow faster than control cells [70]. For this reason, more studies utilizing fulvestrant as opposed to tamoxifen may be merited. Finally, since AIs function by blocking the synthesis of estrogen [67], they should theoretically reduce both genomic and non-genomic estrogen signaling; however, they do not address ligand-independent ER signaling events, which could play a greater role than is currently appreciated.

The study by Badia *et al.* raises the issue of duration of treatment as well. It is possible that longer courses of anti-estrogens may be more effective in ovarian cancer, as suggested in a review by Simpkins *et al.* The authors point out a study that used tamoxifen as an adjuvant therapy for 36 weeks [71], which is shorter than the recommended timeframe for tamoxifen treatment in breast cancer [72]. A meta-analysis of 20 studies of ER-positive breast cancer patients reports reduced recurrence rates through the first 10 years and a one third reduction in mortality through the first 15 years with 5 years of adjuvant tamoxifen therapy [73]. Although current 5-year survival rates of ovarian cancer patients may limit the use of such long-term hormone therapy for some patients with advanced disease, the duration of anti-estrogen treatment after cytoreductive surgery and chemotherapy is one parameter that may merit additional consideration.

Future alternative approaches in clinical study design involving anti-estrogen therapy may be required to best capitalize on endocrine therapy for the treatment of ovarian cancer. Both early and late stage disease may benefit from novel combinatorial treatments, which could potentially include SERMs or SERDs in appropriate patients. However, attention must also be given to factors that contribute to the development of endocrine resistance, which may vary considerably amongst and within tumor subtypes. Further preclinical investigation into the mechanisms of estrogen signaling and the development of endocrine resistance in ovarian cancer is needed to support and define these future experimental studies and clinical trials.

2. Estrogen Signaling Crosstalk

The complexity of estrogen signaling and opportunities for crosstalk with other oncogenic pathways may be key in elucidating why many patients treated with anti-estrogen therapy exhibit *de novo* or acquired resistance to anti-estrogen therapies, which is illustrated in

clinical trials [4-14]. Given the wide variety of alterations observed in ovarian cancer, including genomic copy number changes frequently seen in HGSC and mutations frequently occurring in LGSC and other epithelial subtypes [16], it is not surprising that a number of other well-known and oncogenic signal transduction pathways may be activated in addition to the estrogen signaling pathway. Accordingly, the potential benefit of a given anti-estrogen therapy alone may be masked by the dominant nature of these other oncogenic pathways. Here, we present the potential crosstalk between estrogen signaling and specific oncogenic pathways that serve to highlight both the challenges of and the opportunities for devising future combinatorial treatments to better manage ovarian cancer. Throughout this review, we will discuss studies performed in breast and endometrial cancer as well. These studies and their implications for endocrine resistance are summarized in Table 1. Although anti-estrogen therapy is more effective in breast cancer than ovarian cancer, endocrine resistance presents itself as a significant problem in both diseases [74]; therefore, where sparse research has been performed in ovarian cancer models, we may be able to extrapolate some results from breast cancer studies to ovarian cancer.

2.1 HER/ErbB Family Growth Factor Receptors

The HER (or ErbB) family of growth factor receptors includes HER1 (commonly known as the epidermal growth factor receptor [EGFR]), HER2, HER3 and HER4. Ligand induced dimerization of these receptors by growth factors such as epidermal growth factor (EGF), transforming growth factor- α (TGF α), or heparin-bound EGF (HB-EGF) leads to receptor autophosphorylation and activation of downstream signal transduction pathways, including the MAPK/ERK and PI3K/AKT cascades [75]. EGFR and HER2 are both implicated as important players in ovarian tumorigenesis and progression. EGFR expression is reported in 33-75% of EOC [76], and there is a correlation between EGFR-positivity and poor response to platinum-based chemotherapy, shorter PFS and OS [77, 78]. A meta-analysis of studies comparing patient survival with HER2 levels suggests that increased HER2 levels are also associated with reduced PFS, disease free survival or recurrence free survival in ovarian cancer patients [79].

Several studies corroborate the importance of ER α in the execution of signaling via EGFR [80, 81]. Kalli *et al.* examine EGFR activation in several ER α -positive and ER α -negative ovarian cancer cell lines, and report that although all cell lines have functional EGFR, only the ER α -positive cell line, OV266, has a strong proliferative response to EGF. In this study, the authors speculate on a ligand-independent function for ER α in EGFR signaling events, since the effect is not inhibited with fulvestrant [80, 81].

A requirement for ER α in EGF-stimulated EGFR activation and DNA-synthesis is also demonstrated in MCF7 breast cancer cells [80]. Moreover, EGF- and IGF-1-stimulated uterine proliferation does not occur in ER α knockout mice [82, 83]. These results establish the interdependence of ER α and EGFR signaling, which may be mediated by cytosolic and ligand-independent ER α . ER α is targeted to the cell membrane by palmitoylation of the ligand binding domain (LBD) and/or interactions with scaffolding proteins. This localization at the membrane likely contributes to direct crosstalk between ER α and membrane growth factor receptors, including EGFR [84, 85]. The presence of alternative forms of ER α ,

including ER α 36 and ER α 46, which preferentially localize to the membrane, may further increase opportunities for crosstalk [85]. Furthermore, estradiol, in an ER α -dependent manner, transactivates EGFR in MCF7 breast cancer cells by SRC-dependent activation of matrix metalloproteinases, thereby liberating HB-EGF to activate EGFR [86]. This mechanism, illustrated in Figure 1, represents another potential opportunity for non-genomic estrogen signaling crosstalk with EGFR, and suggests functional overlap of these pathways. However, these interactions have not been well studied in ovarian cancer models, and could represent an underappreciated mechanism of EGFR crosstalk with ER α in this context.

Transactivation of EGFR by estradiol is also shown to occur via membrane-bound GPER1 in multiple tissues [87]. Estradiol increases phospho-ERK1/2 in ER α -negative/GPER1-positive SKBR3 breast cancer cells, but not in ER α -negative/GPER1-negative MDA-MB-231 breast cancer cells. Transfection of GPER1 into MDA-MD-231 cells confers estrogen-responsiveness [41]. Furthermore, this response requires SRC and phosphorylated SHC-adaptor protein (Shc), and is dependent upon transactivation of EGFR via release of HB-EGF [41], similar to the mechanism observed for ER α -induced EGFR transactivation in MCF7 breast cancer cells by Razandi *et al.* [86]. These data demonstrate that estrogen signaling may work in synchrony with EGFR activation to promote breast cancer progression. Likewise, these signaling mechanisms may also be active in ovarian tumors.

EGFR and estrogen signaling crosstalk is further highlighted in ovarian cancer cells. Albanito *et al.* report that in BG-1 cells, both GPER1 and ER α are required for transactivation of EGFR and subsequent phosphorylation of ERK1/2, and suggest that the requirements for ER α and GPER1 may be cell type specific [52]. At least in some cases, it appears that ER α and GPER1 may function together to activate EGFR, since a physical interaction between ER α , GPER1 and EGFR is reported in endometrial and tamoxifen-resistant breast cancer cells [88]. Since tamoxifen is a mixed agonist/antagonist, it is tempting to speculate that tamoxifen may actually promote formation of this non-genomic ER complex.

The role of matrix metalloproteinases in the transactivation of EGFR by GPER1 hasn't been described in ovarian cancer, but seems plausible since GPER1 expression correlates with MMP-9 expression in ovarian tumors [56]. These results point to a role for GPER1 in regulating cancer cell migration and invasion, which is demonstrated in OVCAR5 cells [55]. Studies also show higher GPER1 expression in malignant ovarian tumors than in normal, benign, or "low-malignancy potential" tissue [56, 89], and report the association of GPER1 with reduced 5-year survival [89]. However, an independent study reports no correlation between GPER1 expression and survival [90], and Ignatov *et al.* find GPER1 expression to be positively associated with disease free survival in ovarian cancer [91]. Further studies will be required to determine if these seemingly discrepant outcomes are due to differences in study methodology, immunohistochemical technique, or histological subtype.

GPER1 may also regulate migration and proliferation by activating focal adhesion kinase (FAK) downstream of ERK1/2 and PI3K activation, which occurs in a SRC-dependent, ER α -independent manner in endometrial cancer cells [92]. Interestingly, tamoxifen also elicits this effect in these cells, suggesting another mechanism for tamoxifen resistance

where tamoxifen stimulates pro-tumorigenic pathways by exerting estrogen agonist effects on non-genomic signaling. Taken together, it is clear that more studies are required to address the potential overlap of estrogen and growth factor signaling via EGFR, and the effect of various anti-estrogens on non-genomic estrogen signaling.

Multiple reports show an inverse correlation between ER α levels and HER2 mRNA, protein, or copy number in endometrial, breast and ovarian tumors [93-96]. This inverse correlation between HER2 and ER α supports the notion that hyperactivation of HER2 receptors may play a role in the development of endocrine resistance. In support of this idea, MCF7 breast cancer cell xenografts upregulate growth factor signaling, including HER2, after long-term tamoxifen treatment [97], suggesting a compensatory mechanism which could again be exploited by combinatorial therapy. Concordantly, low levels of HER2 are seen in serous, endometrioid and mixed histology ovarian tumors with improved response to letrozole [9]. Clear cell carcinomas present with lower ER α and increased ER β and HER2-positivity compared to other types of EOC [93], suggesting an association between HER2 and the clear cell phenotype. In corroboration of these results, another study reported that mice with xenografts derived from a mixed clear cell/endometrioid ovarian tumor, HOX424, responded more strongly to anti-HER2 antibodies than mice with xenografts derived from serous tumors [98].

In estrogen-resistant SKOV3 ovarian cancer xenografts, areas with clear cell morphology are reduced upon treatment with anti-HER2 antibodies, along with a concomitant increase in ER α expression. Importantly, combination therapy with letrozole and the anti-HER2 antibody trastuzumab results in increased SKOV3 xenograft tumor regression in comparison with trastuzumab treatment alone, implicating the suppression of HER2 in restoration of anti-estrogen sensitivity [98]. In ER α -positive PEO4 ovarian cancer cells, estradiol-stimulated growth is accompanied by an immediate increase in phosphorylated forms of HER2, ERK, AKT and ER α [99]. It is possible that the eventual downregulation of ER α seen with HER2 constitutive activation could occur by way of a negative feedback mechanism, and thus contribute to anti-estrogen insensitivity. Alternatively, as suggested above, tumor cells could upregulate HER2 as a compensatory mechanism in the absence of productive ER α signaling. It will be important to determine the precise mechanisms underlying the inverse expression of HER2 and ER α in ovarian cancer.

2.2 Insulin-like Growth Factor Receptor

Insulin-like growth factor-I receptor (IGF-IR) functions as a transmembrane receptor tyrosine kinase (RTK) for insulin-like growth factor-I and -II (IGF-I, IGF-II). Multiple IGF binding proteins (IGFBPs) potentiate or inhibit the actions of IGF-I or IGF-II. IGF-IR ligand binding causes phosphorylation of insulin receptor substrate-1 and -2 (IRS1, IRS2), which promote growth by transmitting signals to MAPK/ERK, PI3K/AKT and JAK/STAT in cell type specific manners [100, 101]. In the normal ovary, the IGF-IR pathway is implicated in folliculogenesis, atresia, steroidogenesis, and oocyte maturation in synergy with gonadotropins [102], demonstrating that IGF signaling is important in the intraovarian milieu and could contribute to priming this site for future tumorigenesis. In ovarian tumors and cancer cell lines, IGF signaling regulates proliferation, invasion and metastasis [102].

Elevated serum levels of IGF signaling components detected by western blot or radioimmunoassay and intra-tumoral mRNA and protein levels detected by qPCR or enzyme-linked immunosorbent assay are associated with ovarian cancer progression and poor prognosis, particularly in pre- and perimenopausal women [103-110]; however, some conflicting reports exist [111, 112]. Additionally, Karasik *et al.* report a positive correlation between estradiol and IGF-I levels in invasive malignant cyst fluid from postmenopausal women [110]. Another study reports that IGF-IR signaling proteins correlate with response to the aromatase inhibitor, letrozole [108]. Crosstalk between IGF-IR and ER α is well-studied in breast cancer models, and is implicated as a mechanism of anti-estrogen resistance [101]. These studies could prove useful to inform future directions in ovarian cancer models.

A few studies report crosstalk between IGF signaling and estrogen signaling in ovarian cancer, including the effect of estradiol on IGF signaling components. One group shows that the phytoestrogens resveratrol and genistein inhibit estrogen-induced proliferation of ER α -positive BG-1 cancer cells by suppressing ER α and IGF-IR gene expression and ER α , phospho-IRS1, phospho-AKT and Cyclin D1 protein expression [29, 30]. The authors speculate that resveratrol acts by inhibiting ligand binding of ER α and by preventing ligand bound ER α from interacting with phospho-IRS1 [30]. They additionally show that genistein blocks estrogen-induced BG-1 xenograft progression by way of inhibition of ER α and IGF-IR crosstalk [29]. Another study investigated the effects of estradiol and the xenoestrogen bisphenol A (BPA) on BG-1 cells, revealing that the IGF-I activator IGFBP4 is significantly increased by treatment of either compound [113].

Furthermore, estradiol also increases the number of IGF-I receptors per cell by approximately 50% in cultures from HGSC mesenteric metastases [114]. A study that first reported IGFBP3 mRNA in human ovarian cancer samples shows that estradiol reduces levels of the IGF-I inhibitor, IGFBP3, in ER α -positive PEO4 cells [115]. In support of these studies, Walker *et al.* show that estradiol reduces IGFBP3 and IGFBP5 levels and increases IGFBP4 levels via ER α in PEO4 ovarian cancer cells, which is reversed by tamoxifen treatment. They further link this signature to sensitivity to the aromatase inhibitor letrozole, suggesting that blockade of estrogen production is effective in these tumors partially due to its inhibition of IGF signaling [108]. In parallel, reduced expression of the IGF-I inhibitor, IGFBP5, is a predictor of letrozole response in ovarian cancer patients [9]. Collectively, these studies demonstrate the stimulatory effect of estrogen on IGF signaling in ovarian cancer, and suggest that in cells with intact estrogen signaling, there may be activation of IGF-IR via transcriptional pathways. Conversely, in endocrine resistant cells, IGF-IR signaling may be constitutively active despite downregulation of ER α transcriptional programs.

Crosstalk between the IGF pathway and ER α is also demonstrated in breast cancer cells. IGF-I increases estrogen-dependent transcription [116, 117], increases expression of IGF-IR, IRS1, and IGF-II, and decreases levels of IGFBP3 and the scavenger receptor IGF-IIR [118]. The activation of ER α by IGF (and EGF, as previously discussed) is dependent upon the activator function-1 (AF-1) domain of ER α . IGF-IR activation stimulates ERK1/2 and AKT, which in turn phosphorylate ER α at serine-118 or serine-167, respectively, in the

AF-1 domain [44-46]. The physical association between ER α and IGF-IR is also demonstrated by co-immunoprecipitation of these proteins in the membrane [119]. Another study shows that estradiol rapidly activates the adaptor molecule Shc to facilitate its association with ER α , allowing this complex to associate with IGF-IR and activate MAPK/ERK [120]. Non-genomic crosstalk occurring at the membrane could contribute to endocrine resistance, as demonstrated in MCF7 breast cancer cell xenografts where phospho-IGF-IR is elevated in tamoxifen resistant tumors, despite continued suppression of IGF-IR gene expression by tamoxifen [121]. These mechanisms haven't been sufficiently explored in ovarian cancer, but could potentially contribute to the development of endocrine resistance upon constitutive activation of IGF signaling and reduced reliance on estrogen signaling to stimulate similar effects.

2.3 MAPK Signaling Pathway

Closely intertwined with ER α crosstalk with EGFR, HER2 and IGF-IR is the MAPK signaling pathway, which is an important downstream effector of RTK activation. The cascade relies on RAS and RAF to transmit signals that activate MEK1/2, which in turn phosphorylate extracellular signal-regulated kinases ERK1/2. The activated ERK dimer can regulate cytosolic targets or phosphorylate transcription factors in the nucleus [122]. Several lines of evidence illustrate potential crosstalk between MAPK/ERK and ER signaling, including the previously discussed interactions between growth factor receptors and estrogen signaling, since these receptors activate the MAPK/ERK pathway. Also previously discussed, MAPK can phosphorylate ER α at serine-118 in the AF-1 domain, resulting in activation of ER α [43, 44]. Very little research has been completed on specific molecular interactions between ERs and MAPK/ERK pathway proteins in ovarian cancer; however, as discussed below, MAPK/ERK inhibition is associated with reactivation of signaling via ER α and improved response to anti-estrogens, in parallel to the inverse relationship seen between HER2 and ER α .

In SKOV3 ovarian cancer cells, Hou *et al.* report that MEK inhibition causes ER α overexpression, phosphorylation at serine-118 and an increase in expression of ER α -regulated genes, including TFF1, previously noted for its correlation with improved response to anti-estrogens. Subsequently, combinatorial treatment with fulvestrant and a MEK inhibitor reduces ER α expression and suppresses feedback activation of EGFR and HER2 by the MEK inhibitor. The combinatorial treatment is also synergistically reduces proliferation in vitro and in SKOV3 xenografts [123]. It should be noted that SKOV3 cells are reported to contain a 32 base pair deletion in the gene for ER α , resulting in truncated protein and rendering these cells ER α -positive yet estrogen insensitive [124]. However, Hou *et al.* report estrogen dependency of their SKOV3 cells [122]. It is possible that variations in SKOV3 lines could exist as a result of genetic instability and clonal outgrowth of the cells. While their findings in SKOV3 cells may require further corroboration, these results are in agreement with a study done in breast cancer cells, where inhibition of MAPK/ERK results in increased expression of ER α and restoration of tamoxifen-sensitivity [125].

Again, this inverse relationship between ER α and constitutive MAPK activation could exist due to a negative feedback mechanism, explaining why inhibition of the dominant pathway

may restore estrogen signaling and thus endocrine sensitivity. The results presented here reiterate the point that constitutive activation of oncogenic signaling pathways reduces tumor cell reliance on estrogen signaling, since these pathways overlap functionally, rendering the tumors resistant to anti-estrogen therapy. Targeting these pathways appropriately may allow for increased anti-estrogen sensitivity in the treatment of ovarian cancer.

2.4 c-SRC

C-SRC encodes for a non-receptor tyrosine kinase that regulates proliferation, apoptosis, adhesion, migration and differentiation downstream of activated RTKs [126]. C-SRC is reported to be activated in a majority of serous ovarian tumors [127, 128]. As previously discussed, c-SRC appears to play an important role in estrogen-induced transactivation of EGFR [39, 41]. Studies in breast cancer cells demonstrate that ligand-bound ER α activates c-SRC, which in turn phosphorylates ER α and stimulates ER α -dependent transcription [129]. Moreover, the scaffold protein modulator of non-genomic action of estrogen receptor (MNAR), is required for ER α -SRC interaction in MCF7 breast cancer cells. Activation of c-SRC by estradiol leads to MNAR phosphorylation, promoting its interaction with the PI3K regulatory subunit p85, and subsequent activation of the PI3K/AKT pathway [84]. In addition, MNAR facilitates ER α /SRC interaction, which activates ERK and promotes ER α -dependent transcription [130]. Studies in breast cancer demonstrate improved efficacy of dual c-SRC and estrogen inhibition [131, 132]. Yeh *et al.* also report that a negative regulator of c-SRC, CSK tyrosine kinase, is required for fulvestrant-induced degradation of ER α and increased cell death in MCF7 and T47D breast cancer cell lines [133], further implicating c-SRC in regulating anti-estrogen response.

The role of c-SRC in anti-estrogen response in ovarian cancer has also been recently investigated. Simpkins *et al.* report c-SRC activation by estrogen in an anti-estrogen resistant ovarian cancer ascites-derived cell line, PEO1R. Inhibition of c-SRC with the ABL/SRC family member inhibitor sarcatinib reverses fulvestrant resistance in these cells and sarcatinib in combination with fulvestrant synergistically promotes cell cycle arrest and induction of autophagy. Importantly, this synergistic effect is also striking in PEO1R xenografts, where the authors note little effect with sarcatinib treatment alone and only slower growth with fulvestrant, while combination therapy causes tumor regression [128]. It is important to note, however, that sarcatinib inhibits all SRC/ABL family members. It appears that this pan-inhibition is essential for maximal fulvestrant re-sensitization, since limited results are observed when shRNA against c-SRC is used, due to a compensatory increase in other SRC family members [128]. These results suggest that the interactions between multiple SRC family members and ER signaling are apparently intertwined in a complex and oftentimes redundant weave of signaling downstream of RTKs such as EGFR and IGF-1R, and provide further evidence of ER α crosstalk with these pathways.

2.5 The PI3K Signaling Pathway

The PI3K pathway is also activated downstream of RTKs, and relies on phosphorylation of AKT, which in turn can phosphorylate many targets, including NF κ B, GSK3 β , MDM2 and mTOR. The mTOR kinase complex, the best described target of AKT signaling [134, 135],

is considered to be a master regulator of cellular metabolism/ribosomal biosynthesis, apoptosis, autophagy and cell cycle control [136]. Array Comparative Genomic Hybridization reveals frequent activation of the PI3K/AKT pathway in 93 ovarian tumors, and overexpression of a catalytic subunit of PI3K, PIK3CA, or phospho-AKT correlates with reduced survival in a panel of 522 serous ovarian tumors [137]. Furthermore, mTOR is aberrantly expressed or activated in serous and clear cell carcinomas [138-140], and correlates with phospho-AKT in an ovarian cancer tissue array [138]. Phosphatase and tensin homolog (PTEN), a negative regulator of mTOR, is frequently mutated in endometrioid ovarian tumors [141].

Again, much of what we know about PI3K/AKT and estrogen signaling crosstalk comes from studies in breast cancer. As previously mentioned, activated AKT can phosphorylate ER α at serine-167 in the AF-1 domain and increase ER α -dependent transcription [46]. Likewise, non-genomic estrogen signaling via membrane-bound ERs has the potential to activate multiple downstream signaling cascades, including PI3K/AKT. In HEC-1A endometrial cancer cells, GPER1 activates PI3K to promote cell proliferation [142]. Activation of the PI3K pathway in breast tumors is associated with reduced ER α levels and endocrine resistance [143]. Several studies demonstrate the dependence of estrogen-deprived breast cancer cells on PI3K pathway activation [144], and experimental and clinical studies show that combining PI3K inhibition with an anti-estrogen is more effective than either treatment alone [145]. These results are in agreement with other studies in breast cancer models showing that constitutive activation of growth factor receptor signaling cascades leads to reduced ER α levels and endocrine resistance [145], while inhibition of these pathways can re-sensitize cells to anti-estrogens [125]. Furthermore, these studies illustrate that estrogen deprivation or ER inhibition can produce acquired endocrine resistance by encouraging reliance on other oncogenic cascades. Studies on crosstalk between estrogen signaling and the PI3K pathway in ovarian cancer are limited, but demonstrate that estrogens can activate the PI3K/AKT pathway in ovarian cancer cells [146, 147]. However, further research is needed to determine if downregulation of estrogen signaling occurs with constitutive activation of PI3K, and if this effect can be reversed by inhibition of the PI3K pathway.

As with c-SRC inhibition, consideration of other compensatory mechanisms must be taken into account when selecting appropriate PI3K targeting drugs for study. A positive feedback loop signaling via the alternate mTOR complex, mTORC2, causes a compensatory activation of the PI3K pathway when mTORC1 is inhibited, indicating that inhibition of one component of the PI3K pathway may not be sufficient to produce a significant clinical benefit [144, 148]. A greater understanding of the complexities of estrogen signaling crosstalk with the PI3K/AKT/mTOR pathway in ovarian cancer is required in order to understand how it might be possible to improve patient and inhibitor selection to test combinatorial treatments that target multiple pathways.

2.6 NF κ B

Nuclear factor kappa B (NF κ B) is activated by signals from cytokines such as TNF α , and mediates inflammation, survival and proliferation via transcriptional regulation by NF κ B

dimers [149, 150]. Normally, NF κ B signaling is transient, due to the fact that it upregulates expression of its own negative regulators; however, in a chronically inflamed tumor environment, this signaling pathway can become constitutively active [150], as suggested by increased NF κ B activity in a syngenic mouse model of serous ovarian cancer at late stages of tumor progression [151]. Although it is not as well studied in relationship to ovarian cancer as some other signaling pathways, there are several lines of evidence connecting NF κ B to ovarian cancer. Barlin *et al.* show that the NF κ B network is over-represented in HGSC [152]. Another study finds that protein expression of a positive regulator of NF κ B, I κ B, correlates with poor OS, and that I κ B mediates adhesion and invasion of ovarian cancer cells [153].

The relationship between estrogen signaling and NF κ B signaling is complex. Several studies show that ERs and NF κ B can transrepress each other. ER α can suppress NF κ B in a variety of tissues by reducing NF κ B DNA binding, regulating levels of NF κ B signaling proteins, and modulating NF κ B co-activator functions [154]. An inverse correlation between estrogen signaling and NF κ B signaling is seen in breast cancer cells [155, 156]. In support of these results, re-expression of ER α in an ER α -negative breast cancer cell line, and in an ER α -positive, estrogen-independent breast cancer cell line, reduces NF κ B DNA binding and expression of NF κ B regulatory proteins, and ultimately causes a decrease in cell viability. Additionally, this study reports that the NF κ B subunit p50 associates with ER α on EREs, and that inhibition of NF κ B increases expression of estrogen responsive genes [157]. These data demonstrate that constitutive activation of NF κ B in cancer is one potential mechanism of endocrine resistance, and likely occurs due to direct transrepression of ER α , as opposed to a putative negative feedback mechanism seen with growth factor signaling.

On the other hand, Frasor *et al.* report a mixed relationship between estrogen signaling and NF κ B. They treated ER α -positive MCF7 breast cancer cells with estradiol, TNF α , or both, and measured gene expression profiles in these samples. In agreement with other studies, they find that estrogen represses approximately 41% of TNF α regulated genes, although this number may be underestimated due to the rapid actions of NF κ B. However, TNF α enhances estrogen regulation of 30% of estrogen-regulated genes, and an inhibitor of I κ B blocks the enhancement provided by TNF α , demonstrating the likelihood that it is dependent upon NF κ B. They identify a cluster of genes that exhibit synergy between estrogen and TNF α , and this set includes genes involved in transcriptional regulation, metabolism and apoptosis [158]. In another study, this same group further explores one of these genes, *ABCG2*, a drug transporter which may contribute to chemotherapy resistance, and find that TNF α enhances ER α occupancy at its ERE, which is dependent upon NF κ B binding to a latent NF κ B response element upstream of the ERE [36]. These studies illustrate the complexity of the crosstalk between ER α and NF κ B, which could be explored in ovarian cancer as well.

The crosstalk between estrogen signaling and NF κ B signaling provides opportunities for the development of endocrine resistance. Elevated expression of NF κ B family members is reported in anti-estrogen resistant breast cancer cell lines, and inhibition of NF κ B sensitizes these cells to anti-estrogens, suggesting that repression of estrogen signaling by NF κ B contributes to anti-estrogen resistance [159]. Another study reports that interferon-gamma (IFN γ) restored anti-estrogen sensitivity via IFN regulatory factor 1 (IRF1) suppression of

NF κ B signaling [160]. Thus, suppression of NF κ B signaling could potentially be an additional treatment option to explore in the context of anti-estrogen resistance.

To our knowledge, the only study investigating NF κ B and estrogen signaling crosstalk in ovarian cancer shows that human KGN and COV434 granulosa cancer cells are endocrine resistant due to constitutive activation of NF κ B [161]. However, further research is needed to investigate the role of NF κ B in EOC, either in the context of rendering cells resistant to anti-estrogenic silencing of ER α , or silencing potentially anti-proliferative functions of ER β . These studies would be useful to determine if constitutive activation of this pathway could be responsible for the development of anti-estrogen resistance in certain patients, particularly ER-positive patients with activated NF κ B.

2.7 NOTCH Signaling

NOTCH receptors (NOTCH1, 2, 3 and 4) are transmembrane proteins that interact with multiple ligands, resulting in cleavage of the intracellular domain of the receptor by γ -secretase. This intracellular domain (ICD) translocates to the nucleus and subsequently acts as a transcription factor with various cofactors to regulate differentiation, proliferation and apoptosis [162]. The Cancer Genome Atlas tumor database reveals a 22% incidence of NOTCH pathway alterations in 316 HGSC samples [163, 164], and NOTCH signaling is identified as a significantly altered subnetwork in an unbiased bioinformatic analysis [165].

Recent studies identify crosstalk between Notch signaling and ER α in breast and endometrial carcinoma, and implicate it in the development of endocrine resistance. In endometrial cancer cells, Wei *et al.* show that estrogen promotes NOTCH1 expression in an ER α -positive cell line only, and overexpression of ER α in an ER α -negative line allows these cells to upregulate NOTCH1 in response to estrogen. The estrogen-induced NOTCH1 expression and proliferation is inhibited by the anti-estrogen fulvestrant [166]. Overexpression of NOTCH1 or NOTCH4 ICD induces tamoxifen resistance in T47D breast cancer cells. Additionally, NOTCH4 correlates with expression of protein kinase C- α (PKC α), which is shown to cause tamoxifen resistance in breast cancer [167]. Furthermore, transcriptional analysis of ER α -positive T47D/NOTCH4-ICD overexpressing cells shows clustering with PKC α overexpressing cells, and combinatorial treatment with a γ -secretase inhibitor (GSI) plus tamoxifen synergistically inhibits xenograft tumor growth of these cells [168]. Rizzo *et al.* report similar results – NOTCH1 and NOTCH4 correlate with poor prognosis in breast cancer, and there is significantly more T47D xenograft tumor regression with GSI and tamoxifen co-treatment. Like Wei *et al.*, they also show that estradiol upregulates NOTCH1 expression in ER α -positive cells only, but conversely demonstrate an overall reduction in Notch signaling via downregulation of NOTCH4 and increased cytoplasmic localization of NOTCH1 [169]. Their results again suggest an inverse relationship between NOTCH and estrogen signaling which parallels that seen with other oncogenic signaling cascades.

Further supporting this notion, another study finds that endocrine resistant MCF7 breast cancer cells develop epigenetic silencing of classical ER α -dependent signaling, along with activation of NOTCH signaling. Moreover, the authors report that NOTCH3, via transcriptional regulation of PBX1, regulates expression of genes associated with endocrine

resistance in breast cancer cells and that GSI treatment of these cells reverses this endocrine resistant gene signature [170]. Conversely, a ligand-independent ER α /NOTCH1 crosstalk is reported by Hao *et al.* where activation of NOTCH1 signaling in MCF7 cells increases ER α -dependent transcription in the absence of estradiol. This activation is dependent upon IKK α interaction with an ER α /NOTCH1-ICD transcriptional complex, where NOTCH1-ICD is crucial for IKK α -ER α complex nuclear localization [171]. Collectively, these data illustrate the potential for both a positive and negative relationship between NOTCH and estrogen signaling pathways, suggestive of multiple potential mechanisms of endocrine resistance that could be dependent on cellular context. Further research towards a better understanding of these phenomena may illuminate mechanisms most relevant to endocrine resistance in ovarian tumors.

Interestingly, although NOTCH signaling is increasingly studied in ovarian cancer [172-178], we were unable to identify studies that investigate crosstalk between NOTCH signaling and estrogen signaling in ovarian cancer. The studies from breast and endometrial cancer could potentially help shape future studies in ovarian cancer models, although differences might exist between these tumor types. For instance, the NOTCH1-PBX1 endocrine resistant gene signature is not predictive of outcome in ovarian cancer [170]. Also, NOTCH4, which is known to play a role in breast cancer, may not be as important in ovarian cancer, where *NOTCH3* is the most frequently amplified NOTCH receptor [163, 164]. These differences highlight the need for further research in ovarian cancer, since breast cancer studies may not always be extrapolated to ovarian cancer.

3. Conclusions

To conclude, several oncogenic pathways are activated in ovarian cancer that crosstalk with estrogen signaling, which could potentially lead to anti-estrogen resistance. These studies and their implications are summarized in Table 2, which illustrates the similarities in crosstalk and endocrine resistance mechanisms between ovarian and other estrogen-responsive cancers that were outlined in Table 1. Figure 1 graphically illustrates interactions between estrogen signaling and oncogenic cascades which have been discussed herein. These do not represent all potential actions of ERs, but rather illustrate the complexity of estrogen signaling that should be considered when employing anti-estrogen therapy in ovarian cancer.

Overall, a trend seems apparent where constitutive activation of oncogenic pathways eventually leads to downregulation of ER α levels/suppression of ER α -dependent signaling, or to occur following inhibition of classical estrogen signaling, thereby producing endocrine resistance. Transrepression of ERs by NF κ B can produce the same effect if NF κ B is chronically activated in the inflammatory tumor microenvironment. The transcriptional regulation of genes involved in endocrine resistance, which could occur by way of hyperactive NOTCH signaling, could also be a contributing factor. Moreover, an increase in tamoxifen agonist activity at AP-1 promoters or via crosstalk with non-genomic signaling pathways, despite continued suppression of classical genomic ER signaling, is another potential mechanism for anti-estrogen resistance. The activation of ligand-independent ER actions, which play an important role in the oncogenic pathways discussed, may also be an

underappreciated mechanism for anti-estrogen resistance in ovarian cancer. The non-classical and non-genomic mediated transcriptional actions of ER α are likely involved in the development of anti-estrogen resistance, and gaining a better understanding of the transcriptional profiles mediated by these pathways will be important for our understanding of this process. Although many of these mechanisms have been more thoroughly investigated in breast cancer, their relevance to ovarian cancer is less well established.

Although this review focused on selective pathways frequently discussed in breast and ovarian cancer, other pathways should also be considered. Wnt/ β -catenin signaling, which potentially plays a role in endometrioid EOC [179] and in granulosa cell tumors [180], could also crosstalk with estrogen signaling, and may need to be explored. The role of other membrane receptors (fibroblast growth factor receptor [FGFR], platelet-derived growth factor receptor [PDGF-R], GPCRs and cytokine receptors) could be important, since these also activate tyrosine kinase cascades, as could stress-induced MAPK signaling via JNK or p38-MAPK be explored. Leptin signaling, which is implicated in ovarian cancer [181], can also crosstalk with estrogen signaling in ovarian cancer models via the JAK/STAT pathway [51, 147]. In order to effectively use information from these studies, careful patient selection may be required to identify tumors which could respond to hormonal inhibition or combinatorial treatment with small molecule inhibitors. However, the analysis of estrogen signaling crosstalk is complicated by the crosstalk that occurs within these other oncogenic pathways themselves. Furthermore, drawbacks to targeted therapeutic approaches need to be considered going forward. Ovarian tumors present with a great deal of heterogeneity due to clonal outgrowth [182] and infiltration of stromal cells and tumor-associated fibroblasts, adipocytes and immune cells [183-186], making successful targets more difficult to identify. Xenograft models can better account for this tumor microenvironment and heterogeneity than cell cultures. Towards this end, Baratta *et al.* have developed an in vivo screen using serous ovarian cancer cell xenografts “barcoded” for shRNAs against kinases and potential oncogenes [187]. However, limitations to this approach still exist in that results cannot be generalized to all cell lines and the cells are still passaged in vitro prior to xenografting.

It is also important to continue working to understand targets and mechanisms of estrogen signaling in the ovary, which contribute to establishment of the ovarian milieu that is supportive of tumor growth. We have previously identified the TFIID subunit TAF4B as an ovarian-specific estrogen response gene, which is normally regulated by ER β in granulosa cells, but can also be regulated by ER α in the absence of ER β [188]. We found *Taf4b* expression to be upregulated in epithelial-derived, estrogen-supplemented mouse ovarian tumors [188]. Moreover, the TFIID complex was identified as a significantly altered subnetwork in HGSC [165]. Learning more about the normal functions of estrogen signaling in the ovary could also give us clues as to how this environment supports tumorigenesis. However, it may be important to stratify the mechanisms by which estrogen signaling contributes to ovarian tumor initiation versus progression. If indeed there is an activation of non-classical, non-genomic or ligand-independent ER-mediated pathways that follows anti-estrogen treatment, despite continued repression of classical signaling, there may be elements of classically-mediated ER signaling that are supportive of tumor initiation yet do not play a role in tumor progression.

Much remains to be answered in regards to the importance of estrogen signaling in ovarian cancer. The limited clinical benefit achieved with anti-estrogen therapies is without a doubt a concern, but the possibility remains that if we can improve patient selection for therapy, employ appropriate combinatorial therapy, or identify improved markers for earlier detection, estrogen signaling may still prove to be an effective target in a subset of ovarian tumors.

Acknowledgments

We thank Barbara C. Vanderhyden for productive collaborations that provided the inspiration for this manuscript, for her continued support and guidance, and for helpful insight in the preparation of this manuscript.

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Highlights

- We provide an overview of estrogen signaling in ovarian cancer.
- We discuss clinical benefits and challenges to anti-estrogen therapies.
- We review estrogen signaling crosstalk with major oncogenic signaling cascades.
- We provide insights on overarching mechanisms of endocrine resistance.

Figure 1.

Estrogen receptors (ERs; depicted in red) crosstalk with oncogenic signaling cascades. Cascades are simplified for clarity and to draw attention to examples of their interactions with ERs. TAM = tamoxifen; E2 = estradiol; γ -sec = γ -secretase; NICD = NOTCH intracellular domain.

Table 1

Estrogen Signaling Crosstalk in Other Estrogen-dependent Cancers

	Crosstalk	Implications for endocrine resistance
EGFR	<ul style="list-style-type: none"> •ERα is required for EGFR activation [80]. •Estradiol transactivates EGFR via ERα and/or GPER1 via activation of MMPs and liberation of HB-EGF in MCF7 cells [41, 86]. •TAM stimulates GPER1 activation of FAK downstream of MAPK and PI3K pathways in endometrial cancer cells [92]. 	<ul style="list-style-type: none"> •Functional overlap of ligand-free or non-genomic ER signaling and EGFR signaling •Constitutive activation of EGFR $\rightarrow$ reduced reliance on non-genomic ER growth promoting effects •TAM may increase non-genomic ER signaling crosstalk with EGFR
HER2	<ul style="list-style-type: none"> •Inverse correlation between ERα and HER2 levels in endometrial and breast cancer [94, 95]. •MCF7 xenografts that express high levels of ERα upregulate EGFR, HER2 and p-p38 in response to several months of TAM treatment [97]. 	<ul style="list-style-type: none"> •Negative feedback mechanism that downregulates ERα upon constitutive activation of HER2 •Compensatory increase in HER2 in the absence of active E2 signaling •Inverse relationship could be exploited by inhibiting HER2, which may restore ERα signaling, combined with inhibition of ERα to increase the efficacy of HER2 inhibition.
IGF-IR	<ul style="list-style-type: none"> •IGF-I increases E2-dependent transcription [117]. •IGF-IR activation stimulates MAPK and PI3K pathways [101], which activate ERα in ligand-independent manners [44, 46]. •Estradiol activates Shc to facilitate association of ERα/Shc complex with IGF-IR [120]. •Estradiol increases expression of IGF signaling effectors and decreases IGF inhibitors [reviewed in 118]. •IGF-IR pathway is activated in TAM resistant MCF7 xenografts, despite continued suppression of IGF-IR gene expression by TAM [121]. 	<ul style="list-style-type: none"> •Compensatory upregulation of the IGF-IR pathway upon TAM inhibition of classical E2 signaling •Inhibition of genomic ER mechanisms may allow dominance of non-genomic ER mechanisms that crosstalk with IGF-IR
MAPK/ERK	<ul style="list-style-type: none"> •Inhibition of MAPK/ERK increases expression of ERα and restores TAM sensitivity in breast cancer cells [125]. 	<ul style="list-style-type: none"> •Constitutive activation of MAPK $\rightarrow$ reduced reliance on ERα signaling •MEK inhibition can be used to restore E2 signaling, combined with an anti-E2 to increase the efficacy of the MEK inhibitor
c-SRC	<ul style="list-style-type: none"> •Ligand-bound ERα activates c-SRC, and c-SRC phosphorylates ERα to promote E2-dependent transcription and ERα proteolysis [129]. •c-SRC is involved in mediating non-genomic E2 signaling crosstalk with growth factor signaling cascades [84, 130]. •Improved efficacy of dual c-SRC and E2 signaling inhibition in breast cancer models [131, 132]. •CSK tyrosine kinase, a negative regulator of c-SRC, is required for fulvestrant-induced degradation of ERα, leading to increased cell death in breast cancer cell lines [133]. 	<ul style="list-style-type: none"> •Requirement of c-SRC inhibition for fulvestrant-induced degradation of ERα $\rightarrow$ interaction of c-SRC with ERα protects it from degradation? •Certain non-genomic mechanisms involving c-SRC and tyrosine kinase cascades might not be inhibited by fulvestrant? •Inhibition of c-SRC in combination with E2 signaling $\rightarrow$ more complete blockade of genomic and non-genomic E2 signaling pathways
PI3K/AKT	<ul style="list-style-type: none"> •GPER1 activates PI3K in endometrial cancer cells [142]. •Activated AKT phosphorylates ERα and increases ERα-dependent transcription [46]. •Activation of PI3K in breast cancer cells is associated with reduced ERα levels and endocrine resistance [reviewed in 145]. •E2-signaling deprivation causes dependence on PI3K pathway activation [reviewed in 144]. •Combinatorial PI3K inhibition with anti-E2 is more effective than either treatment alone [reviewed in 145]. 	<ul style="list-style-type: none"> •PI3K functionally overlaps with non-genomic E2 signaling pathways •Constitutive activation of PI3K $\rightarrow$ reduced reliance on ERα •Inhibition of E2 signaling $\rightarrow$ increased dependency on PI3K signaling •PI3K inhibition may lead to restoration of E2 signaling, which can be suppressed by anti-E2s
NFKB	<ul style="list-style-type: none"> •ERα suppresses NFKB signaling in a variety of tissues [reviewed in 154]. •Inverse correlation between NFKB signaling and E2 signaling in breast cancer cells [155, 156]. •Suppression of NFKB signaling restores anti-E2 sensitivity [157, 159, 160]. 	<ul style="list-style-type: none"> •NFKB and ERs frequently transrepress each other •Constitutive activation of NFKB $\rightarrow$ suppression of E2 signaling/anti-E2 resistance
NOTCH	<ul style="list-style-type: none"> •Inverse relationship frequently reported between activated NOTCH signaling and E2 signaling [169, 170]. •Activated NOTCH signaling is associated with endocrine resistance in breast cancer cells [167, 170]. •Positive relationship reported between NOTCH and ligand-independent ERα transcription in MCF7 cells [171]. •Combinatorial treatment of anti-E2 resistant breast cancer cells with a GSI plus TAM synergistically inhibited xenograft growth [168, 169]. 	<ul style="list-style-type: none"> •Both negative and positive relationships reported between NOTCH signaling and E2 signaling •Activated NOTCH may be associated with silencing of ERα transcription, or increase expression of endocrine resistance genes •Combinatorial treatment $\rightarrow$ silence both oncogenic NOTCH signaling and restore endocrine sensitivity

Crosstalk	Implications for endocrine resistance
<ul style="list-style-type: none">• NOTCH3 regulates expression of endocrine resistance genes in breast cancer cells [170].	

Table 2

Estrogen Signaling Crosstalk in Ovarian Cancer

	Crosstalk	Implications for endocrine resistance
EGFR	<ul style="list-style-type: none"> • Ligand-free ERα enhances signaling via EGFR [81]. • GPER1 and ERα participate in transactivation of EGFR and MAPK pathway activation [52]. 	<ul style="list-style-type: none"> • Functional overlap of ligand-free or non-genomic ER signaling and EGFR signaling • Constitutive activation of EGFR $\rightarrow$ reduced reliance on non-genomic ER growth promoting effects • TAM may increase non-genomic ER signaling crosstalk with EGFR
HER2	<ul style="list-style-type: none"> • Estradiol activates HER2 [99]. • Inverse correlation between ERα and HER2 levels in ovarian cancer [93, 96]. • Low levels of HER2 associated with improved response to letrozole [9]. • Inhibition of HER2 in xenografts increases levels of ERα [98]. • Combination therapy with an aromatase inhibitor and anti-HER2 antibodies causes increased xenograft regression compared antibodies alone [98]. 	<ul style="list-style-type: none"> • Negative feedback mechanism that downregulates ERα upon constitutive activation of HER2? • Compensatory increase in HER2 in the absence of active E2 signaling • Inverse relationship can be exploited by inhibiting HER2 to restore ERα signaling, followed by inhibition of ERα to increase the efficacy of HER2 inhibition
IGF-IR	<ul style="list-style-type: none"> • Estradiol has a stimulatory effect on IGF-IR signaling by increasing levels of positive regulators of the pathway and decreasing expression of negative regulators. This gene expression signature is predictive of improved response to aromatase inhibitors [9, 108, 113-115]. • PhytoE2s inhibit E2-induced proliferation of ovarian cancer cells and xenografts by suppressing ERα and IGF-IR crosstalk [29, 30]. 	<ul style="list-style-type: none"> • Functional overlap of non-genomic E2 signaling and EGFR • Constitutive activation of IGF-IR $\rightarrow$ reduced reliance on non-genomic E2 signaling • TAM may enhance signaling through non-genomic pathways that cross-talk with IGF-IR
MAPK/ERK	<ul style="list-style-type: none"> • MEK inhibition causes ERα overexpression and activation, and increases transcription of ERα-regulated genes [123]. • Inhibition of MEK and ERs in combination reduces ERα expression, suppresses feedback activation of EGFR and HER2, and synergistically reduces proliferation of xenografts [123]. 	<ul style="list-style-type: none"> • Constitutive activation of MAPK $\rightarrow$ reduced reliance on ERα signaling • MEK inhibition can be used to restore E2 signaling, combined with an anti-E2 to increase the efficacy of the MEK inhibitor
c-SRC	<ul style="list-style-type: none"> • E2 activates c-SRC in an anti-E2 resistant ovarian cancer cell line [128]. • Inhibition of SRC/ABL family members with sarcatinib in combination with fulvestrant synergistically promotes cell cycle arrest and autophagy in xenografts [128]. 	<ul style="list-style-type: none"> • E2 promotes non-genomic pathways involving s-SRC via mechanisms that are resistant to anti-E2s. • Inhibition of these pathways may be necessary to restore anti-E2 sensitivity in certain patients.
PI3K/AKT	<ul style="list-style-type: none"> • E2s activate the PI3K pathway [146, 147]. 	<ul style="list-style-type: none"> • Activation of the PI3K pathway by E2s mimics that seen with several other oncogenic pathways • Mechanisms regulating endocrine resistance may be applied to PI3K signaling as well
NFKB	<ul style="list-style-type: none"> • NFKB transrepresses ERβ activity in human granulosa cancer cell lines [161]. 	<ul style="list-style-type: none"> • NFKB inhibition may re-activate anti-proliferative ERβ-mediated E2 signaling in some tumor • NFKB inhibition may also re-activate ERα-dependent signaling, which could then be targeted by ERα-specific antagonists
NOTCH	No studies reported.	N/A