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Advanced concepts in estrogen receptor biology and breast cancer endocrine resistance: implicated role of growth factor signaling and estrogen receptor coregulators

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Abstract Estrogen receptor (ER), mediating estrogen-signaling stimuli, is a dominant regulator and a key therapeutic target in breast cancer etiology and progression. Endocrine therapy, blocking the ER pathway, is one of the most important systemic therapies in breast cancer management, but de novo and acquired resistance is still a major clinical problem. New research highlights the role of both genomic and nongenomic ER activities and their intimate molecular crosstalk with growth factor receptor and other signaling kinase pathways in endocrine resistance. These signaling pathways, when overexpressed and/or hyperactivated, can modulate both activities of ER, resulting in endocrine resistance. Thus, these signal transduction receptors and signaling molecules may serve as both predictive markers and novel therapeutic targets to circumvent endocrine resistance. Compelling experimental and clinical evidence suggest that the epidermal growth factor/HER2/neu receptor (EGFR/HER2) pathway might play a distinct role in endocrine resistance, and especially in resistance to selective estrogen receptor modulators (SERMs) such as tamoxifen. Results from preclinical studies of treatment combinations with various endocrine therapy drugs together with several potent anti-EGFR/HER2 inhibitors are very promising, and clinical trials to see whether this new strategy is effective in patients are now ongoing.

Keywords Breast cancer endocrine therapy · Estrogen receptor · Resistance · EGFR/HER2 · Crosstalk

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Introduction: endocrine therapies and resistance

The report from 1896 by Dr. Beatson [8] showing that patients with inoperable breast tumors frequently respond to bilateral oophorectomy early indicated the importance of the association between estrogen and breast cancer, and marked the beginning of the successful journey toward modern management strategies for this malignant disease in which endocrine therapy still occupies a central position. Endocrine therapy was indeed the first targeted therapy conceptualized in the oncology field, long before the target and the therapeutic agents were known [45]. With the identification and cloning of the first receptor, estrogen receptor (ER)α [26], and the recent cloning of the second, ERβ [40], it is now established that most, if not all, of estrogen's biological and physiological effects in both normal and pathological settings are mediated by these two allied receptor subtypes.

The subsequent substantial basic, experimental, and clinical research over the past half century has focused primarily on the therapeutic potential and activity of ER as a ligand-dependent transcription factor in the nucleus. This research has provided indispensable knowledge about the structure and function relationship of the receptor and its interaction with and modulation by both cellular accessory (coregulator) proteins and various ligands including antiestrogens (for a review see [75] and references therein). More recent studies have revealed an additional ER activity that occurs outside the nucleus and involves multiple intimate interactions between ER and growth factor receptors and other signal transduction molecules. Overall, this massive research

has tremendously contributed to the successful development of new strategies and drugs to inhibit the ER pathway. These agents are continually maturing and moving to the clinical setting in the arena of endocrine therapy [10, 90]. The nonsteroidal antiestrogen tamoxifen, which competitively antagonizes ER, has become the "gold standard" endocrine therapy in all stages of ER-positive breast cancer [65]. Several other selective estrogen receptor modulators (SERMs) such as arzoxifene, with more favorable tissue-specific ER effects than tamoxifen [59], as well as selective ER downregulators (SERDs) such as the potent antiestrogen fulvestrant (Faslodex, ICI 182,780), which target and degrade the ER [60, 71], are already in clinical use or at the final stages of clinical development. Parallel to these endocrine strategies, the alternative hormonal tactic of estrogen withdrawal has also progressed, and the third generation of aromatase inhibitors (AIs) (i.e., letrozole and anastrozole) is already challenging tamoxifen as a first-line endocrine therapy for postmenopausal patients [7], although a longer follow-up for these studies, at least in the adjuvant setting, is still needed [92].

Endocrine therapy is currently the most important systemic treatment of ER-positive breast cancer at all stages. The significant decrease of national breast cancer mortality rates over the past 15 years is certainly attributed, at least in part, to the widespread use of tamoxifen during the last 30 years [20]. In the adjuvant setting, a long-term follow-up of patients treated by endocrine therapy suggests that this therapy is curative in many patients [20]. However, despite this encouraging clinical progress, resistance to various endocrine treatments, both de novo and acquired, is still a big problem that limits the use of this type of therapy. Only half of all ER-positive tumors are at first responsive to antiestrogens such as tamoxifen, and even initially responding tumors, at least in the metastatic setting, eventually develop resistance to endocrine treatment, leading to tumor progression and ultimately to patient death [65]. Often, however, tumors resistant to one form of endocrine therapy may still be responsive to other types of endocrine treatments [12]. Clinically, this observation is very important and is the basis for the current strategy in advanced breast cancer, and perhaps in the future also in the adjuvant setting, for the use of sequential endocrine therapies [12]. In the context of this paper, however, it also has a mechanistic significance, suggesting that these resistant tumors are still dependent on the ER pathway and that mechanisms of resistance to various types of endocrine treatment may be somewhat specific.

The underlying principles for endocrine resistance are not yet precisely known. However, compelling evidence suggests that crosstalk between ER and various growth factor and cellular kinase signaling pathways, and especially the epidermal growth factor receptor/HER2/neu receptor (EGFR/HER2) pathway, has a major role in endocrine resistance [64, 76]. Better understanding of the resistance mechanisms to the different types of endocrine therapy and identifying predictive biomarkers for treatment sensitivity and resistance are crucial for rationalizing and individualizing the optimal endocrine therapy for patients and for developing novel strategies to overcome resistance.

ER structure and function in breast cancer pathogenesis and in endocrine therapy

ERs and their estrogen ligands are believed to be the key players in the etiology and progression of breast cancer. Numerous clinical and experimental studies based on genetic, molecular, and histological approaches have repeatedly confirmed this crucial role for ERα (summarized in [75]). The role of ERβ in breast cancer, in contrast, is still controversial [83], although several experimental studies indicate that ERβ can antagonize ERα activity [29] and recent clinical evidence suggests that reduced levels of ERβ protein are associated with resistance to tamoxifen therapy [31]. Advanced molecular and imaging research has revealed that mechanisms of ER action are much more diverse and complex than previously believed. New insights into ER signaling and its operation might explain the pleiotropic effects of the ER pathway in response to multiple signaling inputs, and therefore why ER is such an important factor and therapeutic target in breast cancer.

Nuclear genomic ER activity

ER is predominantly a nuclear protein and shares a common structural and functional organization with all other nuclear receptors (reviewed in [75]). Through its genomic nuclear activity, also known as nuclear-initiated steroid signaling (NISS) [62], ER functions as a ligand-dependent transcription factor and regulates

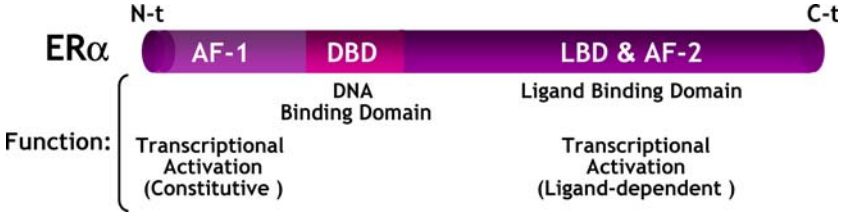

Fig. 1 ERα structure and function organization. ER is a composite of multiple domains. Shown here are the DNA-binding domain (*DBD*), ligand-binding domain (*LBD*), and two major transcriptional activation function (*AF*) domains, AF-1 and AF-2, which reside respectively in the ER amino (*N-t*) and carboxyl (*C-t*) termini

expression of a variety of genes. Many of these gene products directly promote breast cancer cell proliferation and survival. Examples are the insulin-like growth factor 1 receptor (IGFR), the cell cycle regulator cyclin D1, and the antiapoptotic factor Bcl-2 ([37, 48, 73, 76] and references therein). Others are proteins engaged in tumor growth and progression and include factors that are involved in tumor invasion and metastasis or in the activation of tumor stromal components such as the angiogenic vascular endothelial growth factor (VEGF) ([37, 76] and references therein). The antitumor effect of most endocrine therapy agents is largely mediated by countering or eliminating the effect of estrogen on the transcription of this type of genes.

ER is a composite of multiple domains including a DNA-binding domain (DBD) residing in the core of the protein and two major transcriptional activation function (AF) domains, the constitutively active, ligand-independent AF-1 and the ligand-dependent AF-2, residing in the ER amino- and carboxyl-termini, respectively (Fig. 1). Posttranslational modifications, mainly via phosphorylation, of AF-1 can potentiate its constitutive activity [1] and thus seem responsible, in part, for the agonist activity of mixed antiestrogens (i.e., SERMs) [55] and for ligand-independent ER activity. Thus AF-1 phosphorylation and activity status in a given breast tumor is probably an important feature in its sensitivity or resistance to endocrine therapies.

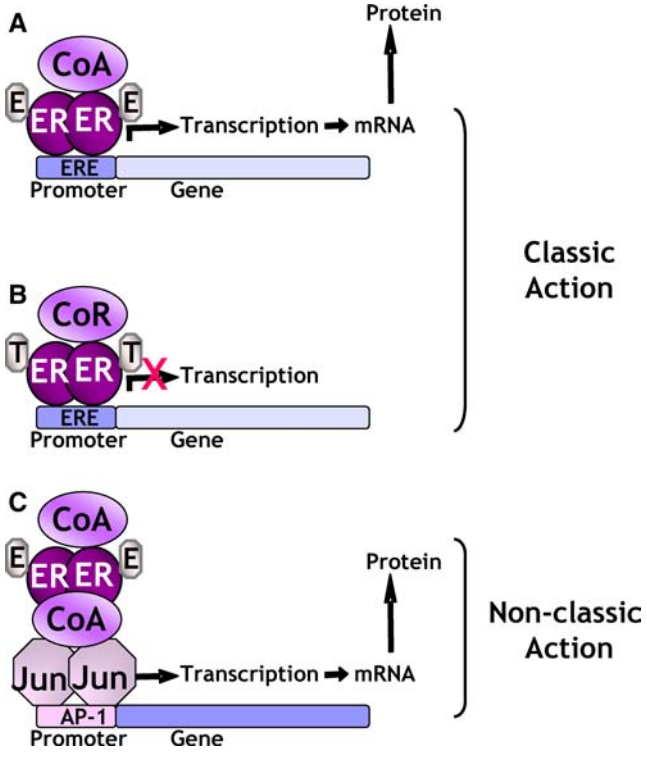

Fig. 2 Nuclear genomic (NISS) ER activity. ER, in its classic action (**A**, **B**), directly binds to DNA sequences called estrogen response elements (*EREs*) residing in the promoter region of target genes, and by recruiting coregulatory proteins regulates gene transcription. Estrogen (E)-bound ER generally recruits coactivators (*CoA*) to induce gene transcription (**A**), while estrogen antagonists such as tamoxifen (*T*) mostly lead to ER association with corepressor complexes (*CoR*), thereby shutting off gene transcription (**B**). In its non-classic action (**C**), ER regulates gene transcription via protein–protein interaction (e.g., with the Fos/Jun family members) at DNA sites responsive to other transcription factors such as AP-1. Together, all of these nuclear ER genomic activities are also called nuclear-initiated steroid signaling (*NISS*)

Partially overlapping with AF-2 is the ligand-binding domain (review in [75]). Ligand binding to ER induces a specific conformational change in the receptor, releases it from an inhibitory complex consisting of several chaperone proteins [33], and triggers receptor dimerization [75]. This change further facilitates the binding of coregulatory proteins [77] that alter ER transcriptional activity on specific consensus DNA elements [also known as estrogen response elements (EREs)] present in the promoter regions of target genes (classic action; Fig. 2A, B). Depending on the nature of the ligand, these coregulatory proteins either enhance (coactivators) or repress (corepressors) ER transcriptional activity [56, 77] via the recruitment of other proteins to the promoter transcriptional complexes that can modulate the chromatin structure by various enzymatic activities such as acetylating histones [56] (Fig. 2A, B). ER can also regulate gene transcription at DNA sites responsive to other transcription factors [44]. In this mode of action, ER, tethering indirectly to promoter sequences via protein–protein interaction, operates more as a coregulator to facilitate the recruitment of specific transcription complexes [44] (non-classic action; Fig. 2C).

While estrogen-bound ER generally recruits coactivators to induce gene transcription, estrogen antagonists, via stimulating a different receptor conformation, mostly lead to ER association with corepressor complexes, thereby shutting off gene transcription (Fig. 2A, B). However, SERMs such as tamoxifen and raloxifene, which by their nature possess mixed agonist/antagonist activity, may either stimulate or antagonize ER function depending on the tissue, cell, and gene context [66]. Adjustments of the estrogen agonist properties of these agents can be adapted over time, and may be an important factor in resistance to certain forms of endocrine therapy. Additionally, recent research suggests that in response to estrogen, ER is also able to inhibit expression of many genes [17]. Whether this downregulation of gene expression is a direct effect that is mediated by the recruitment of corepressor complexes to estrogen-bound ER at selected gene promoter sequences remains to be defined.

Many coregulatory proteins may be present at rate-limiting levels in the nucleus so that changes in their level of expression and/or activity can lead to alterations of ER signaling. In particular, overexpression of coactivators and downregulation of corepressors can negate the inhibitory effects of endocrine therapy, especially in the case of SERMs [46, 81, 86]. The SRC family of coactivators affects a variety of transcription factors including nuclear receptors. Members of this family share com-

mon structures and functions and seem to play important roles in modulating ER activity. When overexpressed in cultured cells, SRC coactivators enhance the agonist activity of SERMs such as tamoxifen on ER-dependent gene transcription, suggesting their potential role in breast cancer endocrine resistance [81, 91].

The third member of the SRC family of coactivators is AIB1 (also known as SRC-3, RAC3, ACTR and p/CIP). AIB1 is overexpressed in >50% and is gene-amplified in about 5–10% of breast tumors [3, 52, 61], strongly suggesting its important role in breast cancer pathogenesis. Genetic experiments in mice clearly demonstrate the absolute requirement of the mouse homolog of AIB1, SRC-3, for normal development of mammary glands. Even more important, a recent report on an AIB1-transgenic model has shown that AIB1 overexpression in mammary epithelial cells leads to the development of malignant mammary tumors, thus establishing the oncogenic nature of AIB1 in breast cancer [87]. Considering the above, we recently conducted a retrospective clinical study to determine the predictive value of AIB1 for response to tamoxifen treatment in breast cancer patients [67]. Interestingly, our findings indeed demonstrate, as we expected, that high AIB1 expression in patients who received tamoxifen adjuvant therapy was associated with inferior disease-free survival (DFS), which is indicative of tamoxifen resistance [67].

Nongenomic (membrane) ER activity

Estrogen and other steroid hormones, in addition to their role as direct modulators of gene transcription mediated by their classic nuclear receptors, can also exert rapid stimulatory effects on a variety of signal transduction pathways and molecules. This rapid and nongenomic activity, also called MISS for membrane-initiated steroid signaling [62], is independent of gene transcription and probably initiated at least in part outside the nucleus. The identity of the receptors responsible for this rapid steroid activity is not completely resolved. In the case of estrogen signaling, most evidence suggests that this activity is mediated by a small fraction of the traditional ER or perhaps by a closely related short-form splicing/translational variant [23, 51] most probably localized in the vicinity of the plasma membrane. However, a role for a genetically unrelated membrane ER has also been postulated [24]. It is now evident that in some tissues, such as bone and endothelial cells, nongenomic activity is the predominant type of estrogen signaling [39, 51].

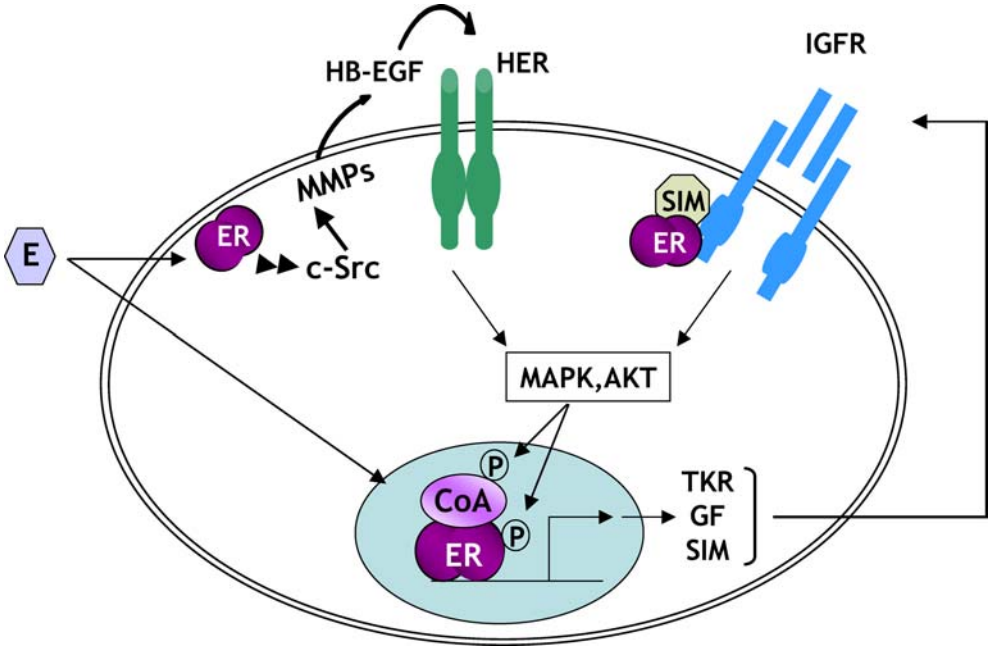

Fig. 3 Nongenomic (MISS) ER activity and its complementary interplay with nuclear genomic ER activity. A small subset of the cellular pool of ER, localized near the plasma membrane, can associate in response to estrogen (*E*) with growth factor tyrosine kinase receptors (*TKRs*) such as IGFR and with signaling intermediate molecules (*SIM*). This interaction activates TKR signaling and its downstream kinases, e.g., p42/44 MAPK (*MAPK*) and AKT. Also, acting as a G-protein-coupled receptor, estrogen (*E*)-bound ER triggers a signaling process that involves c-Src and activation of metalloproteinases (*MMPs*), which then cleave and liberate heparin-binding EGF (*HB-EGF*) that stimulates TKR EGFR (*HER*) and its signaling pathway. TKR-induced kinases phosphorylate (*P*) ER and its coactivators and thereby potentiate genomic ER activity, which results in enhanced gene expression including of genes encoding TKRs, growth factors (*GF*), and signal interaction molecules (*SIM*). These gene products in turn further augment GF-TKR signaling, thus completing the complementary cycle between the two activities of ER. The nongenomic activities of ER are also called membrane-initiated steroid signaling (*MISS*)

The significance of nongenomic ER activity in mediating estrogen signaling to promote cell proliferation and survival in breast cancer cells has also been established. A number of studies using biochemical, immunohistological and genetic methods have further documented the existence and function of cytoplasmic and membrane ER in breast tumor cells [49, 50]. Several mechanisms by which ER couples with components of signaling complexes and triggers their responses have been proposed (Fig. 3). ER, in response to estrogen, can directly and indirectly interact with several growth factor tyrosine kinase receptors such as HER2 and IGFR [15, 35] and thereby activate their kinase/phosphorylation cascades. In HER2-overexpressing breast cancer cells, this HER2 association with membrane ER has been shown to provide resistance to tamoxifen by inhibiting the drug's apoptotic effects [15]. ER also directly associates with key signal transduction adaptors and kinases such as Shc [82], the p85 regulatory subunit of phosphatidylinositol-3-OH kinase (PI3K) [85], and c-Src [58, 94]. These interactions also trigger activation of crucial secondary downstream kinases such as p21Ras/p42/44 MAPK and PDK1/Akt. Finally, ER predominantly residing at the membrane caveolar domains can act as a G-protein-coupled receptor. Ligand induction then triggers a process that involves activation of c-Src and matrix metalloproteinases, which then cleave and liberate heparin-binding EGF (HB-EGF) that stimulates TKR EGFR and its signaling pathway [70].

Kinase cascade signaling induced by nongenomic ER activity can in turn phosphorylate (Fig. 3) and thereby activate various components of the ER pathway as well as other components of the transcriptional machinery, resulting in potentiation of nuclear ER transcriptional activity [78, 84, 85]. Bearing in mind that many target genes of the genomic ER activity are key components of growth factor receptor signaling, it can therefore be concluded that in breast cancer cells the nongenomic and genomic actions of ER appear complementary and even synergistic (Fig. 3). Furthermore, the coexistence and cooperative property of these two activities might help to explain the observed complex pleiotropic effects and dominant role of the ER pathway in breast cancer.

Like the genomic activity of ER, its nongenomic activity is also highly dependent on and regulated by other cellular ER coregulatory proteins and is influenced by the overall operating cell and tumor milieu signal transduction pathways. Several ER coregulatory proteins for nongenomic ER activity have been recently identified. Intriguingly, some of these factors seem to regulate both ER activities, though not necessarily in the same direction. The relatively novel ER-interacting protein modulator of nongenomic activity of estrogen receptor (MNAR)/PELP1 is overexpressed in breast cancer and enhances both genomic ER activity on gene transcription [5, 89, 94] and nongenomic ER activity on the c-Src/p42/44 MAPK phosphorylation cascade by facilitating ER interaction with c-Src [94]. Members of the metastasis-associated gene (MTA) family of ER coregulators, on the other hand, can inhibit genomic ER activity but enhance its nongenomic action. The nuclear MTA1 factor, for example, behaves as a classic corepressor of genomic ER activity via traditional recruitment of corepressor components to ER transcriptional complexes [41, 42]. However, the natural short-form variant MTA1s not only inhibits nuclear ER activity by sequestering ER in the cytoplasm but also concurrently increases nongenomic ER activity [41].

Nongenomic activity, like the genomic activity, is highly dependent on ligand for its activation. In contrast, however, while the genomic activity of ER in breast cancer is usually inhibited by SERMs such as tamoxifen, at least some nongenomic actions of ER are not inhibited and can even be stimulated by the same SERMs [78], thus suggesting that this form of ER activity may well play a significant role in endocrine resistance.

Crosstalk between ER and growth factor receptor pathways in endocrine resistance

Increased growth factor signaling and its crosstalk with ER as a cause of endocrine resistance

While the ER pathway, via both of its activities, upregulates growth factor signaling, the molecular crosstalk between these two pathways is bidirectional. Signaling from multiple signal transduction pathways can modulate both the nongenomic and genomic activities of the ER pathway and their ligand dependency. Many studies have shown that nonsteroid stimuli that elevate intracellular kinase activities such as growth factors ([63] and references therein), neurotransmitters [80], and the signaling molecule cAMP [4] can induce ER-dependent gene transcription even in the absence of ligand or in the presence of tamoxifen ([1] and references therein, [46]). Cellular kinase cascades activated by these signals can then phosphorylate both the ER and its coregulatory proteins. ER itself, mostly at the AF-1 domain, is thought to be subjected to and activated by phosphorylation at multiple sites and by multiple signaling kinases including MAPKs, Akt, c-Src, and the cyclin A-dependent cdk2 [2, 11, 34, 36, 47, 72]. Phosphorylation of ER coregulators is probably no less important in communicating these signal transduction effects on the ER pathway. Phosphorylation of coactivators can enhance their activity on genomic ER action even in the absence of ligand or in the presence of antiestrogens by increasing their subcellular nuclear localization [95], potentiating their ability to interact with the ER and to recruit other transcriptional coregulators to its transcriptional complex [25], and probably also by direct activation of their intrinsic enzymatic activities [53]. Phosphorylation of corepressors, on the other hand, can result in their nuclear export, thereby preventing their access to and inhibition of ER transcriptional complexes in the nucleus [30].

The ER coactivator AIB1, like ER itself, is subjected to phosphorylation and activation by multiple kinases including p42/44 MAPK [25, 95, 96]. The p42/44 MAPK is believed to be activated by the EGFR family member HER2, which is gene-amplified and/or overexpressed in >25% of clinical breast tumors. We hypothesized that tumors with high levels of both AIB1 and HER2 should be especially less responsive to tamoxifen therapy because of increased estrogen agonistic activity of tamoxifen-bound ER. Indeed, in our retrospective clinical study of the predictive role of AIB1 in tamoxifen treatment [67], analysis of the interaction between AIB1 and HER2 showed that those patients whose tumors expressed high levels of both AIB1 and HER2 had the worst outcome with tamoxifen. These clinical data strongly support our hypothesis that increased signaling from the EGFR/HER2 family activates p42/44 MAPK, which in turn activates ER and AIB1. Confirmation of this finding in a larger set of patients is currently ongoing.

Other experimental and clinical evidence amply suggest that overexpression or activation of the EGFR/HER2 pathway leads to endocrine resistance, especially to SERMs such as tamoxifen [9, 54, 78]. Overexpression of HER2 has also been shown in experimental systems to potentiate the membrane ER nongenomic activity in response to both estrogen and tamoxifen [13, 22, 78]. In other words, in tumors with overexpression or hyperactivation of growth factor TKR signaling, nongenomic activity of ER might become prominent, and in turn both estrogen and tamoxifen can act as growth factors via ER to stimulate the TKR signaling cascade. Studying an experimental breast cancer model system of HER2 overexpression in the presence of high AIB1 [78], we recently reported a conceivable mechanism for tamoxifen resistance in these tumors (Fig. 4). In these HER2-overexpressing tumor cells, both estrogen and tamoxifen, via nongenomic ER activities, indeed induce phosphorylation and signal activation of EGFR and HER2. Activated downstream kinases of the EGFR/HER2 pathway then phosphorylate the ER and its coactivator AIB1. This causes tamoxifen to act as an agonist on ER-mediated transcription by recruiting coactivator complexes to gene promoters, in contrast to its antagonist effect in non-HER2 overexpressing tumor cells where it recruits corepressor complexes. Whether estrogen- and tamoxifen-bound ER activate the same set of genes in these HER2-tumors and whether these expression patterns are identical to the pattern of estrogen-dependent genes in tumors with low levels of HER2 are important questions currently under investigation. Several preliminary clinical and experimental data suggest that these patterns are indeed different. In any case, as a result of this increased molecular crosstalk between ER and HER2 pathways, tamoxifen acts also as an agonist on tumor growth and actually stimulates

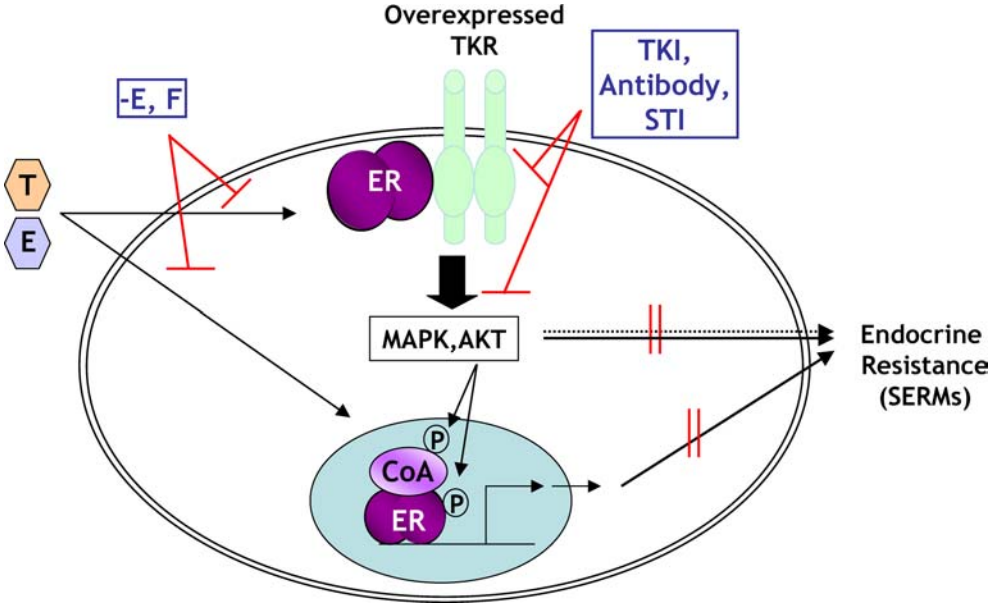

Fig. 4 The role of crosstalk between growth factor receptor pathways and ER signaling in endocrine resistance and novel therapeutic strategies. In tumors with de novo or acquired increased expression or activation of growth factor tyrosine kinase receptors (*TKRs*), both estrogen (*E*) and tamoxifen (*T*) via increased nongenomic ER activities can induce phosphorylation and signal activation of TKRs. Activated downstream kinases in these TKR pathways then can activate ER genomic activity on gene transcription (*arrow*) and/or can directly affect other pathways important for cell proliferation and survival (*dotted arrow*). This causes tamoxifen to act as an agonist on ER-mediated transcription and/or tumor growth (see text for details), resulting in endocrine resistance. These tumors are still highly dependent on the ER pathway and, in contrast to tamoxifen, estrogen deprivation therapy (−*E*) or pure antiestrogens and ER downregulators such as fulvestrant (*F*), either of which block both genomic and nongenomic ER activities, strikingly inhibit the growth of these TKR-activated tumors. Alternatively, targeting TKR signal transduction with selective tyrosine kinase inhibitors (*TKIs*), antibodies, and other signal transduction inhibitors (*STI*) can eliminate tamoxifen's agonist effects, restore its antitumor activity, and overcome de novo and acquired resistance

tumors as a mechanism of de novo resistance [78]. In contrast to tamoxifen, estrogen deprivation therapy, which strategically is equivalent to AIs in postmenopausal women, strikingly inhibits growth of HER2-overexpressing tumors, most probably because it is capable of shutting off both genomic and nongenomic ER activities [78]. Likewise, these tumors are also highly responsive to ER downregulators such as fulvestrant [68], which is devoid of any agonist effect on either genomic or nongenomic ER activities [35, 70] and can degrade the ER protein (Fig. 4).

Acquired resistance to tamoxifen in tumors that originally express low levels of EGFR and HER2, both in the clinical and laboratory settings, can also be associated with increased EGFR/HER2 signaling including HER2 gene-amplification [28, 57, 64]. This again emphasizes the role of this TKR pathway in endocrine resistance and suggests its potential as a therapeutic target to overcome such resistance (Fig. 4). Results from our experimental models [78] as well as others [14, 43, 64, 93] indeed imply that targeting the EGFR/HER2 pathway using specific tyrosine kinase inhibitors or monoclonal antibodies can eliminate tamoxifen's agonist effects and restore its antitumor activity, overcoming de novo resistance and significantly delaying or preventing the development of acquired resistance. Similarly, the involvement of crosstalk between the ER pathway and growth factor receptor signaling, especially the downstream p42/44 MAPK, has also been shown in an experimental model of acquired resistance to estrogen deprivation that is associated with an estrogen-hypersensitivity phenotype [74]. Therefore a related antisignal transduction therapeutic approach might also fit into the setting of this form of endocrine therapy.

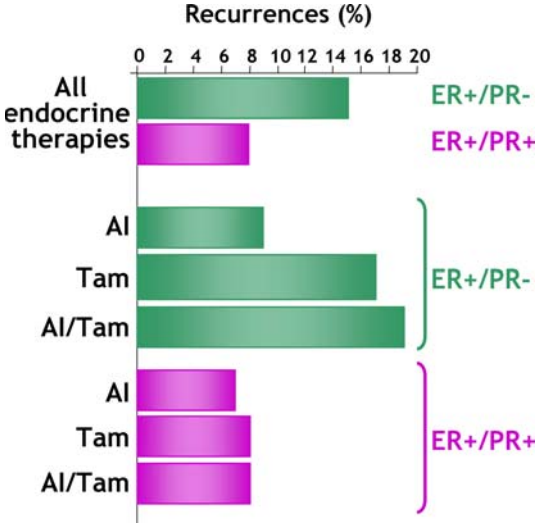

Fig. 5 Subset analysis of breast tumor recurrence in the "ATAC" trial according to hormone receptor status. The percentages of patients with breast tumor recurrence according to the hormone receptor status of the primary tumor (ER^+/PR^+ or ER^+/PR^-) are shown in all three treatment arms together and in the three individual treatment arms: the aromatase inhibitor anastrazole (*AI*), the SERM tamoxifen (*Tam*), and the combination of anastrazole and tamoxifen (*AI*/*Tam*). Adapted from [18]

Clinical implications and future challenges

Results from several recent large randomized retrospective trials strongly support the molecular scenarios portrayed above, especially for the role of the EGFR/HER2 pathway in resistance to SERMs. Two recent randomized studies and one single-arm neoadjuvant study in ER-positive primary breast cancers have demonstrated that EGFR/HER2 overexpression predicts relatively low response rates to tamoxifen and especially high response rates to AIs [21, 79, 97]. Similar results have also recently been reported in the adjuvant setting, showing that the status of all three HER family members (EGFR, HER2, and HER3) predicts for early relapse in ER-positive tamoxifen-treated breast cancer patients [88]. These trials thus support the idea that increased expression or signaling of EGFR/HER2 and perhaps other TKR pathways conveys selective resistance to SERMs such as tamoxifen together with high sensitivity to estrogen deprivation therapies.

It has long been known that ER-positive PR-negative (ER^+/PR^-) tumors are less responsive to endocrine therapy than ER-positive PR-positive (ER^+/PR^+) tumors [69]. A recent report of a retrospective analysis of two large datasets of patients has also confirmed that patients with ER^+/PR^+ tumors benefit much more from adjuvant tamoxifen treatment than those with ER^+/PR^- tumors [6]. Results from the recent large "ATAC" trial, which randomized postmenopausal women to 5 years of adjuvant therapy with the AI anastrazole, tamoxifen, or both, indicated overall a significant superiority for AI therapy [32]. However, subset analyses revealed that the superior effects of anastrazole were largely confined to the group of patients with ER^+/PR^- tumors, while in the ER^+/PR^+ subgroup there was only a small advantage to anastrazole [18]. These subset analyses also show that the inferior outcome in the ER^+/PR^- group was restricted mainly to the tamoxifen treatment groups, and this group of tumors was still fairly responsive to anastrazole (Fig. 5). In light of these results, the old concept that ER^+/PR^- tumors benefit less from endocrine therapy because of having nonfunctional ER signaling is not viable. An alternative explanation is that the ER^+/PR^- phenotype is associated with and probably a result of increased growth factor signaling, which, as described above, leads to selective SERM resistance. Cui et al. [16] have recently shown in an experimental model that increased signaling of TKRs and their downstream PI3K/Akt cascade inhibits PR gene expression in breast cancer cells. Several clinical studies have also suggested that ER^+/PR^- breast tumors are more likely to be positive for HER2

overexpression [19, 38]. Thus PR negativity can be a marker of increased TKR signaling that predicts SERM resistance, and it is this ER^+/PR^- group of tumors that should be treated initially with estrogen deprivation therapy such as AIs and not with SERMs such as tamoxifen.

The precise molecular mechanism responsible for this observed downregulation of PR is at present unknown. As mentioned above, high growth factor TKR signaling can modulate genomic ER activity by increasing ligand-independent AF-1 activity and perhaps also non-classic nuclear activity. This hypersignaling can also augment nongenomic activity, which in some tumors may become the predominant type of ER activity. Of note, ER coregulatory factors such as MTA1 and MTA1s, which can directly inhibit the genomic activity of ER and enhance its nongenomic action, themselves are induced by growth factor signaling [41, 42]. In all of these scenarios, hyperactive growth factor signaling may change somewhat the ''normal/classic'' set of ER-dependent genes. Thus the ''ER-signature'' of gene expression may differ between tumors with low growth factor signaling and tumors with high growth factor signaling, much beyond downregulation of the classic ER-dependent PR gene. Many other classic ER-dependent genes might also be turned off, while other ER-dependent non-classic genes might be turned on. These tumors should not be mistakenly classified as ER-independent tumors, but instead their pattern of gene expression might serve as a predictive signature favoring estrogen deprivation therapy.

Characterization at first tumor presentation of several key tumor biomarkers necessary for treatment decisions (i.e., ER, PR, and HER2) is a part of the standard of care in breast cancer management in many countries. However, almost none of these or other biomarker assays are ever done at the time of tumor progression. The clinical observations that, at progression on tamoxifen therapy, tumors may have gained HER2 amplification and/or overexpression of various signaling kinases [28, 57] and may have lost ER and/or PR [27, 28] strongly argue in favor of integrating a sequential biopsy and biomarker analysis at progression into the practice of breast cancer management.

ER signaling should no longer be assessed independently from the rest of the cellular and tumor signal transduction pathways. Breast cancer is a heterogeneous disease, and distinct signaling kinases and pathways in patients' tumors are probably a key element in their responses to different types of endocrine therapy. New advanced techniques that enable the measurement of hundreds or thousands of genes and proteins simultaneously in any given tumor tissue are now available. With the proper standardization and quality controls, as was systematically done during the development of the now standard immunohistochemistry biomarkers for endocrine therapy, these gene and protein profiles might become a useful clinical tool for personalizing endocrine therapy. Additionally, these multiplexed assays might help us to discover other common important pathways in endocrine resistance, thus pointing at both known and new potential therapeutic targets and biomarkers. With this information at hand, one would be able further to rationalize the appropriate combination of various types of endocrine and signal transduction inhibitor therapies. While studies involving these multiplexed assays are still in the early phases of clinical development, others and we are already conducting clinical trials of treatment combinations with various endocrine therapy drugs together with several potent anti-EGFR/HER2 inhibitors to see whether this new strategy is effective in patients.

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