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Mechanisms of resistance in estrogen receptor positive breast cancer: overcoming resistance to tamoxifen/aromatase inhibitors

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Abstract

Several mechanisms of resistance have been identified, underscoring the complex nature of estrogen receptor (ER) signaling and the many connections between this pathway and other essential signaling pathways in breast cancer cells. Many therapeutic targets of cell signaling and cell cycle pathways have met success with endocrine therapy and remain an ongoing area of investigation. This review focuses on two major pathways that have recently emerged as important opportunities for therapeutic intervention in endocrine resistant breast tumors: PI3K/AKT/mTOR cell signaling and cyclinD1/cyclin-dependent kinase 4/6 cell cycle pathways. Additionally, we highlight individual and combination strategies in current clinical trials that target these pathways and others under investigation for the treatment of ER positive breast cancer.

Introduction

Endocrine therapy has dramatically improved survival in breast cancer patients over the past several decades, however resistance to these therapies remains one of the major causes of breast cancer mortality today [1]. Late recurrence and death from estrogen receptor positive (ER+) breast cancer can occur for at least 20 years after the original diagnosis even after 5 years of adjuvant endocrine therapy [2••]. Identifying mechanisms of resistance and strategies by which to combat these mechanisms is paramount to patient survival.

Several mechanisms of resistance to endocrine therapies have been identified, many centered around the structure, activation, and complex functions of ER, as well as cross-talk between the estrogen signaling network and other cellular pathways. The major form of ER in breast cancer is ERα, encoded by *ESR1*, and the major function of ER is as a transcription factor controlling genes associated with cell survival and proliferation [3]. ER function is influenced by circulating estrogens and related molecules, giving ER-targeted therapies their

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success. Post-translational modifications also influence function, localization, and interaction with other regulators. In addition to ERα, there also exists transcription factor ERβ, encoded by *ESR2*, as well as alternatively spliced and truncated variants of *ESR1*/ERα [4]. The biology of ER is complex and how breast tumors can gain ER function then maintain this despite ER inhibition is not well understood. Thus, the mechanism of action of various endocrine therapies is complicated, varies, and remains an active area of investigation.

Known mechanisms of resistance to hormone therapies are complex and include epigenetic regulation of *ESR1* expression [5,6], *ESR1* mutations [7–12], alternative splicing events [3], *ESR1* truncation and fusion events [13], post-translational modifications [14,15], alterations in the hormone binding domain [7,16], alter-native recruitment sites within the genome [17], differential recruitment of coregulators [18], feedback loops by ER target genes on expression/activity of ER [19●], downstream actions of ER target genes on growth factor pathways and other signaling networks [20,21●●], influences of the tumor microenvironment [22], and many others (Figure 1). The details of these mechanisms are beyond the scope of this review but have been thoroughly described by others [23,24]. The complexity of ER function in tumor cells underscores the heterogeneity of breast cancer biology and demonstrates a necessity for continued basic research and clinical demonstration to effectively target the pathways essential to tumor cell survival.

Much literature exists detailing the mechanisms of resistance. This review highlights two major pathways that have recently emerged as important opportunities for therapeutic intervention in endocrine resistant breast tumors: the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) cell signaling pathway and the cyclin D1/cyclin-dependent kinase (CDK)4/6 cell cycle pathway. Inhibitors to these pathways have been developed, assessed in preclinical studies, and investigated in multiple clinical trials, each with marked benefit toward improving survival but also with specific challenges and limitations discussed below.

PI3K/AKT/mTOR pathway

The PI3K/AKT/mTOR pathway is essential for cell growth and survival, protein synthesis, and glucose metabolism. It is dysregulated in many tumor types, prompting investigation of inhibition of this pathway in many cancer models, including the triple negative subtype of breast cancer [25]. Cross-talk between this pathway and estrogen-mediated signaling is known [26,27], prompting investigation of inhibitors targeting this pathway in ER+ breast tumors. Genomic alterations in *PIK3CA*, the gene encoding the catalytic subunit of PI3K, are common in ER+ tumors [28●,29,30]. It is thought that suppression of the PI3K/AKT/mTOR pathway can lead to increased activity of ER, facilitating resistance, and therefore many groups have used preclinical models to elucidate the mechanisms of PI3K/AKT/mTOR pathway inhibitors and the influence these compounds have on estrogen-mediated signaling [19●,21●●,25,31–33].

Evidence suggests that, while inhibition of the PI3K/AKT/mTOR pathway at all levels results in reduced cell proliferation and survival, the complexity of this signaling pathway

ensures that compensatory mechanisms are activated that confer resistance to single inhibitors [32,33]. For example, inhibition of mTOR results in activation of AKT as well as extracellular signal-regulated kinase (ERK), leading to increased signaling through alternative branches of these pathways [19●,21●●]. Similarly, inhibition of PI3K activates upstream tyrosine kinases, allowing cells to escape the inhibitory effects on cell proliferation [21●●]. AKT, after activation by PI3K, can phosphorylate ER at S167, resulting in ligand-independent activation of ER-mediated transcription [34,35]. Ribas *and colleagues* demonstrated that inhibition of AKT with AZD5363 re-sensitizes cells to tamoxifen, acts synergistically with fulvestrant, and prevents emergence of hormone-independent cells *in vivo*. However, in their model, AKT inhibition activated positive feedback loops driven by MYC, resulting in increased gene expression of human epidermal receptor growth factor 2 and 3 (ERBB2–ERBB3), ERK5, and insulin-like growth factor 1 (IGF1) [19●]. These observations have led to investigation of the use of multiple inhibitors simultaneously and development of novel inhibitors based on alternative iterations of PI3K ligand binding pockets generated by mutations in *PIK3CA*. Cross-talk between the PI3K/AKT/mTOR and ER pathways has immense clinical relevance, but further investigation is required to fully understand the interaction between these pathways. We are not aware of active trials combing a mTOR inhibitor and AKT inhibitor but await the results of combined mTOR inhibitors with PI3K inhibitors.

Several inhibitors of the PI3K/AKT/mTOR pathway have been tested in clinical trials in ER + tumors in conjunction with various endocrine therapies. In 2012, the mTOR inhibitor everolimus was approved by the Food and Drug Administration (FDA) for the treatment of postmenopausal women with advanced ER+/HER2 breast cancer in combination with exemestane. The approval was based on a randomized double-blind multicenter trial (BOLERO-2), where the combination significantly improved disease free survival (DFS) compared to exemestane alone (7.8 months versus 3.2 months) [36]. Recently, the results from a phase 3 trial involving buparlisib, a pan-PI3K inhibitor targeting all four isoforms of class I PI3K (α, β, δ, and γ), were presented and published [37]. Although the efficacy data of buparlisib supports the use of PI3K inhibitors plus endocrine therapy in patients with PIK3CA mutations, the safety profile of buparlisib plus fulvestrant did not support its further development in that setting. Pan-PI3K inhibitors such as buparlisib might be limited by adverse events arising from a broad spectrum of off-target effects [38]. PI3Kα-selective inhibitors are currently under clinical evaluation in combination with endocrine therapy in patients with ER+, HER2-negative breast cancer. Table 1 summarizes the ongoing clinical trials targeting PI3K/AKT/mTOR.

CyclinD1/CDK4/6 pathway

Cell cycle progression is regulated by many proteins, including the cyclins and cyclin-dependent kinases (CDK). Cyclin D1 binds to CDK4/6 to regulate progression through the G1 phase of the cell cycle and is a known downstream target of the PI3K/AKT/mTOR pathway [39]. CDK4/6 has also been shown to crosstalk with the ER signaling pathway [40]. Vora *and colleagues* showed that insensitivity to PI3K inhibitors is evident by persistent RB phosphorylation and can be effectively overcome by combining a CDK inhibitor with a PI3K inhibitor [40]. Since tumorigenesis relies heavily on unchecked progression through

the cell cycle, the cyclinD1/CDK4/6 pathway has emerged as a desirable target in the treatment of many cancers, including breast [41–43]. Originally, inhibitors such as palbociclib were hypothesized to be most efficacious in the triple negative subtype of breast cancer, but early studies identified ER expression as highly correlated with response to palbociclib [44]. Over the past 3 years, multiple large-scale clinical trials have demonstrated that inhibiting CDK4/6 leads to significant clinical benefit when combined with standard endocrine therapies in metastatic ER+, HER2-negative breast cancer [45–52]. Currently CDK4/6 inhibitors, including palbociclib, ribociclib and abemaciclib, are being increasingly employed in clinical practice combined with endocrine therapy. Also, abemaciclib was approved as a monotherapy in ER+, HER2-negative metastatic breast cancer whose disease progressed during or after endocrine therapy [50]. However, CDK4/6 inhibitors typically induce tumor stabilization with only modest increased rates of tumor shrinkage and their cytostatic effects are limited by primary and acquired resistance. Primary and acquired resistance to CDK4/6 inhibitors mediated by loss of the RB1 gene has been demonstrated in preclinical models [53,54●●,55]. In a recent study, the emergence of acquired resistance to palbociclib or ribociclib with the concurrent development of multiple de novo somatic RB1 mutations was shown in metastatic ER+ patients [56]. Table 2 summarizes the ongoing clinical trials with CDK4/6 inhibitors.

Conclusions

Resistance to endocrine therapy remains the most significant challenge in treatment of ER+ breast tumors. Despite this resistance, ER signaling remains biologically significant in these cells, and full understanding of the complex signaling pathways responsible for tumor progression remain to be elucidated. There is a lack of evidence informing optimal sequencing of available therapies in the treatment of advanced ER+ breast cancer [57●]. Currently, the working hypothesis within the field is that multiple signaling pathways must converge on essential biological functions such as cell cycle progression, cell survival, and estrogen-independent ER-mediated transcription, such that effective blockade of tumor progression will only be achieved by combination therapies that affect all compensatory mechanisms and alternative pathways. Breast cancer is a highly heterogeneous disease with novel essential factors constantly being discovered at the preclinical level. This is seen here by combination studies that include hormone therapy with addition of inhibitors targeting RAS/rapidly accelerated fibrosarcoma (RAF)/mitogen-activated protein kinase (MAPKinase), MAPK kinase (MEK), Vascular Endothelial Growth Factor (VEGF), other growth factor receptor inhibitors (e.g. IGF, Insulin Growth Factor; FGFR, Fibroblast Growth Factor Receptor) [23], and other immunotherapy inhibitors [58]. Additionally, more potent and bioavailable selective estrogen receptor degraders (SERD) are being tested to overcome resistance induced by ESR1 mutations [10]. Advances in genomic and other technologies that allow deeper understanding of individual tumors and further investigation into the cross-talk between these signaling pathways have provided a plethora of data that require continued mining, both in the preclinical and clinical settings, to personalize therapeutic regimens for each patient.

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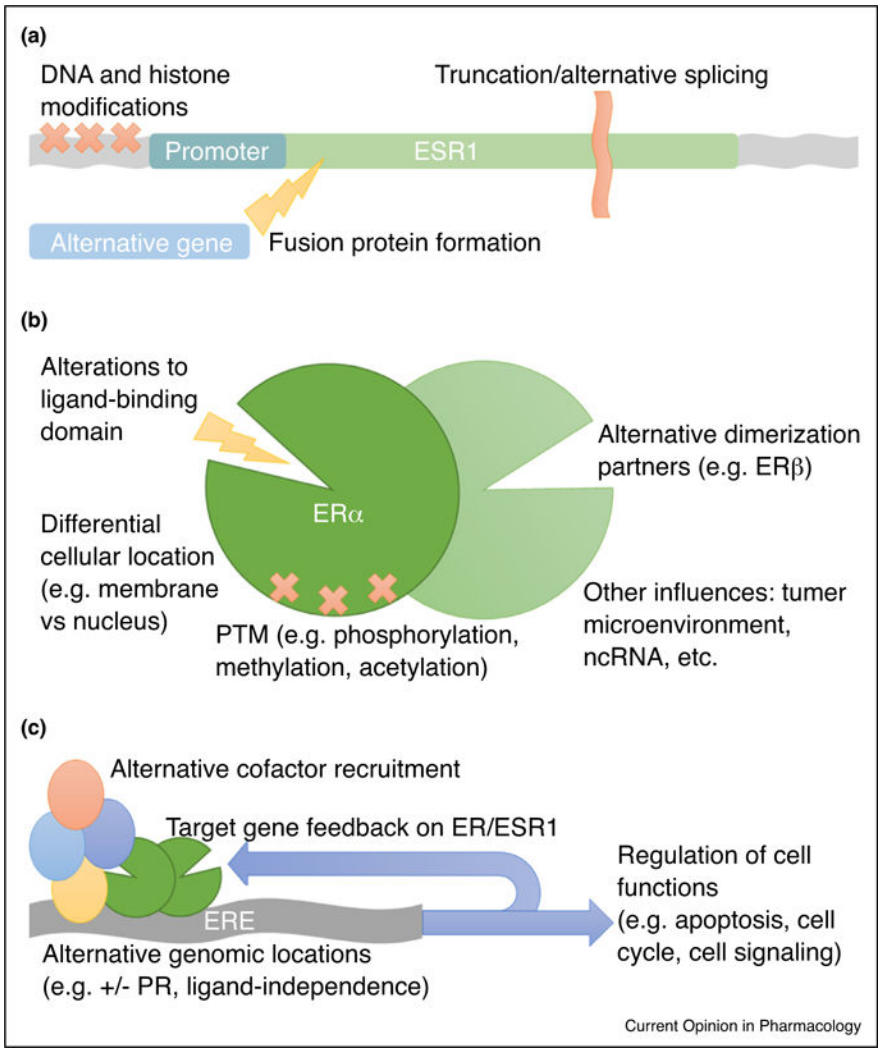

Figure 1.

Diagrammatic representation of known mechanisms of resistance to endocrine therapy. Mechanisms of resistance to endocrine therapy are known to occur at several levels. (a) At the level of ESR1, modifications include epigenetic modification of histone proteins and DNA to alter ESR1 transcription, truncation, alternative splicing events, and fusion events with alternative DNA sequences to produce variations of the ER protein. (b) The events outlined above can result in alterations to the ligand-binding domain and differential cellular localization of the receptor, altering function. Additionally, homo-dimerization or heterodimerization can occur as well as various post-translational modifications (PTM), dependent upon alterations to the proteome of tumor cells and possibly other factors, such as tumor microenvironment and non-coding RNA molecules, resulting in modifications to ER function and target gene selection. (c) Activated ER binds estrogen response elements (ERE) to dictate target genes for transcriptional activation, however alternate recruitment sites are well document and largely dependent upon recruitment of specific cofactors and tethering proteins, such as progesterone receptor (PR). Differential recruitment of cofactors can also lead to repression of transcription rather than activation, modifying the downstream outcome

of ER activation. Target genes can create feedback loops to modify ESR1/ER expression and behavior, including cross-talk with other signaling pathways in the cell. Finally, all of the events outlined above converge on regulation of essential cellular functions, therefore identifying the many opportunities for development of endocrine therapy resistance has profound effects on clinical control of tumorigenesis.

Table 1

Summary of ongoing clinical trials involving PI3K/AKT/mTOR in ER+ breast cancer

Target	Compound	NCT number	Population
PI3K	AZD5363, Paclitaxel	NCT01625286	ER+ MBC
	BKM120 or BYL719 and Capecitabine	NCT01300962	
	BYL719, Letrozole or Exemestane	NCT01870505	
	BYL719, Letrozole	NCT01791478	
	GDC-0032, Letrozole or Fulvestrant	NCT01296555	
	Taselisib, Fulvestrant	NCT02340221	
	Alpelisib, Fulvestrant	NCT02437318	
	Alpelisib, hormone therapy	NCT03056755	PIK3CA-mutant
	BYL719, Fulvestrant	NCT01219699	
	AZD8186, Docetaxel	NCT03218826	PTEN or PIK3CB mutant
	Taselisib, Enzalutamide	NCT02457910	Androgen Receptor+ MBC
mTOR	Everolimus to adjuvant hormone therapy	NCT01805271	Adjuvant ER+
	Everolimus, standard hormone therapy	NCT01805271	
	TAK-228 then Letrozole	NCT02619669	
	TAK-228, Tamoxifen	NCT02988986	ER+ MBC
	Everolimus, Letrozole	NCT01698918	
	AZD2014, Fulvestrant	NCT02216786	
	Everolimus, Exemestane	NCT01783444	
	Everolimus, Tamoxifen	NCT01298713	
mTOR, IGF1R	Ridaforolimus, Dalotuzumab, Exemestane	NCT01605396	ER+ MBC
AKT	AZD5363	NCT03310541	AKT-mutant
	AZD5363, Fulvestrant	NCT01992952	ER+ MBC
	AZD5363	NCT01226316	MBC
	MSC2363318A, with Trastuzumab or Tamoxifen	NCT01971515	
AKT, PD-L1, MEK, VEGF	Ipatasertib, Atezolizumab, Cobimetinib, Bevacizumab	NCT03395899	Neoadjuvant ER+
PI3K, mTOR, CDK	Ribociclib, Everolimus, Exemestane	NCT01857193	ER+ MBC
	Gedatolisib, Fulvestrant, Palbociclib, Goserelin	NCT02626507	
PI3K, CDK	Ribociclib, Fulvestrant and BYL719 or BKM120	NCT02088684	

Target	Compound	NCT number	Population
	Ribociclib, Letrozole, BYL719	NCT01872260	
	Ribociclib, Tamoxifen, Letrozole, Anastrozole, Goserelin	NCT02278120	
mTOR, CDK	Ribociclib, Everolimus, Exemestane	NCT02732119	
	Hormone therapy, hormone therapy with Everolimus, or hormone therapy with CDK4/6 inhibition	NCT02753686	

MBC, metastatic breast cancer; IGF1R, Insulin Growth Factor 1 Receptor; PD-L1, programmed death-ligand 1; MEK, Mitogen-activated protein kinase kinase. Table updated from clinicaltrials.gov on March 11, 2018.

Table 2

Ongoing clinical trials involving CDK inhibitors in ER+ breast cancer

Target	Compound	NCT number	Population
CDK	Ribociclib, Hormone therapy	NCT03285412	Adjuvant ER+
	Ribociclib, Hormone therapy	NCT03078751	
	Ribociclib, Hormone therapy	NCT03081234	
	Palbociclib, Anastrozole, Goserelin	NCT01723774	
	Abemaciclib	NCT02831530	
	Chemotherapy or Ribociclib and Letrozole	NCT03283384	Neoadjuvant ER+
	Fulvestrant with or without Palbociclib	NCT03447132	
	Paclitaxel, Tamoxifen + Palbociclib, Aromatase Inhibitor	NCT02603679	
	+ Palbociclib, Goserelin + Aromatase Inhibitor + Palbociclib		
	Palbociclib, Hormone therapy	NCT01864746	ER+ post-neoadjuvant
	Ribociclib, Letrozole	NCT03439046	ER+ MBC
	Ribociclib, Letrozole	NCT01958021	
	Ribociclib, Letrozole, Goserelin	NCT02941926	
	Ribociclib, Fulvestrant	NCT02422615	
	Endocrine therapy plus CDK 4/6	NCT03425838	
	Ribociclib, Letrozole, Goserelin	NCT03096847	
	Ribociclib or Palbociclib, Fulvestrant	NCT02632045	
	Palbociclib, Letrozole	NCT02499146	
	Palbociclib, hormone therapy	NCT02040857	
	G1T38 with Fulvestrant	NCT02983071	
	Letrozole alone or with Palbociclib	NCT00721409	
	Palbociclib, hormone therapy	NCT03184090	
	Palbociclib, Letrozole	NCT01740427	
	Palbociclib, Exemestane, Goserelin	NCT02917005	
	Palbociclib, Letrozole	NCT02297438	
	Palbociclib	NCT03159195	
	Palbociclib, Tamoxifen	NCT02668666	
	Aromatase inhibitor, Palbociclib	NCT03439735	

Target	Compound	NCT number	Population
	Palbociclib and aromatase inhibitor or Palbociclib and Fulvestrant	NCT03220178	
	Exemestane plus Goserelin with Palbociclib versus Capecitabine	NCT02592746	
	Abemaciclib, Fulvestrant	NCT02107703	
	Abemaciclib	NCT02763566	
	Palbociclib, Letrozole	NCT01684215	
CDK, HER2	Palbociclib, Letrozole, Trastuzumab, Goserelin	NCT02907918	Neoadjuvant ER+/HER2+
	Ribociclib with Trastuzumab or Trastuzumab emtansine	NCT02657343	ER+/HER2+ MBC
	Tucatinib in combination with Palbociclib and Letrozole	NCT03054363	
CDK, PD-1	Ribociclib, PDR001, Fulvestrant	NCT03294694	ER+ MBC
CDK, IGF	Xentuzumab, Abemaciclib, Fulvestrant	NCT03099174	ER+ MBC refractory to CDK4/6
CDK, FGFR	Erdafitinib, Palbociclib, Fulvestrant	NCT03238196	FGFR-amplified ER+

MBC, metastatic breast cancer; PD-1, programmed cell death protein 1; IGF, Insulin Growth Factor; FGFR, Fibroblast Growth Factor Receptor. Table updated from clinicaltrials.gov on March 11, 2018.