Endocrine resistance in breast cancer: biological mechanisms and clinical implications to develop new treatment strategies

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Summary

Breast cancer is one of the most common tumor with more than 1,300,000 cases and 450,000 deaths each year worldwide (1). Breast cancer represents a complex and heterogeneous disease, characterized by different pathological, and histological features related to clinical outcomes. Several studies on gene expression profiles have identified specific molecular portraits conferring different clinical outcomes (2). Actually, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor type 2 (HER2) status have been used as predictive biomarkers to identify high-risk phenotypes and to select the most efficacious therapies. Approximately new 700,000 breast cancers each year are hormone receptor positive and the estrogen deprivation represents a major treatment strategy (3, 4). Furthermore, patients responding to endocrine treatments can develop resistance, that is one of the major challenge for the clinical management of these patients (5). Several mechanisms are involved in the development of endocrine resistance: activation of growth factor signaling pathways, PI3K/Akt/mTOR signaling, altered expression of microRNA, reduced expression of ERs, altered regulation of co-activators of ER, (6-9). Early identification of the biological mechanisms involved in the endocrine resistance can contribute to develop new therapeutic strategies in breast cancer patients.

KEY WORDS: endocrine-resistance, molecular mechanism of endocrine resistance, estrogen receptor, PI3K pathway, HER2 crosstalk, CDK4/CDK6, cyclin and resistance, miRNA.

Introduction

Estrogen is involved in the pathogenesis of breast carcinoma and sustains the growth of breast cancer cells expressing the receptor for this hormone (10). Estrogen receptor (ER) and progesterone receptor (PR) expression is currently the best predictor of response to endocrine therapy in the clinical setting (11). The three available classes of drugs are: SERMs, SERDs, Aromatase inhibitors. Current endocrine therapies have significantly improved clinical outcome in ER-positive breast cancer patients (12-14). Unfortunately, the effectiveness of endocrine therapy may be overcome by the emergence of resistance defined as “acquired” or “de novo” (5). Specific molecules involved in different pathways confer specific sensitivity to hormonal treatments, and therefore may be considered biomarkers of endocrine resistance (6-9). This review evaluates the different mechanisms of resistance and alterations of the pathways involved in this phenomenon, that can be overcome by new treatment strategies.

Methods

This systematic review focused on the mechanisms of resistance to endocrine therapies in breast cancer. A literature research, at December 2013, 1st, was conducted by the Authors using PubMed. The following research terms were used: “endocrine-resistance”, “molecular mechanism endocrine resistance”, “estrogen receptor”, PI3K, HER2, Cyclin D1, CDK4/6, cell cycle and endocrine-resistance, miRNA.

Estrogen receptor: function

Estrogens exert their biological effects by binding their specific receptors, activating ER regulated genes (GENOMIC action) (4) (Fig.1). There are two isoforms of ER, encoded by two different genes: the alpha isoform, that mediates breast cancer cell proliferation and survival, and the beta isoform, whose function is not well defined (15-23). The binding of estrogen to receptor al...
pha in the nucleus results in receptor phosphorylation, dimerization and conformational changes, in the recruitment of co-activators, that enhance the binding of the complexed receptor to the promoter regions of target genes, known as estrogen response elements (EREs), increasing the transcriptional activity (18, 19). The transcription of target genes determines the synthesis of proteins implicated in cell growth control, division, differentiation, and survival, and promotes tumor progression. In addition to this “classical genomic action”, associated with nuclear localization, a small amount of ER in the cell membrane or in the cytoplasmic compartment, is involved in a faster cell signaling cascade, through a direct interaction with intracellular signaling pathways (“non-genomic action”) (20, 6-8) (Fig. 2). More, ER can regulate gene expression without direct interaction with DNA, through interaction with transcription factors (complex activating FOS/JUN), and transcription of genes encoding for vascular endothelial growth factor (VEGF), insulin-like growth factor receptor 1 (IGFR1), insulin receptor substrate 1 (IRS1), transforming growth factor alpha (TGFα) (24-27). ER can also phosphorylate and activate EGFR in a process that involves activation of G protein, Src, MMPs (Fig. 2).

Endocrine therapy: goal

About 75% of breast cancer patients are affected by a hormone receptor positive disease, ER+ and/or PgR+ (4). Drugs interfering with ER activation, by blocking estradiol synthesis or inhibiting estrogen interaction with receptor, play a crucial role in adjuvant and metastatic setting and a developing role in cancer prevention (28, 29). Thus, endocrine therapy represents a milestone in hormone receptor positive breast cancer treatment. Three classes of drugs are currently in use: Selective ER Modulators (SERMs), such as tamoxifen, toremifene, raloxifene with partial antagonist activity; Aromatase Inhibitors (AI), that reduce circulating estrogen levels, such as anastrozole, letrozole, exemestane; Selective ER Down-regulators (SERDs) with complete antagonist action on ER, and induction of receptor degradation, such as fulvestrant (28, 30-34). SERMs. The binding of an anti-estrogen to ER results in its molecular conformation alteration and preferential recruitment of co-repressors, with inhibition of transcriptional activity (29). To note, the recruitment of the co-regulators is a dynamic mechanism, influenced by the relative availability of different co-activators and 

Figure 1 - ER action: genomic and non genomic mode. E, estrogen; ER, Estrogen receptor. ERE, estrogen receptor element; AF 1-2, Transcription activation function; RAPS, Receptor associated proteins; VEGF, vascular endothelial growth factor; IGFR1, insulin-like growth factor receptor 1; IRS1, insulin receptor substrate 1; TGFα, transforming growth factor alpha; Fos, Jun, transcription factors; AP-1, promoter sites.
co-repressors. The effects of agonists and antagonists can be modified by an absolute or relative level of co-regulators proteins, whose availability may confer "de novo" or acquired resistance.

Aromatase inhibitors. Similar results, with a different mechanism of action, can be obtained by AI, that antagonize the ligand of the enzyme aromatase, thus inhibiting the conversion of testosterone and androstenedione to estradiol. By eradicating estrogen production, AI suppress both the genomic and the non-genomic ER action. Third-generation AI are among the front-line treatments for postmenopausal breast cancer patients (35, 36).

SERDs. These drugs act as potent ER antagonists, by inducing rapid receptor turnover, and display no agonist activity in estrogen target tissues. Fulvestrant is an analogue of estradiol and the first in a new class of drugs that are complete antiestrogens and potent downregulators of ER expression, by the induction of an abnormal conformation of the receptor resulting in an accelerated ubiquitination and shuttling to proteasome for degradation (37). The pure antagonistic property of fulvestrant , H12 may contribute to fulvestrant-induced ER degradation (38).

Endocrine resistance: “de novo” versus acquired

About 25% of ER+/PR+ tumors, 66% of ER+/PR– tumors, 55% of ER–/PR+ tumors fail to respond to endocrine therapy or develop early resistance for unclear reasons (39). More than 50% of metastatic patients do not respond to first line endocrine therapy, and the majority of responders finally relapse (40, 41). Thus, not all hormone receptor-positive patients respond to endocrine therapy and can be considered therefore resistant. At a clinical level endocrine resistance can be defined as “de novo” or “acquired”, even if it determined by a series of genetic alterations involved (11-13). “De novo” resistance occurs rapidly in the course of metastatic or adjuvant treatment or within 12 months from the end of adjuvant therapy, while acquired resistance occurs later.

Mechanisms of endocrine resistance and clinical implications

The only marker of absolute endocrine-resistance is the absence of ER, but different mechanisms are involved in the emergence of endocrine resistance:
HER amplification/overexpression and resistance

A marker of endocrine resistance, as well as of biological aggressiveness, is HER2 gene overexpression (42, 43). Preclinical and clinical studies have shown an inverse correlation between ER expression and HER2 (44). HER signaling effectors can reduce the expression of both ER mRNA and protein expression. For example, Akt inactive FOXO3, a key regulator for ER transcription (45). More, effectors of HER2 pathway may lead to a degradation of ER-mediated MAPKs. As a consequence of this negative control by the pathway of HER2 on ER expression levels, tumor cells are resistant or at least less endocrine sensitive. For the same reason, in preclinical models the block of overexpressed HER2 pathway may restore hormone sensitivity (46-49). More, the HER family seems to play a central role in the development of “de novo” or acquired resistance. (5, 15, 16, 50). HER signalling can also potentiate ER genomic and non genomic signalling resulting in impairment of endocrine sensitivity. ER+/MCF7 breast cancer xenografts with acquired resistance to tamoxifen exhibit increased level of HER2, IGF-1R and EGFR (46). When c-erbB2 or other growth factor receptors pathways are activated, a phosphorylation cascade from the membrane to the nucleus, phosphorylate and active ER in the absence of the binding of the ligand. The cell becomes hormone refractory. Thus, ER can be phosphorylated and activated in a ligand-independent way by Growth Factor Receptor (EGFR, HER2, IGF-1) or stress-related kinase as PI3K/Akt, MAPK, resulting in the development of endocrine-resistance. These same kinase can also phosphorylate coactivators such as AIB1, also know as SRC3, resulting in a switch from antagonist to agonist of SERM action. The cross-talk between ER and the HER2 pathway may mutually affect their expression and activity. More, in clinical trials, ER positive expression is associated with reduced response to HER2 target-therapy in ER+/HER2+ breast cancer patients. (51). Estrogen signaling can also increase TGFα and IGF1 expression, and down regulate HER1 expression. (52-56). This confirm the crosstalk between ER and HER pathways, potentially responsible for resistance not only to endocrine therapy but also to anti-HER2 treatments. As direct consequence of the extensive crosstalk between this pathways in breast cancer ER+/HER2+, a promising strategy to prevent or overcome endocrine and anti-HER2 resistance, is combining treatments that simultaneously block this pathways. In TanDEM, phase III study, in ER+/HER2+ metastatic breast cancer patients trastuzumab addiction with Aromatase Inhibitor (AI) anastrozole compared with AI alone, had a longer progression free survival (PFS) (57). In a phase III of ER+/HER2+ metastatic breast cancer patients, comparing letrozole with and without lapatinib, the combination treatment resulted in a clinical benefit (58). In the neoadjuvant setting, trastuzumab/lapatinib/letrozole association in locally advanced ER+/HER2+ breast cancer patients, determined 21% pathological complete response. These data suggest that “chemotherapy-free” treatments blocking both ER and HER2 pathways can be considered a valid treatment option in a selected subgroup of patients, also “unfit” for chemotherapy. Since the molecular expression and signaling of tumor cell can change despite effective targeted treatment due to the activation of escape pathways, re-biopsy of recurrent lesion, where feasible, should be considered in order to optimized therapeutic strategies (59).

PI3K/Akt/mTOR pathway activation and resistance

PI3K/Akt/mTOR pathway activation is an alternative molecular mechanism allowing cancer cells proliferation and survival, even in with ER inhibition, thus conferring endocrine resistance. This signaling pathway is dysregulated in over 70% of hormone receptor positive breast cancer, prevalently due to mutations in the catalytic domain of PI3K or loss of function of PTEN, an endogenous inhibitor of the pathway. Among the PI3Ks family, PI3KIA is prevalently involved, whose catalytic subunit is encoded by the PIK3CA gene. The prognostic role of PIK3CA mutations is not yet known, even if they seem to be associated with an improved outcome (60). Signaling cascade is activated by the binding of an extracellular growth factor (e.g. EGF, IGF-1) to the specific membrane receptor, leading to its dimerization and autophosphorylation and recruitment of adaptor proteins, such as the insulin receptor substrate (IRS)1 and 2, PI3K is recruited to the phosphorylated receptor tyrosine kinase and activated, but it can be also directly activated by the Ras protein. Once activated, PI3K phosphorylates PIP2 (phosphatidylinositol 4,5-bisphosphate) to PIP3 (phosphatidylinositol 3,4,5-trisphosphate), which can be dephosphorylated by PTEN with consequence negative signaling regulation. PIP3 promotes the translocation of Akt to the cell membrane and induces a conformational change to its structure, leading to its phosphorylation; nevertheless, its full activation requires an additional phosphorylation on a different domain, mediated by the mTORC2 complex (61). Activated Akt is able to dissociate from the membrane, translocate to the cytoplasm and the nucleus and then activate proteins implicated in metabolism, proliferation, survival and angiogenesis. The subsequent step is the activation of the mTOR complex, a serine/threonine kinase which forms the mTOR complex 1 (mTORC1), when complexed with regulatory-associated protein of mTOR (raptor), and the mTOR complex 2 (mTORC2), when complexed with rapamycin-insensitive companion of mTOR (rictor).
mTORC1 has two major down-stream effectors, 4E-BP1 and S6K1 (ribosomal S6 kinase 1), involved in proliferation and survival; mTORC2 activates Akt. Activated S6K1 phosphorylates and destabilizes IRS1 and IRS2 in insulin-like growth factor (IGF)-responsive cells. mTOR inhibition can then block the negative feedback on IGF-1 receptor signaling, with increasing Akt downstream signaling activity (62).

Preclinical studies showed the existence of an active crosstalk between the ER and the PI3K/Akt/mTOR pathway: Akt and PI3K can induce estrogen-independent activation of ER through its direct phosphorylation. Hyperactivation of the PI3K/Akt/mTOR pathway determines resistance to endocrine therapy, while its inhibition at different levels allows the restoration of sensitivity to aromatase inhibitors and tamoxifen, as demonstrated in preclinical studies on cells in culture in absence of estrogen for long time (long-term estrogen deprivation, LTED) (63, 64).

The first identified allosteric inhibitor of mTOR was rapamycin, isolated from a soil sample on Easter Island (Rapa Nui) in 1975, even if its target, mTOR, was identified only in 1991. Rapamycin showed the capability to inhibit the growth of different cancer cell lines and xenografts, but its poor hydrophilicity and chemical instability limited clinical implementation and required the development of synthetic rapamycin analogs (rapalogs) with a more favorable pharmacological profile: everolimus (RAD001), temsirolimus, and deforolimus. Inhibitors of other components of the pathway have been subsequently developed: allosteric Akt inhibitors, kinase inhibitors of Akt and kinase inhibitors of PI3K. Inhibition of mTOR may lead to a paradoxical activation of PI3K. Therefore, dual kinase inhibitors, targeting both mTOR and PI3K, are under investigation.

The clinical safety and efficacy of everolimus was first investigated in the neoadjuvant setting. In a randomized, double-blinded phase II trial, by Baselga et al., 270 postmenopausal women with operable ER-positive BC were randomly assigned to receive 4 months of neoadjuvant treatment with letrozole associated with everolimus or placebo: response rate was 68% versus 59% (p=0.062), with a reduction in Ki67 expression in 57% 30% patients (p <0.01), in the everolimus and placebo arms, respectively.

In the metastatic setting, in the phase III BOLERO-2 study, 724 postmenopausal women with advanced ER-positive HER2-negative BC, with recurrence or progression after prior non-steroidal AI therapy, were randomly assigned to receive exemestane associated with everolimus or placebo. PFS was 6.9 months in the everolimus arm and 2.8 months in the placebo arm, (hazard ratio (HR) for progression or death, 0.43; p=0.001). Median PFS was 10.6 months and 4.1 months, respectively, according to central assessment (HR 0.36; p=0.001) (65). The most common grade 3 or 4 reported adverse events were stomatitis (8% in the everolimus vs 1% in the placebo group), anemia (8% vs <1%), dyspnea (4% vs 1%), hyperglycemia (4% vs <1%), fatigue (4% vs 1%), and pneumonitis (3% vs 0%) (66). At 18 months median follow-up, a highly significant prolongation of PFS was reported with the addition of everolimus to exemestane: median PFS 7.8 vs 3.2 months (HR 0.45) and 11.0 vs 4.1 months (HR 0.38) by local and central review, respectively. Similar treatment benefits were seen across all prospectively planned patient subgroups defined by baseline demographic and clinical factors (67).

To date, the personalization of treatment is required as an absolute necessity. A better biological characterization of primary tumor and metastasis is critical to try to individualize more precisely as possible cancer therapy, through the use of innovative drugs (68-70). The sampling of tissues is essential to analyze a range of biological markers and test their prognostic and/or predictive significance, to best treat our patients.

**Cell Cycle and endocrine resistance**

Alteration of cell cycle regulation is a common event in neoplastic cells. Since ER regulates the expression of many genes involved in the progression of cell cycle, its inhibition by anti-estrogens may modify these mechanisms. A commonly deregulated checkpoint is the pathway involving Cyclin D/CDK4/CDK6/Rb (71, 72). The complexes Cyclin D1/CDK4 and Cyclin D3/CDK6 phosphorylate the Rb family proteins, and promote the E2F transcription factors, activating in turn genes encoding proteins involved in the cell cycle progression (73). In many tumors, such as breast cancer, the genes encoding for Cyclin D1, CDK4, CDK6 are upregulated. In other cases, the tumor suppressor gene Rb is inhibited by Loss of heterozygosity or mutations (68-73). Patients harbouring ER + with loss of Rb tumors have a shorter recurrence free survival. Furthermore, the activation of CDK4/CDK6/E2F promotes endocrine-resistance and CDK4/6 inhibition abrogates endocrine resistance (74, 75). In a phase II study in ER+/HER2+ metastatic breast cancer patients, the addiction of the inhibitor PD0332991 (Palbociclib) to letrozole demonstrated a benefit in median PFS, 26.1 vs 7.5 months, HR 0.37 (76-78). These data should be confirmed in the ongoing phase III study. Cancer cell sensitivity to CDK4/6 inhibitors may be predicted using genetic biomarkers, such as loss of Rb that seems to confer insensitivity to CDK4/6 inhibition. Conversely, amplification of the gene encoding Cyclin D1, or loss of the gene encoding p16, may be indicative of cells with enhanced CDK4/6 pathway.

**miRNA and endocrine resistance**

MicroRNAs (miRNAs) are endogenous non-coding RNA molecules of 18-25 nucleotides in length (79). The discovery of miRNAs as regulators of gene expression is an important event in tumor biology (80). It is estimated that miRNAs regulate 1/3 of all human transcripts, and about 1100 known miRNAs control approximately 16,230,000 target sites. Alterations of miRNAs seem to modify the process of initiation and progression of cancer (81). MiRNAs can act as oncogenes or tumor suppressor genes, and may silence gene ex-
expression even at post-transcriptional level. They may act by altering the expression of estrogen-responsive genes, but also at a post-transcriptional level regulating pathways involved in endocrine resistance in breast cancer (82). MiRNA may be predictive of the development of resistance and can be used as predictive/prognostic biomarkers in the clinical management of breast cancer patients (83). MiRNA are stable molecules, well preserved in formalin fixed paraffin embedded tissue or fresh snap-frozen specimens. They can play a relevant role as potential biomarkers. Altered expression of specific miRNA seems to be related with tamoxifen resistance and seem to predict sensitivity and clinical outcome in breast cancer patients (84-86). Different miRNA profiles were identified both in cells sensitive and resistant to tamoxifen by microarray analyses. Since it is not always easy to obtain tumor tissue from patients progressing during hormonal treatment, circulating blood was tested, and circulating miRNAs in serum were detected for the first time in large B cell lymphomas patients (87). In subsequent studies 26 miRNAs were identified as diagnostic markers in breast cancer patients (88). These considerations should lead to significant improvement in the managements of endocrine resistance in breast cancer patients. The detection of genes/pathways targeted by miRNA can further improve the knowledge of the mechanism of resistance to endocrine therapies and the development of new therapeutic agents.

Conclusions

The resistance to endocrine therapy involves multiple biological mechanisms. Breast cancer is a highly heterogeneous disease both at clinical and molecular levels, thus treatment strategies should be personalized. The molecular portrait of each tumor should be carefully defined in order to select the appropriate therapeutic approach. Furthermore, new therapeutic strategies should consider the association of endocrine therapy with drugs targeting key molecules involved in the development of endocrine resistance. This may be the most promising approach to prevent and/or revert endocrine resistance and optimize clinical outcome in breast cancer patients.

References

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