Multidrug-resistant breast cancer: current perspectives

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Abstract: Breast cancer is the most common cancer in women worldwide, and resistance to the current therapeutics, often concurrently, is an increasing clinical challenge. By understanding the molecular mechanisms behind multidrug-resistant breast cancer, new treatments may be developed. Here we review the recent advances in this understanding, emphasizing the common mechanisms underlying resistance to both targeted therapies, notably tamoxifen and trastuzumab, and traditional chemotherapies. We focus primarily on three molecular mechanisms, the phosphatidylinositide 3-kinase/Akt pathway, the role of microRNAs in gene silencing, and epigenetic alterations affecting gene expression, and discuss how these mechanisms can interact in multidrug resistance. The development of therapeutics targeting these mechanisms is also addressed.

Keywords: PI3K/Akt, epigenetics, miRNA, ER, HER2, triple negative

Introduction

Breast cancer affects 1.38 million women worldwide per year, making it the most common cancer in women,¹ and although the implementation of screening programs and the development of new therapeutics in the last 20 years have significantly reduced mortality rates in the Western world, resistance to these therapeutics is a growing problem.² Resistance can be de novo but may also be acquired. Indeed, 30% of women with early-stage breast cancer have recurrent disease, and resistance to therapeutic agents can occur in at least a quarter of all cases.³ ⁴ The incidence of resistance to therapeutics increases with disease progression,⁵ and this refractivity contributes to breast cancer having the highest mortality rate, 12.9/100,000 population in the USA in 2008, after lung cancer.¹ ⁵

Breast cancer is a heterogeneous disease and is divided clinically into three basic subtypes determined by the expression of hormone receptors (estrogen and progesterone), human epidermal growth factor receptor 2 (HER2), and triple-negative breast cancer, which expresses none of these receptors.⁶ Further subdivisions are now recognized, including luminal A, luminal B, basal-like, and HER2-enriched,⁷ and, recently, work has classified breast cancers into ten subtypes.⁸ However, to aid clarity, we will use the three basic clinical subtypes in this review. Each subtype has a different treatment strategy. For hormone receptor-positive breast cancer, the frontline treatment is endocrine therapy, whereas for HER2-positive cancers, it is trastuzumab (Herceptin; Roche, Basel, Switzerland). These specific therapies are often used in conjunction with traditional cytotoxic chemotherapeutics, such as taxanes and anthracyclines, which are the frontline therapies for triple-negative breast cancer.⁴ The mechanisms underlying resistance to these different therapeutics are multiple, complex, and not mutually
exclusive, and have recently been excellently reviewed for each of the three subtypes individually. Here we will provide an overview of selected mechanisms underlying the drug resistance in each of these different groups, highlighting common mechanisms, specifically focusing on the role of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, miRNAs, and epigenetic alterations in drug resistance in breast cancer and how these interactions can contribute to a multidrug-resistant phenotype. The identification of potential targets for new therapies or as adjuvants to restore sensitivity to current treatments will be emphasized.

**Hormone receptor-positive breast cancer**

The predominant hormone receptor expressed in breast cancer is the estrogen receptor (ER), which is activated by the binding estrogens, predominantly estradiol. Hormone receptor-positive breast cancer also expresses progesterone receptors; however, these receptors have been somewhat neglected as therapeutic targets, as the first generation of antagonists had severe side effect profiles. Progesterone receptors have traditionally been considered as downstream targets of ERs, and their mechanistic roles in resistance to endocrine therapy underexplored. Consequently, we will limit our discussions here to ER-positive breast cancer (for further information on progesterone receptors in breast cancer we would like to draw the reader’s attention to the recent review by Brisken). Activation of the ER leads to a transcriptional response, both in genes with and without ER response elements, and also nontranscriptional cellular responses, all of which favor cell proliferation and survival (see Figure 1). There are two ER forms, α and β. High expression levels of the latter have been correlated with good clinical prognosis, but as the role of this receptor in breast cancer has not been as widely studied as the ERα, we will focus on the former (we refer the reader to the recent review by Haldosén et al for further information). ERα-positive breast cancer accounts for approximately 70% of all breast cancer cases. The expression levels of ERα determine patient response to endocrine (antiestrogen) treatment and can be used as predictor of

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**Figure 1** Estrogen, HER2 signalling, and the PI3K/Akt pathway in drug-resistant breast cancer.

**Notes:** ER can activate gene transcription by nuclear translocation following ligand binding (1) or as a result of receptor phosphorylation in the absence of ligand (2). ERs may also be found associated with the plasma membrane in the presence of SRC and other adaptor proteins. Here, ligand binding triggers nongenomic effects via activation of signaling pathways, including the PI3K/Akt and the Ras/MAPK pathways (not shown) (3). These pathways are also activated by ligand binding to the GPR30 (4) and by growth factor binding to receptor tyrosine kinases, including HER2, inducing autophosphorylation and downstream signaling (5). The PI3K/Akt pathway (6) as indicated is a convergence point in the mechanisms implicated in drug resistance in the three types of breast cancer discussed here, as pathway hyperactivity frequently occurs with multiple downstream effects (7).

**Abbreviations:** BAD, Bcl-2-associated death promoter; DMNTs, DNA methyltransferases; ER, estrogen receptor; GF, growth factor; GPR30, G-protein coupled receptor 30; GSK3B, glycogen synthase kinase 3 beta; HER2, human epidermal growth factor receptor 2; IGF-1R, insulin-like growth factor receptor 1; MAPK, mitogen-activated protein kinase; mTORC1, mammalian target of rapamycin complex 1; P-gp, P-glycoprotein; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; RTK, receptor tyrosine kinase; SRC, steroid receptor coactivator.
disease outcome. The frontline treatment for this type of breast cancer is endocrine therapy, predominantly tamoxifen or fulvestrant, which are antagonists of ER in breast tissue (tamoxifen has agonist actions in other tissues⁴). In postmenopausal women, tamoxifen/fulvestrant is often used in conjunction with aromatase inhibitors (eg, anastrozole or letrozole) that reduce estrogen synthesis and lower the recurrence rate compared with tamoxifen alone. However, many patients with metastatic ER-positive disease develop resistance to endocrine therapy, so understanding the molecular mechanisms that lead to refractory disease may identify ways to circumvent resistance and restore responses to endocrine therapies. The mechanism of estrogen-induced cell responses (Figure 1) means there are many levels at which resistance to endocrine therapy can occur (excellently reviewed by Bianco and Gevry⁶). Here we will explore those that mediate tamoxifen resistance, as this is the best studied, and many of these mechanisms apply to other endocrine therapies.

Molecular mechanisms of tamoxifen resistance

Tamoxifen metabolism
Tamoxifen itself is a prodrug that requires bioactivation to the major metabolite, endoxifen, to be active against ER. This process involves two members of the cytochrome P450 (CYP) family, CYP2D6 and CYP3A4. Both isoforms have several common polymorphisms. To date, none of the common CYP3A4 polymorphisms has been associated with altered tamoxifen metabolism. However, the CYP2D6 polymorphisms are well characterized and affect its catalytic activity. These polymorphisms are categorized into four groups, from the ultrarapid metabolizers with increased activity down to poor metabolizers with no CYP2D6 activity. Recent work has correlated CYP2D6 metabolizer status with response to tamoxifen treatment, with poor metabolizers having greater tumor progression than extensive metabolizers. Thus, determining CYP2D6 metabolizer status before tamoxifen treatment would be beneficial, allowing patients with poor metabolizer status to be treated with altered doses or other endocrine therapies, and consequently not categorized as resistant to endocrine therapy.

ERα activity
Phosphorylation is a common mechanism of post-translational modification to regulate enzyme activity, and has been linked with drug resistance in some cancers, including gemcitabine-resistant pancreatic cancer. Phosphorylation regulates ERα activity and plays a role in tamoxifen resistance, specifically phosphorylation of the serine 305 residue by protein kinase A or p21-activated kinase-1. Both of these kinases show enhanced activity in tamoxifen-resistant breast cancer. This phosphorylation changes the action of tamoxifen from antagonist to agonist; thus, the presence of tamoxifen now leads to the formation of an active transcription complex. The mechanisms underlying this change of response are not fully understood, but phosphorylation of S305 induces an altered orientation of binding between ERα and the steroid receptor coactivator-1, allowing the recruitment of RNA polymerase II and ERα-mediated gene transcription. This occurs without changes in the overall levels of binding; thus, in the presence of tamoxifen, estrogen-dependent gene transcription can be induced, and in patients with overactive protein kinase A or p21-activated kinase-1, treatment with tamoxifen could enhance tumor progression. To date, though, no studies have explored this mechanism in patient tissues.

Recently, a third isoform of ER has been identified, a 36kDa protein transcribed from an alternative start site and lacking the transactivation domains of the full-length (66kDa) ER, ER-α36. This isoform has a dominant negative effect on ERα activity, inhibiting both estrogen-dependent and independent effects, and levels are increased in tamoxifen-resistant MCF-7 cells and have been associated with poorer disease-free survival in ER-positive and ER-negative breast cancer. The binding of both estrogen and tamoxifen to ER-α36 stimulates activation of mitogen-activated protein kinase (MAPK)/extracellular signal regulated kinase (ERK) pathway, leading to cell proliferation and thus contributing to tamoxifen resistance.

In addition to the MAPK/ERK pathway, ERα can activate, by nontranscriptional mechanisms, the PI3K/Akt pathway (see Figure 1 for details), and alterations that increase the activity of this signaling pathway can lead to tamoxifen resistance. However, ERα is not the only receptor that can activate this pathway. It is also activated by a number of growth factor receptors, including fibroblast growth factor receptors (FGFR) 1 and 3 and insulin-like growth factor receptor 1 (IGF-1R). Both of these receptors have been shown to contribute to tamoxifen resistance via PI3K/Akt signaling and MAPK signaling. Recent work suggests that microRNAs (miRNAs) also have important roles in modulating the PI3K/Akt pathway in response to tamoxifen treatment. Sachdeva et al identified miR-101 as being able to confer tamoxifen-resistant and estrogen-independent growth on MCF-7 cells by suppressing membrane-associated guanylate kinase inverted 2 expression and reducing the activity of phosphatase and tensin homolog (PTEN), a negative regulator of the PI3K/Akt pathway.
regulator of the PI3K/Akt pathway. Aberrations to the PI3K/Akt pathway and the roles of miRNAs in drug resistance in breast cancer are discussed in more detail in the sections PI3K/Akt pathway and miRNA-mediated resistance.

Aberrant expression of ERα target genes can lead to tamoxifen resistance. For example, overexpression of cyclin D1 drives cancer cell proliferation, circumventing the normal cell-cycle control and deregulating ERα-dependent gene responses. Such alterations to the expression of ERα-dependent genes often arise from epigenetic changes, which occur in multiple formats (see section Epigenetic regulation for more details), the net effects of which are increased or decreased gene transcription. For example, transcription of the antiapoptotic gene BCL-2, an ERα target gene, is increased in tamoxifen-resistant cells, due to histone demethylation, thus favoring cell survival and disease progression.

HER2-positive breast cancer
HER2 is a receptor tyrosine kinase and a member of the ErBb family. Dimerization of HER2 as homo- or heterodimers with other ErBb family members, in both the presence and absence of ligand, leads to receptor activation and downstream signaling (see Figure 1). In mammary tissues, HER2 activation promotes cell survival and proliferation. Amplification of the HER2 (ErhB2) gene occurs in approximately 20% of breast cancers, and 8% of ERα-positive breast cancers are also positive for HER2. ERα/HER2-positive breast cancer is associated with a poorer clinical outcome due to a tendency to display intrinsic resistance to endocrine therapy, which may result from interplay between the downstream signaling pathways.

The development of trastuzumab (Herceptin), a monoclonal antibody that binds HER2, has improved clinical outcomes for patients with HER2 amplification. Trastuzumab induces a cytostatic signal, G1 arrest, and induction of an immune response to destroy the cancer cell. However, de novo resistance to trastuzumab occurs in approximately 65% of cases, and resistance develops in approximately 70% of patients who initially respond. A number of causative mechanisms have been proposed (for an in-depth review, please see Pohllmann et al and Vu and Claret), including altered binding, upregulation of downstream pathways, and alterations to the immune response induced.

Molecular mechanisms of trastuzumab resistance
Epitope masking
Resistance to trastuzumab can occur due to epitope masking altering the binding to HER2. Two possible epitope masking candidates have been identified to date. Mucin 4, a large O-glycosylated membrane-associated protein, is one possible candidate, as it is upregulated in JIMT-1 cells that are trastuzumab resistant. The ratio of trastuzumab binding to HER2 binding sites was a fifth of normal on these cells, and trastuzumab–HER2 binding was increased by RNAi knockdown of Mucin 4. The second candidate is the CD44/hyaluronan polymer complex, and, again, RNAi knockdown of CD44 or chemical inhibition of hyaluronan synthesis resulted in increased trastuzumab–HER2 binding in JIMT-1 cells. This effect is postulated to be due to the bulky nature of the complex sterically hindering trastuzumab binding and alterations to trastuzumab internalization, thus conferring resistance, as trastuzumab cannot suppress HER2 signaling.

HER2 signaling
Blockade of HER2 signaling with trastuzumab can be bypassed, to some degree, by the upregulation of other ErBb family members and increased heterodimer formation. In addition, in up to 30% of HER2-enriched breast cancers, an amino-terminal truncated form of HER2 is expressed, p95-HER2. This form of HER2 possesses constitutive kinase activity, triggering downstream signaling, but lacks the trastuzumab binding site, thus generating trastuzumab resistance. In these cases, treatment with another monoclonal antibody, pertuzumab, which blocks dimer formation, or with lapatinib, which inhibits the tyrosine kinase activity of HER2 dimers, may restore clinical responsiveness to anti-HER2 therapy. However, even if HER2 signaling is effectively blocked, cancer proliferation may continue, as downstream pathways are activated by alternative routes, as described for tamoxifen resistance, again via IGF-1R, which is often overexpressed in trastuzumab-resistant cells, and its inhibition can restore sensitivity to trastuzumab in SKBR3 cells. Signals from IGF-1R are transduced, in part, by the PI3K/Akt pathway, which is also activated by HER2 and ERα. Trastuzumab treatment can induce alterations to this pathway, giving rise to resistance via sustained Akt activation. One mechanism that may mediate this is the upregulation of c-Met, which physically interacts with HER2, enhancing growth in HER2-overexpressing cells. This effect is abrogated upon treatment with a c-Met inhibitor such as SU11274. Trastuzumab treatment also induces upregulation of a number of miRNAs, notably miR-21, that can influence the PI3K/Akt pathway by inhibiting PTEN. In trastuzumab-resistant cells, overactivity of the PI3K/Akt pathway leads to epigenetic changes, including repression of FoxO, and
subsequently the increased transcription of the antiapoptotic
gene survivin.\textsuperscript{46} It can also downregulate p27\textsuperscript{Kip1}, which
has been proposed as a critical downstream effector of
trastuzumab, leading to increased cyclin-dependent kinase
2 expression and cell proliferation.\textsuperscript{42} The aberrations in this
pathway contributing to multidrug-resistant breast cancer are
discussed further in the section PI3K/Akt pathway.

**Altered immune response**

As a monoclonal antibody, trastuzumab-induced cell death
is partially mediated by antibody-dependent cell-mediated
cytotoxicity, a process by which natural killer cells monocytes
bind to the Fc region of trastuzumab with Fc\gamma receptors
and initiate tumor cell death.\textsuperscript{47} Fc\gammaRIIIa has a well-known
polymorphism at position 158, where either a valine or a
phenylalanine is expressed, which has been linked to clin-
cal prognosis. Indeed, the latter is less effective at inducing
antibody-dependent cell-mediated cytotoxicity upon trastu-
zumab binding to HER2 and shows poorer clinical outcome
than the valine/valine genotype in patients.\textsuperscript{48} This mechanism
of resistance is specific to monoclonal antibody therapies
and not related to the common mechanisms discussed in
the section Common mechanisms leading to drug-resistant
breast cancer.

**Triple-negative breast cancer**

Triple-negative breast cancer does not express ER\alpha or pro-
gestrone receptors (PR) or show HER2 amplification, and
accounts for 10\%–20\% of breast cancers.\textsuperscript{49} To date, there
are no targeted therapies for this breast cancer subtype.
Consequently, frontline treatments are limited to surgical
approaches and chemotherapeutics, with the taxanes and
anthracyclines being the chemotherapeutics of choice.\textsuperscript{30}
Taxanes (eg, paclitaxel) act as mitotic poisons by stabilizing
microtubules, leading to abnormal spindles and induction of
apoptosis,\textsuperscript{51} whereas anthracyclines (eg, doxorubicin) act as
DNA intercalators and topoisomerase II inhibitors and they
can also generate reactive oxygen species via semiquinone
formation.\textsuperscript{52} These chemotherapeutics are often given in
conjunction with the targeted therapies for the other sub-
types of breast cancer discussed previously, and thus cellular
alterations induced by targeted therapies can contribute to
chemotherapeutic resistance and vice versa.

**Molecular mechanisms of resistance to chemotherapeutics**

Resistance to both the taxanes and the anthracyclines
predominantly arises from decreased drug intracellular
concentrations due to increased efflux.\textsuperscript{12} This increased
efflux is mediated by a small number of adenosine triphos-
phate (ATP)-binding cassette proteins that utilize ATP hydro-
lysis to translocate a variety of substrates, and increased
expression of these correlates with poor clinical prognosis
in breast cancer irrespective of subtype.\textsuperscript{53} The taxanes and
anthracyclines are substrates for P-glycoprotein (P-gp), the
best studied of the ABC transporters responsible for drug
efflux and encoded by the \textit{MDR1} gene (\textit{ABCB1}), as well as
other transporters, notably multidrug resistance-associated
protein 2 (MRP2) and breast cancer resistance protein, that
contribute to resistance to chemotherapeutics.\textsuperscript{54} We will
focus on P-gp here as an example, as many of the mecha-
nisms discussed may be applicable to MRP2 and breast
cancer resistance protein. Resistance to one chemotherapeu-
tic commonly means resistance to many, often structurally
distinct compounds, as P-gp can transport a diverse range
of molecular structures. Indeed, the tamoxifen metabolite
endoxifen is a P-gp substrate.\textsuperscript{55}

**Alterations to drug efflux levels**

The primary mechanism underlying increased efflux is
upregulation of the drug transporter proteins. This phe-
nomenon occurs in a wide variety of cancers and has con-
sequently been excellently reviewed by Chen and Sikic.\textsuperscript{12}
Subsequently, we will cover this topic briefly here to high-
light the commonalities with tamoxifen and trastuzumab
resistance, specifically focusing on miRNAs, the PI3K/
Akt pathway, and epigenetic alterations (discussed in more
detail in the section Common mechanisms leading to drug-
resistant breast cancer).

Recent work has identified miRNAs as having an
important role in multidrug resistance, with over 50 dif-
ferent miRNAs linked to this phenotype (see Kutanzi
et al\textsuperscript{56} for a review). A number of these miRNAs, including
miR-19, miR-21, and miR-205, target PTEN, suggesting
that the PI3K/Akt pathway is important in resistance to
chemotherapeutics as well as for the more targeted thera-
pies discussed previously. Indeed, use of the Akt inhibitor
perifosine in multidrug-resistant MCF-7 cells improved
response to doxorubicin treatment.\textsuperscript{57} It remains to be seen
whether this is via a direct effect of the PI3K/Akt pathway
on \textit{MDR1} expression or an indirect effect possibly via a
reduction in cell survival signaling. In addition to altered
miRNA levels, chemotherapeutic-resistant cancers, both in
vitro and in patients, show diminished epigenetic repression
of \textit{MDR1} with promoter hypomethylation and histone H3
lysine 9 acetylation.\textsuperscript{58–60}
Common mechanisms leading to drug-resistant breast cancer

As can be seen from these discussions, the three clinical subtypes of breast cancer have distinct therapeutic approaches, but the molecular mechanisms that give rise to refractory disease have common facets, notably alterations to the PI3K/Akt pathway, miRNA levels, and epigenetic modulation of gene transcription. These common facets will now be discussed in more detail, together with their potential as targets for adjuvant therapies to circumvent drug resistance and restore clinical responsiveness.

PI3K/Akt pathway

The PI3K/Akt pathway is an important signaling mechanism regulating many cellular responses, including cell proliferation and survival (Figure 1) in normal as well as neoplastic breast tissue. It forms a convergence point between all three clinical subtypes of breast cancer, and aberrations in this pathway occur in 70% of breast cancers irrespective of subtype. As highlighted previously, aberrations in this pathway are important in resistance to both tamoxifen and trastuzumab, especially as this pathway forms a crosslink between HER2 signaling and ERα-regulated gene transcription, and have also been linked to MDR1 upregulation and resistance to chemotherapeutics. Thus, understanding this pathway is paving the way for new adjuvant treatments in resistant breast cancer. A number of changes can occur, but all result in sustained pathway activity. Common aberrations include activating mutations or amplification of any of the PI3K subunits p110α, p110β, or p85α or loss of PTEN activity and its inhibition of PI3K, via inactivating mutations, over-expression of miRNAs, or promoter hypermethylation. Both of these scenarios result in increased Akt phosphorylation and sustained Akt activation, the net effects of which are inhibition of apoptosis, transcription of ERα-dependent genes, and cell proliferation. A major downstream effector of Akt activation that mediates a number of these responses is mammalian target of rapamycin complex 1 (mTORC1).

miRNA-mediated resistance

In the last decade it has become clear that alterations to miRNA expression levels can contribute to cancer prognosis and outcome. miRNAs are small, noncoding RNAs or approximately 22 nucleotides, which bind to mRNA, preventing translation and accelerating mRNA deadenylation and subsequent degradation, thus having a gene silencing effect. Several miRNAs have been associated with drug resistance in breast cancer (see Table 1), and these target a variety of genes, including PTEN, ESRI (ERα), FoxO3, and DNA (cytosine-5)-methyltransferases (DNMTs). The mechanisms that lead to miRNA upregulation in drug-resistant breast cancer are currently unclear, but they have powerful effects. One miRNA that is overexpressed

Table 1 miRNA associated with drug resistance in breast cancer

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Expression change associated with resistance</th>
<th>Target genes</th>
<th>References</th>
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<tbody>
<tr>
<td>Lin 28</td>
<td>Upregulated</td>
<td>CDKN1A (p21), RBL, Let-7</td>
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<td>miR-10a</td>
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<td>PTEN, PTCD4</td>
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<td>92</td>
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<td>miR-22</td>
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<tr>
<td>miR-29a</td>
<td>Upregulated</td>
<td>PTEN</td>
<td>92,94</td>
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<td>miR-30c</td>
<td>Downregulated</td>
<td>TWIF1</td>
<td>95</td>
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<td>miR-31</td>
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<td>PKC epsilon (P5KCE)</td>
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<td>E2F3</td>
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<td>BMI1, ABC5C</td>
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<td>miR-181</td>
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<td>miR-181a</td>
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<td>ABCG2 (BCRP)</td>
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<td>ZEB1, CDH1 (E-cadherin), PTEN, NTRK2 (TrkB), BMI1</td>
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<td>miR-633</td>
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<td>HSPG2 (hypomethylated)</td>
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Notes: A number of miRNAs have shown altered expression levels in drug-resistant forms of breast cancer in both cells and patients. The table contains those reported since 2011 (for prior studies we refer the reader to Kutanz et al[62] together with their delineated target genes.

Abbreviations: BCRP, breast cancer resistance protein; miRNA, micro-RNAs; PTCD4, programmed cell death 4; P-gp, P-glycoprotein; PTEN, phosphatase and tensin homolog.
in both trastuzumab-resistant cells and cells resistant to chemotherapeutics is miR-21, which targets PTEN and results in sustained PI3K/Akt pathway activity as discussed previously. It also downregulates the apoptotic gene programmed cell death 4 (PDCD4), allowing cancer cells to evade apoptosis. This protein is also inactivated by phosphorylation by S6K1, a downstream effector of the PI3K/Akt pathway. Another prominent miRNA that appears to be important in drug resistance in both ERα-positive and triple-negative breast cancer is miR-221, which targets the cell-cycle inhibitory protein p27Kip1, among others. Thus, it can be seen that miRNAs have important roles in mediating drug resistance in breast cancer. However, the mechanisms leading to miRNA overexpression are not yet fully understood.

### Epigenetic regulation

There are three main interlinked mechanisms by which epigenetic modulation leads to transcriptional regulation, chromatin remodeling, modification of nucleosome composition, and modification of epigenetic marks, all of which have been implicated in resistance to breast cancer therapies. ATP-dependent chromatin remodeling allows transcriptional complexes to access the highly coiled genomic DNA to initiate gene transcription. This can be achieved by selected transcription factors, known as pioneer factors. One such family, the Forkheads (Fox), is highly involved in breast cancer. Indeed, FoxA1 controls approximately 50% of ERα target genes, and its expression, along with that of FoxP1, has been correlated to a favorable response to tamoxifen treatment. In contrast, FoxM1 has a role in trastuzumab and paclitaxel resistance, as knockdown increases drug sensitivity in multidrug-resistant cell lines. FoxO1 expression is also associated with chemotherapeutic and tamoxifen resistance, as it regulates the transcription of both the MDR1 (P-gp) and ABC2 (MRP2) drug efflux pumps. The nuclear translocation of another FoxO isoform, FoxO3a, is inhibited by phosphorylation by Akt, which acts to drive cell proliferation and tamoxifen resistance, as FoxO3a has cytostatic actions via p27 upregulation and cell-cycle inhibition and by decreasing the expression of ERα-regulated genes. The biology of this complex family of transcription factors is not fully understood, but it has become clear that the balance of expression of the different isoforms is important, and further studies are needed to fully delineate their roles in drug-resistant breast cancer.

Nucleosomes are duplicates of the canonical histones H2A, H2B, H3, and H4 contained in a DNA loop, and modification by substitution with noncanonical histones regulates chromatin compaction and thus the transcription factor access to the genomic DNA. One such noncanonical histone implicated in ERα-positive breast cancer is H2A.Z, which stabilizes the nucleosome at the promoter of ERα-dependent genes. Overexpression of H2A.Z increased cell growth in MCF-7 breast cancer cells in the presence of tamoxifen and the absence of estrogen, suggesting that this histone can drive cell growth in an ERα-independent manner, and high expression levels of H2A.Z in patients have been associated with poor clinical prognosis. Post-translational modification of histone residues, predominantly by methylation and acetylation, also contributes to alterations in gene transcription. Methylation of lysine 27 of histone H3 governs the ligand dependency of ERα-mediated transcription of the BCL-2 gene, an important driver of the antiapoptotic response of breast cancer cells. Both tamoxifen-resistant and triple-negative breast cancer have been shown to have demethylation of H3K27 and consequent constitutive activation of BCL-2 and cancer cell survival. Changes to histone acetylation also occur in breast cancer, and hyperacetylation of histones H3 and H4 of the HER2 promoter may contribute to trastuzumab resistance by driving increased HER2 expression. Histone hyperacetylation is also involved in the overexpression of MDR1 (P-gp) in chemotherapeutic-resistant breast cancer, and the levels of acetylation of the lysine 9 residue of histone H3 are elevated by two orders of magnitude in drug-resistant MCF-7 cells.

In addition to histone residues, DNA bases can also be methylated, specifically at CpG sites, which are often found to be associated with gene promoter elements. The formation of methylated CpGs is catalyzed by the DNMT enzymes, and methylation levels are inversely correlated with gene transcription. Both hypermethylation and hypomethylation occur in drug-resistant breast cancer, as seen in Table 2. PTEN is frequently hypermethylated in drug-resistant breast cancer, and this silencing is maintained by a positive feedback as reduced PTEN levels lead to increased Akt activity and increased activity of DNMT1, the DNMT that methylates the PTEN promoter expression, thus bypassing HER2 or ERα induction of PI3K/Akt pathway activation. Hypermethylation of the ESR1 (ERα) promoter by DNMT3B contributes to tamoxifen resistance by reducing expression of tamoxifen’s target, ERα. In contrast, hypomethylation and increased expression
of MDR1 (P-gp) play an important role in resistance to chemotherapeutics. Thus, it is clear that DNA methylation status has important roles in mediating drug resistance in breast cancer and, to this end, Rhee et al have recently published an integrated analysis correlating DNA methylation status with gene expression data for the different subtypes of breast cancer. This study, together with the comprehensive molecular analysis of the subtypes compiled by the Cancer Genome Atlas Research Network, will allow other genes with altered methylation states to be identified and their roles in drug resistance explored, especially for drug-resistant HER2-positive breast cancer, as this area has not been explored in this subtype.

**Future perspectives for the treatment of drug-resistant breast cancer**

The studies discussed here delineating the molecular mechanisms underlying drug resistance in breast cancer, in terms of both single and multidrug resistance, have identified a

**Table 2 Genes with altered methylation status in drug-resistant breast cancer**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Hypermethylated/hypomethylated</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCB1 (MDR1)</td>
<td>P-glycoprotein</td>
<td>Hypomethylated</td>
<td>57,58,111–114</td>
</tr>
<tr>
<td>ABCB1 (MDR1)</td>
<td>P-glycoprotein</td>
<td>Hypermethylated</td>
<td>111</td>
</tr>
<tr>
<td>CDH1</td>
<td>E-cadherin</td>
<td>Hypermethylated</td>
<td>113</td>
</tr>
<tr>
<td>CDK10</td>
<td>Cyclin-dependent kinase 10</td>
<td>Hypermethylated</td>
<td>116</td>
</tr>
<tr>
<td>CXXR4</td>
<td>Stromal cell-derived factor 1 receptor</td>
<td>Hypermethylated</td>
<td>115</td>
</tr>
<tr>
<td>ESR1</td>
<td>ERα</td>
<td>Hypermethylated</td>
<td>113,115</td>
</tr>
<tr>
<td>ESR2</td>
<td>ERβ</td>
<td>Hypermethylated</td>
<td>117,118</td>
</tr>
<tr>
<td>FTH1</td>
<td>Ferritin heavy chain</td>
<td>Hypomethylated</td>
<td>115</td>
</tr>
<tr>
<td>FOXX1</td>
<td>Forkhead box protein k1</td>
<td>Hypomethylated</td>
<td>115</td>
</tr>
<tr>
<td>GSTP1</td>
<td>Glutathione S-transferase π1</td>
<td>Hypomethylated</td>
<td>57,113</td>
</tr>
<tr>
<td>HIC1</td>
<td>Hypermethylated in cancer 1</td>
<td>Hypermethylated</td>
<td>113</td>
</tr>
<tr>
<td>IL2</td>
<td>Interleukin 2</td>
<td>Hypermethylated</td>
<td>115</td>
</tr>
<tr>
<td>LEP</td>
<td>Leptin</td>
<td>Hypermethylated</td>
<td>115</td>
</tr>
<tr>
<td>MGMT</td>
<td>6-O-methylguanine-DNA methyltransferase</td>
<td>Hypermethylated</td>
<td>57,119</td>
</tr>
<tr>
<td>NAT1</td>
<td>N-acetyltransferase 1</td>
<td>Hypermethylated</td>
<td>120</td>
</tr>
<tr>
<td>CDKN1A</td>
<td>p21</td>
<td>Hypermethylated</td>
<td>121</td>
</tr>
<tr>
<td>TP73</td>
<td>p73</td>
<td>Hypermethylated</td>
<td>115</td>
</tr>
<tr>
<td>PLAU</td>
<td>Plasminogen activator urokinase</td>
<td>Hypomethylated</td>
<td>57,113</td>
</tr>
<tr>
<td>PGR</td>
<td>Progesterone receptor</td>
<td>Hypermethylated</td>
<td>122</td>
</tr>
<tr>
<td>TFF1</td>
<td>p52 (trefoil factor 1)</td>
<td>Hypermethylated</td>
<td>123</td>
</tr>
<tr>
<td>DUSP7</td>
<td>Dual-specific phosphatase 7</td>
<td>Hypermethylated</td>
<td>123</td>
</tr>
<tr>
<td>GDF15</td>
<td>Growth differentiation factor 15</td>
<td>Hypermethylated</td>
<td>123</td>
</tr>
<tr>
<td>PSAT1</td>
<td>Phosphoserine aminotransferase 1</td>
<td>Hypermethylated</td>
<td>124</td>
</tr>
<tr>
<td>PTEN</td>
<td>PTEN</td>
<td>Hypermethylated</td>
<td>78</td>
</tr>
<tr>
<td>RAB6C</td>
<td>WTH3</td>
<td>Hypermethylated</td>
<td>113,125,126</td>
</tr>
<tr>
<td>RASAL1</td>
<td>Ras protein activator-like 2 (GAP1)</td>
<td>Hypermethylated</td>
<td>113</td>
</tr>
<tr>
<td>ARHGGEF2</td>
<td>Rho/rac GEF2</td>
<td>Hypermethylated</td>
<td>115</td>
</tr>
<tr>
<td>RASSF1</td>
<td>Ras association domain containing protein 1</td>
<td>Hypermethylated</td>
<td>113</td>
</tr>
<tr>
<td>RFC1</td>
<td>Replication factor C1</td>
<td>Hypermethylated</td>
<td>127</td>
</tr>
<tr>
<td>SDK2</td>
<td>Sidekick 2</td>
<td>Hypermethylated</td>
<td>115</td>
</tr>
<tr>
<td>SULF2</td>
<td>Sulfatase 2</td>
<td>Hypermethylated</td>
<td>113</td>
</tr>
<tr>
<td>TGM2</td>
<td>Tissue transglutaminase</td>
<td>Hypermethylated</td>
<td>113,115</td>
</tr>
<tr>
<td>UTRN</td>
<td>Utrophin</td>
<td>Hypermethylated</td>
<td>115</td>
</tr>
</tbody>
</table>

**Note:** Hypomethylation results in gene overexpression as DNA methylation reduces gene transcription; consequently, hypermethylation effectively leads to gene silencing.

**Abbreviations:** eR, estrogen receptor; PTEN, phosphatase and tensin homolog.
number of pathways that offer potential routes to circumvent resistance to the current therapies.

The PI3K/Akt/mTORC1 signaling axis offers targets for therapeutic interventions, and a number of clinical trials are ongoing using PI3K, AKT, mTOR, or dual inhibitors in combination with endocrine or chemotherapies (Table 3). However, caution is required, as the clinical response may depend on the specific aberration and the subtype of breast cancer, as inhibition of Akt may induce apoptosis by release of Bcl-2-associated death promoter (BAD) inhibition, but Akt inhibition can also permit FoxO3a nuclear translocation, potentially leading to the transcription of ERα-dependent genes encouraging cell proliferation. Also, upregulation of growth factor receptors (eg, FGFRs and IGF-1R) may favor activation of other signaling cascades, such as the MAPK pathways, which could be exacerbated by inhibition of

Table 3 Inhibitors of the PI3K/Akt pathway currently undergoing clinical trials

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Breast cancer selection criteria</th>
<th>Combination therapies</th>
<th>Phase</th>
<th>Trial identifiers</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZD5363</td>
<td>Akt</td>
<td>ER+</td>
<td>Paclitaxel/none</td>
<td>I</td>
<td>NCT01625286, NCT01226316</td>
</tr>
<tr>
<td>GSK2110183</td>
<td>Akt</td>
<td>Drug resistant</td>
<td>None</td>
<td>I</td>
<td>NCT01476137</td>
</tr>
<tr>
<td>GSK2141795</td>
<td>Akt</td>
<td>Not stated</td>
<td>None</td>
<td>I</td>
<td>NCT00920257</td>
</tr>
<tr>
<td>MK2206</td>
<td>Akt</td>
<td>ER+</td>
<td>Lapatinib ditosylate/paclitaxel/\ anastrozole/letrozole/exemestane/\ fulvestrant/none</td>
<td>II</td>
<td>NCT01245205, NCT01277757, NCT01776008, NCT01344031</td>
</tr>
<tr>
<td>HER2+</td>
<td></td>
<td></td>
<td></td>
<td>I</td>
<td>NCT01705340, NCT01281163</td>
</tr>
<tr>
<td>Not stated</td>
<td>Akt</td>
<td>Paclitaxel</td>
<td></td>
<td>I</td>
<td>NCT01263145</td>
</tr>
<tr>
<td>Triciribine Phosphate Monohydrate</td>
<td>Akt</td>
<td>Not stated</td>
<td>Palbociclib/\ doxorubicin/\ cyclophosphamide</td>
<td>I/II</td>
<td>NCT01697293</td>
</tr>
<tr>
<td>BY 80–6946</td>
<td>PI3K</td>
<td>Not stated</td>
<td>Paclitaxel</td>
<td>I</td>
<td>NCT01411410</td>
</tr>
<tr>
<td>BKM120</td>
<td>PI3K</td>
<td>ER+</td>
<td>Fulvestrant/letrozole</td>
<td>I, III</td>
<td>NCT01339442, NCT01248494, NCT01633060, NCT01610284</td>
</tr>
<tr>
<td>HER2</td>
<td></td>
<td></td>
<td>Lapatinib/\ trastuzumab/\ capecitabine</td>
<td>I/II</td>
<td>NCT01589861, NCT01132664</td>
</tr>
<tr>
<td>Trastuzumab-resistant HER2+</td>
<td></td>
<td></td>
<td>Trastuzumab + paclitaxel</td>
<td>I, II</td>
<td>NCT01285466, NCT01816594</td>
</tr>
<tr>
<td>HER2–</td>
<td></td>
<td></td>
<td>Paclitaxel</td>
<td>II</td>
<td>NCT01572727</td>
</tr>
<tr>
<td>Triple-negative</td>
<td></td>
<td></td>
<td>Postchemotherapeutics</td>
<td>I, II</td>
<td>NCT01629615</td>
</tr>
<tr>
<td>GDC-0941</td>
<td>PI3K</td>
<td>ER+/HER2–</td>
<td>Letrozole/fulvestrant</td>
<td>I</td>
<td>NCT01791478, NCT01219699</td>
</tr>
<tr>
<td>Not stated</td>
<td>PI3K</td>
<td>Trastuzumab, paclitaxel, bevacizum</td>
<td>Fulvestrant</td>
<td>I</td>
<td>NCT00960960</td>
</tr>
<tr>
<td>ER+</td>
<td></td>
<td></td>
<td>Letrozole</td>
<td>II</td>
<td>NCT01437566</td>
</tr>
<tr>
<td>XL147</td>
<td>PI3K</td>
<td>ER+ HER2+</td>
<td>Letrozole</td>
<td>I</td>
<td>NCT01082068</td>
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<tr>
<td>CC-223</td>
<td>mTOR</td>
<td>ER+/HER2+/–</td>
<td>Endocrine therapies (tamoxifen)/\ bevacizumab, lapatinib</td>
<td>II, III</td>
<td>NCT01298713, NCT01805271</td>
</tr>
<tr>
<td>Everolimus</td>
<td>mTOR</td>
<td>ER+, AI-resistant</td>
<td>Fulvestrant/\ \ chemotherapeutics/exemestane/\ Paclitaxel, trastuzumab</td>
<td>II, III</td>
<td>NCT01779120, NCT01088893, NCT00863655 and NCT01626222</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>mTOR</td>
<td>HER2+</td>
<td>Vinorelbine</td>
<td>II</td>
<td>NCT01520103</td>
</tr>
<tr>
<td>Temsirolimus</td>
<td>mTOR</td>
<td>HER2+</td>
<td>Gemcitabine, cisplatin</td>
<td>I</td>
<td>NCT01939418</td>
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<tr>
<td>Ridaforolimus</td>
<td>mTOR</td>
<td>HER2–</td>
<td>Trastuzumab</td>
<td>II</td>
<td>NCT00411788</td>
</tr>
<tr>
<td>BEZ235</td>
<td>PI3K/mTOR dual inhibitor</td>
<td>HER2+</td>
<td>Letrozole/everolimus</td>
<td>I</td>
<td>NCT01248494, NCT01482156</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TRiple-negative/\ triple negative</td>
<td>Dalotuzumab, exemestane</td>
<td>II</td>
<td>NCT01234857</td>
</tr>
<tr>
<td>GDC-0980</td>
<td>PI3K/mTOR dual inhibitor</td>
<td>ER+</td>
<td>Paclitaxel, trastuzumab</td>
<td>I</td>
<td>NCT01285466</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TRiple-negative/\ triple negative</td>
<td>Trastuzumab</td>
<td>II</td>
<td>NCT01495247</td>
</tr>
<tr>
<td>XL765</td>
<td>PI3K/mTOR dual inhibitor</td>
<td>ER+ HER2–</td>
<td>Fulvestrant</td>
<td>II</td>
<td>NCT01437566</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Letrozole</td>
<td>II</td>
<td>NCT01082068</td>
</tr>
</tbody>
</table>

Notes: The alterations to the PI3K/Akt pathway are common factors in the different subtypes of breast cancer and also have important roles in mediating drug resistance (see text for details). Subsequently, this pathway is a promising therapeutic target to overcome drug resistance with a variety of compounds in clinical trials. Data obtained from the ClinicalTrials.gov database. Abbreviations: AI, aromatase inhibitor; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; mTOR, mammalian target of rapamycin complex 1.
PI3K/Akt/mTORC1. In addition, the effects of this pathway on transcription via epigenetic changes need to be considered to prevent the selection of tumor subpopulations that are resistant to therapy, especially the FoxO family, as targeting these transcription factors directly is not a viable option currently, due to their complexity. Thus, the combination of therapies needs to be carefully considered and appropriate for the cancer subtype.

Overcoming drug resistance that results from P-gp overexpression may require a different approach to that of the PI3K/Akt pathway, as several P-gp inhibitors have been trialed without satisfactory clinical outcomes.83 To this end, a number of compounds that are microtubule stabilizers, like the taxanes, but are not P-gp substrates have been trialed with promising clinical results (see Nobili et al85 for details). Other drugs that actually exploit P-gp overexpression are being considered, notably NSC73306, the cytotoxic capacity of which correlates with P-gp expression levels. The more P-gp expressed, the more toxic NSC73306 is. NSC73306 does not appear to be a P-gp substrate. Understanding the way this drug utilizes P-gp overexpression may help identify mechanisms to circumvent multidrug resistance involving P-gp.

As yet, no therapeutics targeting specific miRNAs have made it into the clinic. However, one antagonist to miR-122 is undergoing Phase II clinical trials for use in hepatitis C,84 suggesting that miRNAs could be valid therapeutic targets in breast cancer. To this end, therapeutics targeting miR-21 and miR-221, which are implicated in drug resistance in breast cancer, are being commercially developed for use in hepatocellular carcinoma and other cancers,85,86 and so it may not be too long before clinical trials of miRNA inhibitors for the treatment of multidrug-resistant breast cancer become a reality.

Reversing the deleterious alterations to epigenetic regulation that are associated with drug resistance in breast cancer is a challenging proposition. However, histone deacetylase inhibitors are showing promise in all subtypes of breast cancer,87,88 but caution is required, as these approaches may alter the expression of ERα, HER2, and MDR1, as discussed previously, which could actually enhance drug resistance. It will be interesting to see how the PI3K/Akt/mTOR inhibitors perform in the ongoing clinical trials to ascertain whether such global approaches are useful for circumventing resistance to frontline therapies. Further studies are required before therapeutics targeting other common mechanisms highlighted here, miRNA upregulation and epigenetic alteration, may be targeted, as these are not yet as well understood.

**Disclosure**

The authors report no conflicts of interest in this work.

**References**


