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Resistance to HER2-targeted therapies: a potential role for FOXM1

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SUMMARY

Despite the tremendous efficacy of trastuzumab against HER2-overexpressing metastatic breast cancers, a significant fraction of women demonstrate progressive disease during treatment. Multiple mechanisms have been proposed to mediate trastuzumab resistance. In this mini-review, we discuss the evidence supporting FOXM1 as a mediator of resistance and potential new therapeutic target in trastuzumab-refractory breast cancer. FOXM1 expression is significantly elevated in multiple breast cancer data sets. Some studies suggest a direct correlation between FOXM1 and HER2 expression levels. In addition, overexpression of FOXM1 reduces the sensitivity of HER2-positive breast cancer cells to trastuzumab or lapatinib. Conversely, knockdown or pharmacological inhibition of FOXM1 rescues resistance to HER2-targeted therapies. Current pre-clinical information supports further investigation of the role of FOXM1 in trastuzumab-resistant breast cancer.

Keywords

breast cancer; foxm1; her2; lapatinib; resistance; trastuzumab

Introduction

Breast cancer remains one of the most common cancers worldwide and is a major cause of cancer-related deaths among adult females in the United States. Molecular techniques, such as immunohistochemistry (IHC) and fluorescence in situ hybridization, are routinely performed to characterize breast tumor biopsies [1]. Gene expression profiling has identified

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at least five major subtypes of breast cancer- luminal A (ER+ and/or PR+, HER2–, low Ki67), luminal B (ER+ and/or PR+, HER2+ or HER2– with high Ki67), ER– and HER2+, triple negative (TNBC) and basal-like (ER–, PR–, HER2–) [2–7]. Histopathological and molecular classifications improve the abilities to predict outcomes and direct appropriate targeted treatment options to patients.

Among newly diagnosed breast cancer patients, an estimated 15–20% of breast tumors demonstrate overexpression of the human epidermal growth factor receptor 2 (HER2) receptor tyrosine kinase [8–10]. *HER2* amplification is associated with a more aggressive tumor biology [11] and an increased incidence of metastasis [12] due to the constitutive activation of numerous downstream signaling networks involved in migration, cell-cycle regulation, proliferation, inhibition of apoptosis, and angiogenesis [13, 14]. The increased expression of this cell-surface molecule specifically in tumor cells and its association with unfavorable outcomes in patients with breast cancer provide rationale for selectively inhibiting this molecular target. The first anti-HER2 antibody to be translated to clinical use was trastuzumab [15], which is currently the main first-line therapy for patients with HER2-overexpressing breast cancer. Trastuzumab binds to domain IV of the HER2 extracellular domain and disrupts downstream PI3K signaling [16] and Ras-MAPK signaling [17]. Trastuzumab-mediated tumor regression appears to be partially dependent on the abilities to block angiogenesis [18, 19], induce antibody-dependent cellular cytotoxicity [20, 21], and suppress invasion and metastasis [22, 23], which may be related to the ability to target a HER2-positive stem cell population [24, 25].

Despite the tremendous efficacy of trastuzumab against HER2-overexpressing metastatic breast cancers, a significant fraction of women demonstrate progressive disease during treatment. There are many proposed mechanisms of resistance. One potential mechanism is masking of the HER2 epitope to which trastuzumab binds, which has been described as a result of overexpression of the mucin cell-surface protein MUC4 [26]. Compensatory signaling and receptor cross-talk have also been proposed as mechanisms through which HER2 signaling is sustained in resistant cells; for example, the insulin-like growth factor-I receptor [27, 28] and the hepatocyte growth factor receptor MET [29] have been shown to cluster and crosstalk with HER2. Increased signaling through the PI3K pathway is recognized as one of the most clinically relevant mechanisms of resistance and may occur due to down-regulation of PTEN [30], hyperactivating mutations in the catalytic subunit of PI3K [31], or subsequent to increased upstream growth factor receptor signaling. Further downstream, reduced expression or cellular relocalization of the p27 protein [16, 32–35] or increased expression of anti-apoptotic regulators, including Bcl-2 [36], have been described in models of trastuzumab resistance. Another potential mechanism is up-regulation of ligands that increase phosphorylation of HER2, such as the EGFR ligand TGF- α [37], HER3 ligand heregulin [37], and the cytokine growth differentiation factor 15 [38]. There are additional mechanisms of trastuzumab resistance that have been proposed, many of which have been comprehensively discussed in a number of excellent, recent reviews [39–42].

Attempts to overcome trastuzumab resistance have resulted in new therapeutic strategies targeted against HER2, including the small-molecule dual EGFR/HER2 kinase inhibitor

lapatinib [43] [44]. Single-agent lapatinib reduces tyrosine phosphorylation of HER2 [45] and inhibits downstream signaling through PI3K and MAPK in trastuzumab-resistant cells [46, 47]. In addition, lapatinib monotherapy induces apoptosis and increases sensitivity to radiation in trastuzumab-resistant cells [48]. Clinical studies investigating the combinatorial effects of trastuzumab and lapatinib in HER2-overexpressing breast cancers demonstrated synergistic enhancement of trastuzumab-mediated antibody-dependent cellular cytotoxicity [49]. Lapatinib is currently approved as a second-line therapy in combination with chemotherapy for trastuzumab-refractory metastatic breast cancer [50]. However, a majority of patients who received prior trastuzumab therapy demonstrate resistance to lapatinib. Thus, improved understanding of the molecular mechanisms contributing to resistance to both trastuzumab and lapatinib is critical for developing new therapies and for identifying those who are most likely to respond to currently available agents.

FOXM1 and breast cancer

Forkhead box M1 (FOXO1) is a member of the forkhead family of transcription factors [51]. There are more than 100 proteins in the forkhead family, which represents a subgroup of the helix-turn-helix class of transcription factors; this name refers to the winged nature of the DNA-binding domain, which is flanked by two side loops [51]. There are three known isoforms of human FOXO1, which are referred to as FOXO1a, FOXO1b, and FOXO1c; these isoforms result from alternative splicing of the transcript of the 25-kb *foxo1* gene, which contains 10 exons and is found at chromosomal location 12p13.33 [51]. The FOXO1 isoforms are characterized by a highly conserved DNA-binding domain, an N-terminal repressor domain, and a strong transactivation domain. FOXO1b and FOXO1c recognize and activate transcription from consensus sequence 5'-A-C/T-AAA-C/T-AA-3' [51]. Both are regulated by MEK signaling, but FOXO1c has two Erk1/2 phosphorylation sites, S330 and S703 [51]; thus, FOXO1c may be more dependent upon MEK signaling. The functions of FOXO1 are related to the functions of its target genes, of which there are more than 200; these targets regulate the majority of cancer-related processes, including proliferation, invasion, angiogenesis, senescence, stem cell function, and DNA repair (Figure 1) [51]. Normal physiological functions include regulation of replication, mitosis, and repair. Readers are guided to a recent, outstanding review article [51] for in-depth details regarding the normal and cancer-related functions and structure of FOXO1.

FOXO1 is associated with poor prognosis in breast cancer [52]. FOXO1 transcript levels are significantly elevated in multiple breast cancer tissue data sets (Figure 1). Studies indicate that FOXO1 plays a role in most subtypes of breast cancer, not just HER2-overexpressing forms. Triple negative breast cancer (TNBC), FOXO1 overexpression protects cancer cells from DNA double-strand breaks, by interacting with NFκB to promote doxorubicin chemoresistance [53]. Accordingly, it has been shown that FOXO1 inhibition decreases transcription of DNA repair genes and restores Doxorubicin sensitivity in TNBC [53]. Constitutive overexpression of FOXO1 in MCF-7 breast cancer cells promotes acquired cisplatin resistance, by enhancing the expression of the DNA damage response genes; breast cancer-associated gene 2 (BRCA2) and X-ray cross complementing group 1 (XRCC1) [54]. Furthermore, in ERα positive and negative breast cancer cells, FOXO1 has been shown to interact with the coactivator CARM1 to regulate ERα transcription [55].

Increased FOXM1 levels amplify estrogen-mediated mitogenic actions and promote endocrine therapy resistance in ER α positive breast cancer [56]. FOXM1 inhibition has been demonstrated to decrease expression of ER α -regulated genes, suppress estrogen-induced breast cancer cell proliferation, and restore tamoxifen sensitivity [55, 56].

Studies investigating FOXM1 as a downstream target of HER2 signaling have demonstrated a direct correlation between HER2 and FOXM1 expression levels *in vivo* and *in vitro* [57, 58]. Stable overexpression of FOXM1 in HER2-overexpressing cell lines effectively diminished trastuzumab sensitivity, increased colony formation, and inhibited lapatinib-induced cytotoxicity [57, 59]. Interestingly, inhibition of EGFR/HER2 with lapatinib had no observable effect on FOXM1 protein levels in lapatinib-resistant lines. In contrast, combined inhibition of MEK signaling plus lapatinib diminished nuclear FOXM1 levels [58]. Consistent with these findings, inhibition of Raf/MEK/ERK signaling delays G2/M transition and inhibits expression of FOXM1 target genes [60]. Additionally, treatment of sensitive and resistant breast carcinoma lines with the anti-EGFR tyrosine kinase inhibitor gefitinib reduces FOXM1 and HER2 phosphorylation only in sensitive cell lines [61]. Moreover, FOXM1 blocks paclitaxel-induced apoptosis due to reduced levels of the microtubule-destabilizing protein stathmin in HER2-positive breast cancer cells [57]. Aberrant FOXM1 signaling can promote a drug-resistant phenotype, characterized by activation of anti-apoptotic proteins Bcl-2, up-regulation of genes important for homologous recombination, and promotion of epithelial to mesenchymal transition (EMT) [62–64]. Furthermore, FOXM1 has been shown to sustain TGF β -induced formation of a SMAD3/SMAD4 nuclear transcription complex that up-regulates the downstream EMT target SLUG to promote breast cancer metastasis [65, 66]. This process may also be mediated by growth differentiation factor 15 (GDF15), a divergent member of the TGF β superfamily, which promotes invasion, EMT, and is increased in the setting of acquired trastuzumab resistance [38]. Collectively, these studies provide validity for further investigation into the mechanisms of FOXM1-mediated chemoresistance in HER2-positive breast cancer.

Targeting FOXM1 in trastuzumab-resistant breast cancer

FOXM1 deregulation is a potential diagnostic and prognostic biomarker of oncogenic potential in several malignancies [67]. Aberrant HER2 signaling constitutively activates multiple downstream signaling pathways, including PI3K/Akt, and ERK, which enhance FOXM1 signaling [68]. FOXM1 regulates proliferation, mitosis, metastasis, tumor development, and progression in breast cancer [14–20]. Thus, FOXM1-mediated trastuzumab resistance may occur through a variety of molecular mechanisms. Treatment of HER2-positive breast cancer cells with thiostrepton, a selective inhibitor of FOXM1 mRNA, causes increased sensitivity to lapatinib. Furthermore, thiostrepton diminishes proliferation, invasiveness, and transformation, and induces apoptosis in breast cancer cells that express FOXM1, regardless of HER2 overexpression status, indicating that FOXM1-targeting is a relevant approach for multiple subtypes of breast cancer [54, 59]. Knockdown of FOXM1 with thiostrepton in micelle-nanoparticles administered to MDA-MB-231 breast cancer xenografts reduced tumor growth rates and increased apoptosis [69]. Additionally, co-administration of thiostrepton and lapatinib reduces the survival of HER2-positive breast cancer cells. The natural nontoxic agent 3,3'-diiodolymethane (DIM) combined with

trastuzumab causes down-regulation of Akt, NF κ B, and FOXM1 in breast cancer cells [70]. DIM enhances trastuzumab efficacy by selectively reducing FOXM1 expression and inhibiting tumor growth without toxicity [71]. Injection of FOXM1-specific siRNA into tumor xenografts suppresses tumor growth and reduces expression of FOXM1 transcriptional targets [72]. Similarly, treatment of resistant and sensitive breast cancer cells with the ARF-derived peptide, which is a FOXM1 inhibitor, decreases proliferation and restores sensitization to trastuzumab [57]. Knockdown of FOXM1 with shRNA diminishes proliferation, anchorage independence, and tumorigenesis of breast cancer cells *in vitro* and *in vivo* [73]. These studies demonstrate the therapeutic potential of co-targeting FOXM1 in drug-refractory breast cancer.

Future perspective

Battling the clinical challenge of drug resistance requires an understanding of the molecular mechanisms that facilitate escape from targeted therapies. FOXM1 is overexpressed in many breast cancers, including the HER2-overexpressing and triple-negative subtypes. However, the extent to which FOXM1 contributes to the development or progression of individual subtypes of breast cancer remains unknown. Specific inhibition of FOXM1 may have substantial clinical impact in these subtypes, including trastuzumab-refractory metastatic breast cancers. However, further translational and clinical investigations into the mechanisms through which HER2 regulates FOXM1 are needed to determine the true suitability of FOXM1 as a therapeutic target. Further, the mechanisms employed by FOXM1 to promote progression of HER2-positive cancers are not completely defined; knowledge of these mechanisms is needed to develop more effective targeted therapies or combination treatments. Finally, pharmacological approaches to target FOXM1 are lacking. Although the thiazole antibiotic and proteasome inhibitor, thioistrepton, effectively knocks down FOXM1 expression, its clinical utility is limited by the fact that it is insoluble in aqueous solution. Attempts to encapsulate thioistrepton in micelles achieved enhanced apoptosis and reduced tumor growth of FOXM1-expressing TNBC cells in culture and as xenografts [74]. Future efforts should focus on the development of FOXM1-targeted therapies, including nanoencapsulation of FOXM1-targeted siRNA, DNazymes, or proteasome inhibitors. Targeting FOXM1 for degradation should ultimately improve responses to existing cancer therapies, such as trastuzumab, and should delay progression of breast cancer.

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KEY POINTS

- HER2 is overexpressed in 15–20% of metastatic breast cancers.
- Trastuzumab resistance eventually develops in a majority of patients.
- FOXM1 transcript levels are significantly elevated in multiple breast cancer tissue data sets.
- There is a direct correlation between HER2 and FOXM1 expression levels.
- FOXM1 overexpression reduces sensitivity to HER2-targeted treatments.
- FOXM1 knockdown increases sensitivity to HER2-targeted therapies.

Figure 1. The role of FOXM1 in normal cells and the effects of its deregulation in tumor cell chemo-resistance

FOXM1 has several mechanisms of activation. Under normal conditions FOXM1 transcriptional activity and expression are tightly regulated. FOXM1 controls a variety biological process, by driving the transcription of target genes that regulate cell cycle progression/arrest, cellular responses to oxidative stress, DNA damage and cell death. In tumor cells, FOXM1 homeostatic regulation is compromised due to dysregulation of cell signaling pathways. For example, deregulation of Wnt, PI3K, and/or ras/MEK/ERK signaling has been shown to increase FOXM1 expression and activation. Sustained FOXM1 signaling promotes increased expression of FOXM1 target genes and evasion of cell death in tumor cells resulting in chemo-resistance and tumorigenesis. Thus, FOXM1 antagonizes the effects of chemotherapy by upregulating DNA repair, self-renewal, proliferation, and migration.

Abbreviations: Bcl-2, B cell lymphoma 2; Bmi-1, B lymphoma Mo-MLV (Moloney-murine leukemia virus) insertion region-1; BRCA2, Breast cancer 2, early onset; Chk1, Check point kinase; Cdk, Cyclin-dependent kinase; ERK, extracellular signal regulating kinase; IGF1, insulin-like growth factor-1; JNK, c-jun NH₂-terminal kinase; MAPK, mitogen-activated

protein kinase; MEK, MAPK kinase; MMP2, MMP9, matrix metalloproteinase; MnSOD, manganese superoxide dismutase; TNF α , tumor necrosis factor α ; VEGF, vascular endothelial growth factor; WNT, wntless-type; XRCC, X-ray repair cross-complementing [68, 75, 76].

A

B

C

Figure 2. Increased FOXM1 transcript levels in breast cancer tissues

(A) FoxM1 transcript levels in breast cancer tissues versus normal breast tissues are shown for the Curtis breast data set retrieved from Oncomine; this data set included 2,136 samples examined on the Illumina Human HT-12 V3.0 R2 Array consisting of 19,273 measured genes. Fold change = 2.21, ** $p < 0.005$ (B) FoxM1 transcript levels in breast cancer tissues versus normal breast tissues are shown for the TCGA breast data set retrieved from Oncomine; This data set included 593 samples; the array measured 20,423 genes, but the name of the array platform was not provided in the Oncomine database. Fold change = 5.213, ** $p < 0.005$ (C) FoxM1 transcript levels in breast cancer tissues versus normal breast tissues are shown for the Richardson breast 2 data set retrieved from Oncomine; This data set included 47 samples examined on the Human Genome U133 Plus 2.0 Array consisting of 19,574 measured genes. Fold change = 17.629, ** $p < 0.005$; [Citation: www.oncomine.org, April 2014, Compendia Bioscience, Ann Arbor, MI]