Targeting a Novel ER/HOXB7 Signaling Loop in Tamoxifen-Resistant Breast Cancer

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Summary: The majority of patients with breast cancer present with an estrogen receptor–positive (ER+) tumor, and the endocrine agent tamoxifen is a mainstay for their treatment. Unfortunately, however, resistance remains a major problem because most patients who respond eventually have a recurrence. Thus, an enduring challenge in the breast cancer field is to identify mechanisms underlying tamoxifen resistance. Jin and colleagues describe a novel ER/HOXB7 signaling loop in tamoxifen-resistant breast cancer models. Importantly, they reveal that targeting this signaling loop has great promise as an approach to treat patients with tamoxifen-resistant breast cancer.

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See related article by Jin et al., p. 944 (9).

The approximately 70% of breast cancer patients whose tumors are estrogen receptor–positive (ER+) are treated with endocrine agents, including tamoxifen, the first clinically successful ER modulator (SERM), and the more recently introduced agents fulvestrant, an ER degrader (SERD), and aromatase inhibitors that block estradiol production. Endocrine agents have increased the survival of hundreds of thousands of breast cancer patients since the introduction of tamoxifen into the clinic in the mid-1970s (1). Unfortunately, tumor recurrence caused by acquired resistance often occurs (reviewed in refs. 2 and 3). Therefore, it is crucial to gain an understanding of the mechanisms underlying endocrine therapy resistance.

ER biology is quite complex, and it is likely that multiple, nonexclusive mechanisms contribute to endocrine resistance. For example, loss of ER, which is observed in 15% to 20% of recurrences, is an obvious resistance mechanism (4). Receptor tyrosine kinase (RTK) overexpression has also been proposed to contribute to endocrine resistance. Indeed, breast tumors with high expression and activity of EGFR and ERBB2 are less sensitive to tamoxifen (5). Moreover, the subgroup of patients with breast cancer with ER+ tumors and the ERBB2 ampiclon generally do not respond to tamoxifen (6). These clinical data suggest that ERBB RTK signaling can circumvent the requirement for ER signaling. Recent findings demonstrate that mutations of ER (encoded by ESR1) are also linked to acquired resistance. Genomic sequencing efforts revealed that metastatic tumors have a higher frequency of ESR1 mutations than primary tumors (~20% vs 0.5%). The ESR1 missense mutations identified in metastatic disease generally lead to ligand-independent constitutive activation of the receptor and are thought to be acquired during treatment (2).

Biochemical analyses of some of the mutant ERs suggest that treatment of these patients with higher doses of current endocrine agents or with newer, more potent agents might provide clinical benefit (7).

In this issue of Cancer Discovery, Jin and colleagues follow up on their 2012 Proceedings of the National Academy of Sciences article (8), in which they showed that the HOXB7 transcription factor renders breast cancer cells resistant to tamoxifen through activation of the EGFR pathway. Their new article (9) provides novel mechanistic insight into the regulation of HOXB7 in tamoxifen-resistant breast cancer models and proposes novel approaches to target tamoxifen-resistant breast cancer.

Interestingly, their data indicate that a direct interaction between HOXB7 and ER is crucial for the upregulation of ER target genes in tamoxifen-resistant cells. By performing communoprecipitations, they revealed a physical interaction between these two proteins. Moreover, chromatin immunoprecipitation (ChIP) experiments demonstrated increased binding of both HOXB7 and ER at EREs in known ER target genes, such as MYC, GREB1, and CCND1, suggesting that HOXB7 binds to these sites in association with the ER. Of note, binding between HOXB7 and ER was shown to be enhanced upon E2 or tamoxifen treatment.

An important question that the authors answer, then, is “Why do these tamoxifen-resistant cells have increased HOXB7 levels?” To summarize the answer, the authors demonstrated that MYC is stabilized by phosphorylation mediated by ERBB2–EGFR signaling. Subsequently, stabilized MYC represses the expression of miR-196a, a known repressor of HOXB7 (10), resulting in increased HOXB7 levels (Fig. 1).

One interesting aspect of this work is the direct relation between HOXB7 and ERBB2–EGFR expression and activation. The authors convincingly showed increased levels of phosphorylated ERBB2 and EGFR in cells that overexpress HOXB7. Furthermore, their data suggest that ERBB2 levels are directly regulated by ER/HOXB7 in tamoxifen-resistant cells. This is based on ChIPs revealing increased HOXB7 and transcriptional cofactor binding at an estrogen response element (ERE) site in the ERBB2 locus of HOXB7-overexpressing tamoxifen-resistant MCF7 cells. In addition, quantitative
PCR data show an increase in ERBB2 mRNA upon tamoxifen treatment. Data demonstrating increased HOXB7 and ER binding upon E2 or tamoxifen treatment are lacking; therefore, it still remains a question whether or not ERBB2 is regulated in a similar manner to the other ER/HOXB7 targets described in the article. Moreover, it cannot be ruled out that HOXB7 controls ERBB2 and EGFR level and phosphorylation by other direct or indirect mechanisms.

Another important finding in the HOXB7-overexpressing tamoxifen-resistant models is the stabilization of MYC by ERBB2/EGFR signaling, leading to repression of miR-196a, which in turn represses HOXB7. Overexpression of miR-196a was shown to reduce expression of ER targets and could reverse resistance to tamoxifen. In agreement, in vivo xenograft studies, using miR-196a–overexpressing tamoxifen-resistant BT474 cells, revealed a highly significant decrease in primary tumor growth.

Importantly, uncovering this new ER/HOXB7 signaling loop implies that targeting the ER-associated HOXB7, either directly, or indirectly by ERBB2, MYC, or miR-196a, might have potential for treating tamoxifen-resistant breast cancer (Fig. 1). To test targeting different nodes of this loop, the authors used several tamoxifen-resistant xenograft models. In BT474 xenografts, a model for ER+/ERBB2 amplicon–positive breast cancer, HOXB7 knockdown almost totally blocked tumor outgrowth. This block was associated with a strong decrease in ERBB2 and EGFR levels and a subsequent decrease in AKT signaling. As there are currently no drugs

Figure 1. Targeting the ER/HOXB7 signaling loop in tamoxifen-resistant breast cancer. HOXB7 interacts with tamoxifen (TAM)-bound ER and coactivators, inducing ER target gene expression including MYC and ERBB2. HOXB7 binds the EGFR promoter directly, increasing EGFR transcription. Increased signaling through ERBB2 and EGFR leads to MYC phosphorylation and stability. MYC represses miR-196a transcription, a repressor of HOXB7. Red boxes represent potential treatment strategies to target the ER/HOXB7 signaling loop: ERBB2 by trastuzumab, EGFR/ERBB2 by lapatinib, MYC by 10058-F4 or 10074-G5, miR-196a by nanoparticles containing miR-196, and ER by fulvestrant.
directly targeting HOXB7, the authors targeted MYC using the inhibitor 10058-F4 and targeted ERBB2 using trastuzumab. As anticipated, treatment of BT474 xenografts with trastuzumab caused a strong reduction in tumor growth. MYC inhibition reduced primary tumor growth only slightly, but, interestingly, the combination of 10058-F4 and trastuzumab revealed synergy and tumor stasis. In another model, HOXB7-overexpressing MCF7 cells, treatment with fulvestrant resulted in complete remission. These data suggest that patients with breast cancer with high HOXB7 levels might be the target population for fulvestrant treatment after tamoxifen resistance emerges. It would be interesting to examine how the BT474 xenograft model responds to fulvestrant combined with the other inhibitors. Overall, these preclinical findings reveal that tamoxifen-resistant tumors expressing high levels of HOXB7 can be targeted at several nodes of the ER/HOXB7 signaling loop.

Finally, the authors show analyses using several independent databases harboring information on endocrine therapy–treated patients with ER+ breast cancer. These analyses revealed that patients expressing high HOXB7 have a worse probability of overall survival. Interestingly, elevated HOXB7 in combination with high ERBB2 and MYC showed an even stronger decrease in the probability of overall survival. In summary, their findings imply that it could be beneficial to select tamoxifen-resistant patients based on these three markers, for rationalized targeting of the ER/HOXB7 signaling loop.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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