

activation domains (AF-1 and AF-2)—but retains the DNA-binding domain—and its overexpression identifies individuals with breast cancer who are less likely to benefit from tamoxifen treatment, it is important to clarify whether this variant is also a target for CUEDC2 and, if so, what the outcome of their interaction would be^{8,9}.

The ability of CUEDC2 to influence tamoxifen action by regulating ER- α and progesterone receptor may additionally affect tissues other than breast, such as the endometrium, in which tamoxifen shows estrogenic effects and is associated with increased incidence of cancer¹⁰.

Finally, the study by Pan *et al.*⁴ also suggests a positive correlation between CUEDC2 and HER2. Because overexpression of HER2 confers endocrine resistance and the estrogen-ER- α and tamoxifen-ER- α complexes repress HER2 (refs. 11,12), further studies will clarify whether CUEDC2-mediated downregulation of ER- α is responsible for the increased HER2 expression in tamoxifen-resistant tumors. The nature of the CUEDC2-HER2 correlation and the potential clinical role of CUEDC2 as a biomarker in predicting the response of breast cancers to HER2-specific antibodies and inhibitors will also need further investigation in the future.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. Early Breast Cancer Trialists' Collaborative Group. *Lancet* **365**, 1687–1717 (2005).
2. Massarweh, S. & Schiff, R. *Endocr. Relat. Cancer* **13**, Suppl. 1, S15–S24 (2006).
3. Musgrove, E.A. & Sutherland, R.L. *Nat. Rev. Cancer* **9**, 631–643 (2009).
4. Pan, X. *et al. Nat. Med.* **17**, 708–714 (2011).
5. Zhang, P.J. *et al. EMBO J.* **26**, 1831–1842 (2007).
6. Zhao, J.J. *et al. J. Biol. Chem.* **283**, 31079–31086 (2008).
7. Livasy, C.A. *et al. Mod. Pathol.* **19**, 264–271 (2006).
8. Barone, I., Brusco, L. & Fuqua, S.A. *Clin. Cancer Res.* **16**, 2702–2708 (2010).
9. Shi, L. *et al. J. Clin. Oncol.* **27**, 3423–3429 (2009).
10. Shang, Y. *Nat. Rev. Cancer* **6**, 360–368 (2006).
11. Arpino, G., Wiechmann, L., Osborne, C.K. & Schiff, R. *Endocr. Rev.* **29**, 217–233 (2008).
12. Hurtado, A. *et al. Nature* **456**, 663–666 (2008).

ERasing breast cancer resistance through the kinome

Amber B Johnson & Bert W O'Malley

Estrogen receptor α (ER α)-positive breast cancers often find a way to circumvent endocrine therapies such as tamoxifen. A newly described ER α kinase, lemur tyrosine kinase-3 (LMTK3), may provide both a diagnostic advance and a new therapeutic target to fight these resistant, aggressive tumors.

Over the decades of research aimed toward understanding the intricacies of ER α regulation, milestones have been reached in the treatment of breast cancer. These therapies, which include selective ER α modulators, such as tamoxifen, selective ER α downregulators, such as fulvestrant, and aromatase inhibitors have had an indisputable positive impact on the survival of patients with ER α -positive breast cancers. Nonetheless, resistance to these endocrine therapies often occurs and remains a crucial problem for patients with breast cancer^{1,2}.

Resistance has been attributed to various causes, including drug metabolism, ligand-independent activation of receptors and induction of growth-activating pathways involving transcriptional coactivators. For example, the antiestrogen tamoxifen confers agonist-like properties when bound to ER α in specific cellular and promoter contexts³. Overexpression of coactivators such as steroid receptor coactivator 3 (SRC-3), which itself can be hyperactivated by elevated growth signals from overexpressed growth factor receptors such as human epidermal growth factor 2 (Her2), is a potential mechanism of resistance in these tissues^{4,5}. Tamoxifen

treatment can further exacerbate these signaling pathways by increasing coactivator levels within the cells⁶.

Modifications of ER α by various kinases, such as protein kinase A⁷ and mitogen-activated protein kinase⁸, can also promote ligand-independent activation of ER α and tamoxifen resistance⁹. Studies have shown that breast cancers with elevated levels of growth factor signaling cascades and coactivators, such as these mentioned, are associated with tamoxifen resistance and poor survival^{4,8}. Thus, there is an unmet clinical need to develop therapies that will treat these resistant tumors.

In this issue of *Nature Medicine*, Giamas *et al.*¹⁰ used an siRNA screen of the kinome to uncover targets that may help develop new therapies to combat endocrine-resistant, ER α -positive breast cancers. The screen specifically targeted 691 kinases and kinase-related genes, followed by measurement of expression of the ER α target mRNA, *TFF1*, in the ER α -positive breast cancer cell line MCF-7. From this screen, they identified LMTK3 as a positive regulator of ER α 's transcriptional activity¹⁰.

Knockdown of LMTK3 selectively inhibited the growth of ER α -positive breast cancer cell lines and not ER α -negative cell lines, indicating that LMTK3-targeting siRNA may have antigrowth effects by regulating ER α activity. The authors showed multiple modes of LMTK3-mediated ER α regulation, including

LMTK3-mediated phosphorylation of ER α *in vitro*, LMTK3-mediated protection of ER α from ubiquitin-mediated proteasomal degradation and LMTK3-dependent transcription from the promoter of the gene encoding ER α , *ESR1* (Fig. 1). Thus, targeting LMTK3 in breast cancers would hit ER α at two levels: mRNA synthesis and protein stability. As multiple kinases regulate ER α at the protein level through phosphorylation, the tumor cell may compensate for the loss of a single ER α kinase. However, blocking LMTK3 would have the additional benefit of downregulating ER α mRNA expression, as well, thus probably making this type of treatment more effective.

The authors show how LMTK3 regulates *ESR1* transcription by positively regulating FOXO3, a known transcriptional activator of *ESR1* (ref. 11). Protein amounts of FOXO3 were reduced upon knockdown of LMTK3, and overexpression of LMTK3 increased FOXO3 occupancy at the *ESR1* promoter in MCF-7 cells. Overexpression of FOXO3 also rescued the decrease in *ESR1* transcription observed with loss of LMTK3.

Previous evidence has demonstrated that protein kinase C (PKC) phosphorylates and activates AKT, which, in turn, phosphorylates FOXO3 and targets it for degradation¹². Giamas *et al.*¹⁰ found that LMTK3's kinase domain inhibits PKC's ability to phosphorylate some of its substrates. Whereas the

Amber B. Johnson and Bert W. O'Malley are in the Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas, USA. e-mail: berto@bcm.edu

authors did not directly show the expected inhibition of AKT phosphorylation by expression of the LMTK3 kinase domain, they did show that loss of LMTK3 increased phosphorylation of AKT and other unidentified PKC targets¹⁰. These data reveal a crucial pathway that is altered by LMTK3; however, the means by which LMTK3 inhibits PKC's activity is yet to be determined. For example, does LMTK3 directly phosphorylate and inactivate PKC?

The authors found that loss of LMTK3 leads to a decrease in total ER α protein¹⁰. This decrease was partially rescued by treatment with the proteasome inhibitor MG132, and an increase in ubiquitination of ER α was observed in MCF-7 cells with reduced LMTK3 abundance. Prior *in vitro* phosphorylation of ER α by incubation with the LMTK3 kinase domain protected ER α from degradation in an *in vitro* proteasomal degradation assay. This suggests that LMTK3-mediated phosphorylation of ER α protects it from ubiquitin-mediated, proteasomal degradation; however, including a phosphorylation site mutant of ER α would better support this conclusion. Additionally, as PKC can promote ER α degradation through the proteasome¹³, LMTK3 also protects ER α from degradation indirectly by inhibiting PKC activity.

Although there are still open questions regarding the exact mechanism of LMTK3-mediated ER α stability and increased gene transcription, the authors clearly showed that knockdown of LMTK3 decreased phosphorylation of ER α , as well as the coactivator SRC-3 (ref. 10), events associated with resistance to endocrine therapies such as tamoxifen^{9,14}. The additional loss of SRC-3 in resistant cells gives the authors' approach even greater therapeutic potential. Thus, the authors investigated whether loss of LMTK3 can render tamoxifen-resistant cells sensitive and found that transfecting LMTK3-targeting siRNA into these cells inhibited proliferation in the presence of tamoxifen¹⁰. To assess the efficacy of LMTK3-targeting siRNA to combat tumors *in vivo*, the authors injected it into human MCF-7 breast carcinoma tumors grown in nude mice, which clearly revealed that knockdown of LMTK3 reduces tumor growth.

To determine whether there is a specific correlation between LMTK3 abundance and poor prognosis in human breast cancers, tissue arrays were scored for LMTK3 by immunohistochemistry. High amounts of LMTK3 in the nucleus correlated with shorter disease-free survival and shorter overall survival. Notably, high

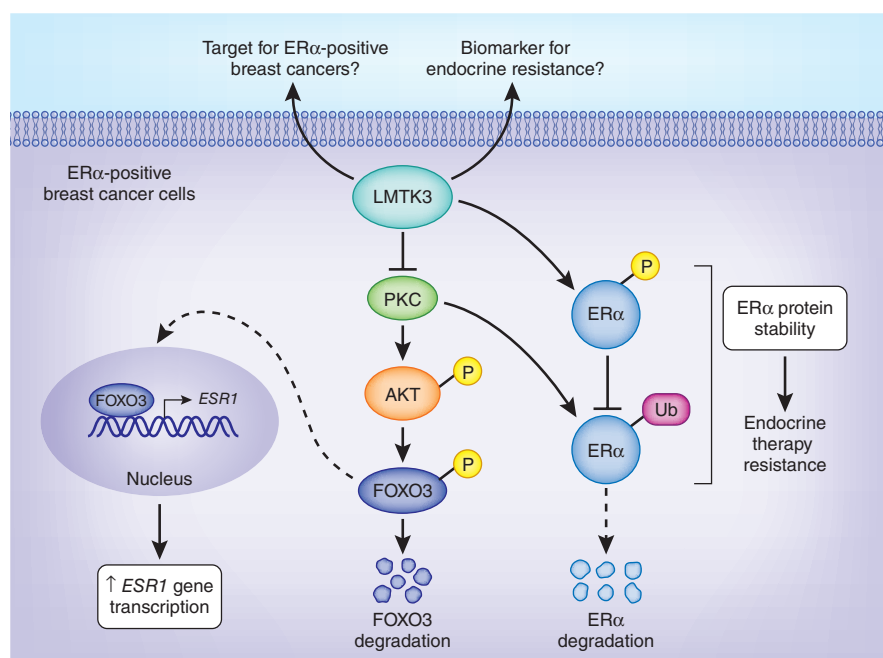


Figure 1 LMTK3 positively regulates *ESR1* gene transcription, protein levels and phosphorylation, promoting resistance to endocrine therapies. LMTK3 enhances transcriptional activation of *ESR1* by inhibiting PKC, whose activity results in phosphorylation of AKT, which in turn phosphorylates FOXO3, targeting it for degradation. LMTK3 also directly phosphorylates ER α protein, which has been shown to contribute to resistance to endocrine therapies such as tamoxifen. LMTK3 protects ER α from ubiquitin (Ub)-mediated proteasomal degradation directly through phosphorylation and indirectly through inactivation of PKC.

LMTK3 levels also correlated with poor response to endocrine therapies, but there was no specific association between LMTK3 abundance and response to chemotherapy. Select alleles of the *LMTK3* gene also correlated with tumor recurrence and poor overall survival. These data indicate that LMTK3 may serve as a new marker for ER α -positive cancers that are likely to become resistant to endocrine therapies. It will be interesting to learn how these polymorphisms affect the synthesis or function of LMTK3.

Giamas *et al.*¹⁰ have found an important new regulator of ER α and endocrine therapy resistance. From their studies, it is clear that LMTK3 regulates *ER1* gene transcription, protein stability and phosphorylation (Fig. 1). Studies on the molecular mechanisms of resistance to endocrine therapy are a crucial, ongoing area of breast cancer research that may yield better future therapies that are patient specific, such as has been shown previously in the case of Herceptin (trastuzumab) for Her2-overexpressing breast cancers¹⁵. The study by Giamas *et al.*¹⁰ also indicates that screening for specific *LMTK3* alleles could be an important tool to identify individuals

likely to be resistant to endocrine therapies. LMTK3 is therefore another potential target for treating ER α -positive breast cancers in patients that are resistant to typical, first-line endocrine therapies.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

- Orlando, L. *et al. Cancer Treat. Rev.* **36** Suppl 3, S67–S71 (2010).
- Ali, S. & Coombes, R.C. *Nat. Rev. Cancer* **2**, 101–112 (2002).
- Romano, A. *et al. Mol. Cell. Endocrinol.* **314**, 90–100 (2010).
- Osborne, C.K. *et al. J. Natl. Cancer Inst.* **95**, 353–361 (2003).
- Jordan, V.C. & O'Malley, B.W. *J. Clin. Oncol.* **25**, 5815–5824 (2007).
- Lonard, D.M., Tsai, S.Y. & O'Malley, B.W. *Mol. Cell. Biol.* **24**, 14–24 (2004).
- Zwart, W. *et al. EMBO J.* **26**, 3534–3544 (2007).
- Kato, S. *et al. Science* **270**, 1491–1494 (1995).
- Sarwar, N. *et al. Endocr. Relat. Cancer* **13**, 851–861 (2006).
- Giamas, G. *et al. Nat. Med.* **17**, 715–719 (2011).
- Guo, S. & Sonenshein, G.E. *Mol. Cell. Biol.* **24**, 8681–8690 (2004).
- Belguise, K. & Sonenshein, G.E. *J. Clin. Invest.* **117**, 4009–4021 (2007).
- Marsaud, V., Gougelet, A., Maillard, S. & Renoir, J.M. *Mol. Endocrinol.* **17**, 2013–2027 (2003).
- Xu, J., Wu, R.C. & O'Malley, B.W. *Nat. Rev. Cancer* **9**, 615–630 (2009).
- Hudis, C.A. *N. Engl. J. Med.* **357**, 39–51 (2007).

Marina Corral