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*J Clin Invest.* 2012;122(8):2771-2773. <https://doi.org/10.1172/JCI65133>.

### Commentary

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# Targeting PES1 for restoring the ER $\alpha$ /ER $\beta$ ratio in breast cancer

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**Alteration of the ER $\alpha$ /ER $\beta$  balance is a critical step in breast cancer development and progression, and selective restoration of the activity of estrogen receptors has been proposed as one of the major therapeutic approaches for breast cancer. In this issue of *JCI*, Cheng et al. show that, by differentially modulating the stability of ER $\alpha$  and ER $\beta$ , PES1 increases the ER $\alpha$ /ER $\beta$  ratio and triggers breast tumor growth. These findings highlight PES1 as a potential target for the treatment of breast cancer.**

Alteration in the expression of estrogen receptors (ERs) has been detected in breast cancer tissues, and differences in the levels of ERs are correlated with the clinical outcome (1, 2). Insights into the mechanisms that regulate expression and activity of ERs have suggested novel approaches for the prognosis and treatment of breast cancer. Apart from the development of selective ER $\alpha$  and ER $\beta$  agonists and antagonists that stimulate or inhibit the activity of the receptors, current research is focusing on alternative strategies that target the signaling of ERs beyond the ligand-ER interaction. Several factors that control the expression and turnover of ERs, including methylases, microRNAs, kinases, and ubiquitin ligases, are under investigation (3). These studies aim to discover novel biomarkers that can complement ER status in the prognosis and prediction of therapeutic response as well as identify new drug targets for restoring the levels of ERs in cancer tissues.

Estrogens mediate their effects on cell growth and differentiation within the mammary gland by signaling through ER $\alpha$  and ER $\beta$  (4, 5). After activation in response to estrogen binding, ERs can elicit different transcriptional responses by acting as transcription factors themselves and/or interacting with and regulating the activity of other transcription factors. They can also interact with and alter the activity of one another. Their ligand-binding domains and activating function 1 domains (AF1 domains), which interact with coactivators,

share a medium and low degree of homology, respectively. This explains their different affinity to ligands and coregulatory proteins and, at least partly, their distinct biological actions (6). It is well known that ER $\alpha$  expression is associated with aberrant proliferation and the development of malignancy, and ER $\alpha$  level is the principal predictor for the response of breast cancers to endocrine therapy. In contrast, ER $\beta$  has been shown to inhibit breast cancer cell proliferation, migration, and invasion (3, 7). Although there is still a controversy regarding the prognostic and predictive role of ER $\beta$  expression in breast cancer, most of the studies that have analyzed a large number of samples with well-validated antibodies have shown correlations of wild-type receptor (ER $\beta$ 1) with better clinical outcome (8, 9).

## PES1 governs the ER subtypes

In this issue of *JCI*, Cheng et al. show that Pescadillo ribosomal biogenesis factor (PES1), a breast cancer-associated gene 1 (BRCA1) C-terminal (BRCT) domain-containing protein, may play a crucial role in the development and the response of breast cancer to systemic therapy (10). It has previously been reported that PES1 is expressed at higher levels in primary breast cancers compared with PES1 expression in normal mammary tissues (11). Furthermore, knockdown of PES1 slows down the proliferation of breast cancer cells. PES1 stimulates cell proliferation by promoting both ribosome biogenesis and cell cycle progression (11–13). Now Cheng et al. hypothesize that PES1 induces breast tumor growth by regulating steroid hormone signaling through control of the turnover of both ER $\alpha$  and ER $\beta$ . They

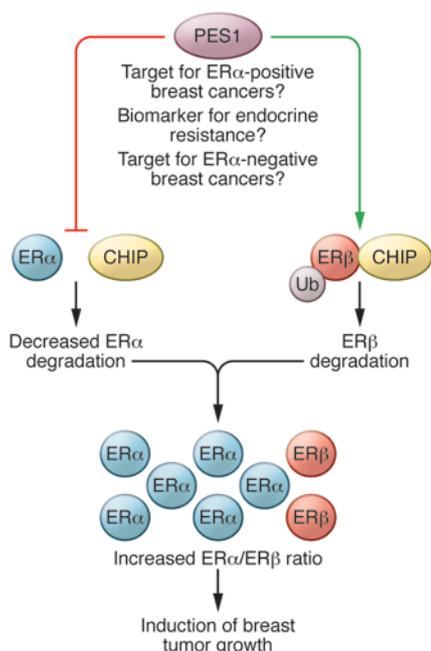
found that PES1 enhances the stability of ER $\alpha$  while simultaneously targeting ER $\beta$  for degradation, thereby increasing the levels of ER $\alpha$  and decreasing those of ER $\beta$ . The authors believe that this alteration in the ER $\alpha$ /ER $\beta$  expression ratio explains the correlation between increased PES1 levels and increased breast tumor growth and the better response to tamoxifen treatment.

Cheng et al. manipulated PES1 expression in a series of breast cancer cell lines that were positive for both ER $\alpha$  and ER $\beta$ , positive only for ER $\alpha$ , or negative for both receptors. Treatment of these cells with selective ER $\alpha$  or ER $\beta$  agonists revealed that PES1 exerted differential actions on the transcriptional responses of the ER subtypes; it increased the transcriptional activity of ER $\alpha$  and decreased that of ER $\beta$ , resulting in increased expression of estrogen-responsive genes that are known to promote cell proliferation and survival. The authors found that PES1 altered the transcriptional activity of the ERs by regulating their protein stability. Ubiquitylation assays and treatment of the cells with proteasome inhibitors showed that increased levels of PES1 protected ER $\alpha$  from proteasome-mediated breakdown while targeting ER $\beta$  for degradation through the same pathway. Furthermore, the authors found that the E3 ubiquitin ligase carboxyl terminus of Hsc70-interacting protein (CHIP) is responsible for the PES1-mediated alteration in the ubiquitylation and degradation of the ERs (Figure 1 and ref. 10).

To further understand the function of PES1, the authors carried out pull-down and coimmunoprecipitation experiments with mutants with different domain deletions of PES1, ER $\alpha$ , and ER $\beta$  and identified the ER AF1 and AF2 domains as those responsible for the PES1 interaction. Using the same mutants, they identified the regions of PES1 responsible for the CHIP-dependent alteration of ER $\alpha$  and ER $\beta$  degradation. The authors also showed that the PES1-mediated increase in the ER $\alpha$ /ER $\beta$  expression ratio is associated

**Conflict of interest:** The authors have declared that no conflict of interest exists.

**Citation for this article:** *J Clin Invest*. doi:10.1172/JCI65133.



**Figure 1**

A model of PES1 activity in breast cancer. PES1 promotes mammary tumor growth and plays a role in sensitivity to endocrine therapy by regulating the stability of ERs. PES1 reduces the interaction of ER $\alpha$  with the E3 ubiquitin ligase CHIP, thereby blocking ubiquitylation and degradation of ER $\alpha$ . In contrast, by inducing the interaction of CHIP with ER $\beta$ , PES1 targets ER $\beta$  for degradation. This leads to an increase in the ER $\alpha$ /ER $\beta$  protein ratio that has been shown to influence breast tumor growth. Since ER $\alpha$  levels determine tamoxifen sensitivity in breast cancer, PES1 may have a role in predicting response to endocrine therapy. These findings also suggest that PES1 may represent a novel therapeutic target in breast cancer and may serve as a useful biomarker for endocrine therapy resistance. Ub, ubiquitin.

with enhanced proliferation and tamoxifen sensitivity of breast cancer cells when they grow either *in vitro* or *in vivo* in mice (10).

### PES1 as biomarker for endocrine resistance

Cheng et al. investigated the clinical significance of PES1 by analyzing protein levels of PES1 and other biomarkers in normal and breast cancer tissues. They found that PES1 levels correlate positively with ER $\alpha$  and negatively with ER $\beta$ . Increased expression of PES1 was also associated with better survival in patients with breast cancer who received tamoxifen treatment. Although a role for PES1 in predicting endocrine resistance emerges from the analysis of the breast cancer samples of this study, further investigation of the patterns of expression of PES1 and correlation with ER $\alpha$  expression and clinical outcome in the ER $\alpha$ -positive tamoxifen-treated patients as well as multivariate analysis would be required to indicate the utility of PES1 as a biomarker. In addition, a comparison of PES1 expression between ER $\alpha$ -positive and ER $\alpha$ -negative breast cancers would help to clarify whether lack of PES1 is associated with the loss of ER $\alpha$  in a significant proportion of breast cancers.

### Multiple actions of PES1 in breast cancer

The authors provide substantial evidence that strengthens their major conclusion that PES1 promotes mammary cell growth by regulating the stability of ERs and increas-

ing the ER $\alpha$ /ER $\beta$  ratio in the tissue. Given that increased proliferation of the mammary cells is implicated in breast cancer initiation, tumor growth, and response to systemic therapy, the study by Cheng et al. proposes a new target for the development of drugs, which through a selective restoration of ER $\alpha$  and ER $\beta$  level and activity, can prevent cancer development and progression and improve existing endocrine therapy.

In contrast to other factors that have previously been shown to influence breast cancer development and response to therapy by regulating only one of the two ER subtypes, PES1 is unique in regulating the expression and activity of both receptors. At first glance, this implies that PES1 influences specific biological responses in which both ER subtypes are involved. However, the distinct biological functions elicited by ER $\alpha$  and ER $\beta$  and the ER subtype-specific expression patterns detected in breast cancer imply multiple roles for PES1 in breast cancer biology and therapy, depending on the breast cancer subtype and the disease stage. ER $\beta$  has been shown to regulate migration and invasion and is expressed in ER $\alpha$ -negative breast cancers (9, 14). Therefore, it is of clinical interest to clarify whether PES1 can impact metastasis and survival in triple-negative cancers by down-regulating ER $\beta$ . In addition, since both ERs have been reported to regulate the expression of members of the ERBB family of receptor tyrosine kinases, including human epidermal growth factor receptor-2 (ERBB2, also known as HER2) (9, 15, 16), it is important to elu-

cidate whether PES1 might affect the levels of HER2 and thus have a clinical role as biomarker in predicting the response of HER2-positive breast cancers to HER2-specific antibodies and inhibitors.

The study by Cheng et al. also shows an inverse correlation between PES1 and ER $\beta$  in breast cancers, in which PES1 is correlated with the response to tamoxifen treatment. Honma et al. have associated ER $\beta$  with survival in patients who received tamoxifen (8). However, the role of ER $\beta$  in endocrine therapy in breast cancer still remains unclear. Further studies will clarify whether ER $\beta$  is involved in PES1-dependent alteration of the tamoxifen response in breast cancers.

Finally, it remains to be elucidated whether the ability of PES1 to affect tumor growth by regulating the ER $\alpha$ /ER $\beta$  expression ratio influences other tissues, such as prostate and colon, in which perturbation of the balance between ER $\alpha$  and ER $\beta$  has been associated with increased incidence of cancer (14, 17).

### Acknowledgments

Jan-Åke Gustafsson is indebted to the Robert A. Welch Foundation for an endowment.

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## miR-122 regulates hepatic lipid metabolism and tumor suppression

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**In this issue of *JCI*, two independent groups describe the effects of germline and liver-specific deletion of *Mir122a*, the predominant liver miRNA. Their findings reveal a critical role for miR-122 in fat and cholesterol metabolism but suggest that other metabolic actions of the liver are independent of miR-122. Knockout mice also displayed hepatic inflammation, fibrosis, and a high incidence of hepatocellular carcinoma, suggesting that miR-122 has a tumor suppressor role in hepatocytes.**

MicroRNAs (miRNAs) are small, non-coding RNA molecules that regulate the expression of complementary messenger RNAs. Since their initial discovery in 1993 in *Caenorhabditis elegans*, more than 1,400 miRNAs have been detected in the human transcriptome. In addition to regulating physiologic processes, miRNAs have also been implicated in numerous disease states. The broad function of miRNA in the liver was investigated by studying mice with conditional deletion of *Dicer1* in hepatocytes (1, 2). Despite the lack of mature miRNA in this model, the liver was able to perform the essential functions of blood glucose regulation, albumin production, and bilirubin metabolism. However, over time, it became clear that miRNA plays an important role

in fat metabolism, inflammation, and cell cycle regulation in the liver, as these animals developed progressive hepatic steatosis, hepatitis, apoptosis, and hepatocellular carcinoma (HCC) (1, 2).

miR-122 is the predominant liver miRNA, making up 70% of the total miRNA population (3). The activity of miR-122 has previously been assessed through antisense oligonucleotide-mediated knockdown, implicating miR-122 in cholesterol and fat metabolism (4, 5). Although HCC was not observed in the time frame of these studies, several groups have reported tumor suppressor activity for miR-122, based on decreased miR-122 levels in tumor tissue and inhibitory effects of miR-122 in tumorigenicity assays (6). However, knockdown experiments are limited by their transient nature and the potential for off-target effects.

In this issue of *JCI*, Hsu et al. and Tsai et al. present definitive evidence of miR-122

function using genetic deletion in mice (7, 8). Mice with germline or conditional deletion of *Mir122a* in the liver were viable and fertile. However, as the animals aged, they developed steatohepatitis, liver fibrosis, and HCC. These groups define direct roles for miR-122 in both fat metabolism and tumor suppression, although it is less clear whether the link to fibrosis is directly or indirectly related to miR-122 loss. Thus, although miR-122 cannot be construed as a “master regulator” of liver function — as the mutant mice have generally normal liver function — it is a critical checkpoint both in hepatic fat production and hepatocellular proliferation (Figure 1).

### miR-122 regulates fat and cholesterol metabolism

Temporary miR-122 inhibition has been shown to reduce serum cholesterol via downregulation of genes involved in cholesterol biosynthesis such as HMG-CoA reductase (4). This is recapitulated in the genetic models: the serum lipid profiles of both liver-specific knockouts (LKO) and germline knockouts (KO) show a 30% reduction in total cholesterol, LDL, HDL, and serum triglyceride (TG). However, the livers of both KO and LKO mice also have progressive steatohepatitis (7, 8), a feature

**Conflict of interest:** The authors have declared that no conflict of interest exists.

**Citation for this article:** *J Clin Invest.* doi:10.1172/JCI63966.