Kinome-wide Functional Screen Identifies Role of PLK1 in Hormone-Independent, ER-Positive Breast Cancer

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Abstract

Estrogen receptor (ER) α-positive breast cancers initially respond to antiestrogens but eventually become estrogen independent and recur. ER+ breast cancer cells resistant to long-term estrogen deprivation (LTED) exhibit hormone-independent ER transcriptional activity and growth. A kinome-wide siRNA screen using a library targeting 720 kinases identified Polo-like kinase 1 (PLK1) as one of the top genes whose downregulation resulted in inhibition of estrogen-independent ER transcriptional activity and growth of LTED cells. High PLK1 mRNA and protein correlated with a high Ki-67 score in primary ER+ breast cancers after treatment with the aromatase inhibitor letrozole. RNAi-mediated knockdown of PLK1 inhibited ER expression, estrogen-independent growth, and ER transcription in MCF7 and HCC1428 LTED cells. Pharmacologic inhibition of PLK1 with volasertib, a small-molecule ATP-competitive PLK1 inhibitor, decreased LTED cell growth, ER transcriptional activity, and ER expression. Volasertib in combination with the ER antagonist, fulvestrant, decreased MCF7 xenograft growth in ovariectomized mice more potently than each drug alone. JUNB, a component of the AP-1 complex, was expressed 16-fold higher in MCF7/LTED compared with parental MCF7 cells. Furthermore, JUNB and BCL2L1 (which encodes antiapoptotic BCL-xL) mRNA levels were markedly reduced upon volasertib treatment in MCF7/LTED cells, while they were increased in parental MCF7 cells. Finally, JUNB knockdown decreased ER expression and transcriptional activity in MCF7/LTED cells, suggesting that PLK1 drives ER expression and estrogen-independent growth via JUNB. These data support a critical role of PLK1 in acquired hormone-independent growth of ER+ human breast cancer and is therefore a promising target in tumors that have escaped estrogen deprivation therapy. Cancer Res; 75(2); 1–10. ©2014 AACR.

Introduction

Most breast cancers express estrogen receptor α (ERα) and are driven by estrogen (1–3). Targeting the function of ER with receptor antagonists such as tamoxifen, fulvestrant, and aromatase inhibitors (AI), such as anastrozole and letrozole, are effective treatments for patients with this subtype of breast cancer (4–7). However, a significant number of patients with ER+ tumors, particularly those with advanced disease, exhibit primary and acquired resistance to antiestrogens (8–11). Most mechanisms that mediate this resistance are not yet fully understood. Therefore, identifying those mechanisms and strategies to interfere with them is needed to prevent or delay acquired endocrine resistance and improve patient outcome. We and others have modeled secondary resistance to treatment with AIs, the equivalent of estrogen deprivation in postmenopausal patients, by generating long-term estrogen-deprived (LTED) cells, whereby ER+ breast cancer cells have adapted to estrogen deprivation and acquired robust growth under hormone-free conditions (12–14). In this study, we used ER+ MCF7/LTED and HCC1428/LTED breast cancer cells, which overexpress ER and retain estrogen-independent ER binding to DNA and ER transcriptional activity (15).

To identify targetable molecules that drive hormone-independent growth and ER transcription in LTED cells, we performed a screen using a library with siRNA pools targeting 720 kinases. We used two metrics in this assay: (i) luciferase activity as assessed by using an estrogen-response-element (ERE)-regulated reporter and (ii) cell viability measured by the Alamar Blue assay. In this screen, we identified Polo-like kinase 1 (PLK1) as the top hit whose downregulation decreased both ER transcriptional activity and cell viability. PLK1 is a serine/threonine kinase that is highly expressed during the G2 phase of the cell cycle where it controls the metaphase-to-anaphase transition and mitotic exit (16–18). PLK1 is one of three isoforms of PLKs...
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(PLK2 and PLK3; refs. 19, 20) and has been shown to be overexpressed in several human cancers (21–25). Currently, PLK1-specific small-molecule inhibitors are in clinical trials in patients with advanced cancer (26, 27). Herein, we discovered that genetic and pharmacologic inhibition of PLK1 decreased ER expression, estrogen-independent ER-mediated transcriptional activity, and growth of ER+ xenografts alone and in combination with fulvestrant. These results provide a basis for combinations of PLK1 inhibitors with ER downregulators in patients with hormone-independent ER+ breast cancer.

Materials and Methods

Cell lines and reagents

Parental cancer cell lines were from ATCC and maintained in improved minimum essential medium (IMEM)/10% FBS (Gibco) as previously described (28). LTED cells were generated and maintained in phenol red-free IMEM with 10% dextran/charcoal-treated FBS (DCC-FBS) as previously described (28). B1Z536 and volasertib were obtained from Selleckchem. Fulvestrant was obtained from the Vanderbilt Hospital Outpatient Pharmacy (Nashville, TN). FOXM1-E1 construct was a gift from Junjie Chen (Yale University; New Haven, CT; ref. 29). The myristoylated-PLK1 construct (Addgene plasmid 20859) was a gift from William Hahn (Dana Farber Cancer Institute; Boston, MA; ref. 30).

RNAi screen

MCF-7/LTED cells were transfected with the Dharmacon RTF Protein Kinase siRNA library (14) as described in Supplementary Methods. Secondary validation was performed with four independent siRNAs against GSG2, RPS6KA2, and PLK1 (Qiagen).

qRT-PCR

Cells maintained in 10% DCC-FBS were transfected with control or PLK1 siRNA oligonucleotides (Thermo Scientific) with Lipofectamine RNAiMAX for 48 to 72 hours. Cells were harvested and their RNA extracted using the RNeasy Mini Kit (Qiagen). Using the iScript CDNA Synthesis Kit (Bio-Rad), 1 μg of RNA was reverse transcribed to cDNA and real-time PCR reactions were conducted in 96-well plates using the iCycler iQ (Bio-Rad) and primers obtained from SA Biosciences (Qiagen). RNA was extracted from MCF7 and MCF7/LTED cells that had been treated with volasertib or transfected with PLK1 siRNA; cDNA was applied to the Estrogen Receptor Signaling PCR Array (SA Biosciences). Fold changes in gene expression were determined using the web-based software from SA Biosciences (http://www.qiagen.com/us/products/genes%20and%20pathways/data-analysis-center-overview-page/). Genes identified in the array were verified by qRT-PCR.

Cell proliferation assays

Cells seeded in triplicate in 12-well plates (2.5 × 105 cells/well for MCF-7/LTED and 4 × 104 cells/well for other lines) were treated in 10% DCC-FBS. Media and inhibitors were replenished every 3 days; after 5 to 10 days, adherent cells were trypsinized and counted using a Coulter Counter or fixed/stained with crystal violet. Crystal violet-stained plates were scanned using an Odyssey Infrared Imaging System (LI-COR Biosciences) and the staining intensity was quantified using the manufacturer’s analysis software. For the combination studies of volasertib and fulvestrant, MCF7 and MCF7/LTED cells were seeded in 96-well black plates followed by the Alamar Blue Assay after 72 hours as described in the Supplementary Methods.

Immunoblot analysis

Cells were lysed with NP40 lysis buffer (150 mmol/L Tris, pH 7.4, 100 mmol/L NaF, 120 mmol/L NaCl, 0.5% NP-40, 100 μmol/L sodium vanadate, and 1× protease inhibitor cocktail; Roche). Lysates (40 μg) were resolved by SDS-PAGE and transferred to nitrocellulose membranes; these were first incubated with primary antibodies at 4°C overnight or at room temperature for 2 hours, followed by incubation with horseradish peroxidase–conjugated anti-rabbit and anti-mouse secondary antibodies (Santa Cruz Biotechnology) for 1 hour at room temperature. Immunoreactive bands were visualized by enhanced chemiluminescence (Thermo Scientific). All primary antibodies were from Cell Signaling Technology except the ERα antibody (Santa Cruz Biotechnology).

Transcriptional reporter assays

Cells were plated as above and transfected with pGLB-MERE (encodes estrogen response element-regulated Firefly luciferase), pGL4.23 vectors (Peak2 or Peak5 Firefly luciferase; ref. 28) and pTK-Renilla (encodes TK-driven Renilla luciferase; Promega) plasmids. Cells were then treated as above; luciferase activity was measured 16 to 20 hours later using the Dual Luciferase Kit (Promega) according to the manufacturer’s instructions utilizing a Moonlight 3010 Luminometer (Analytical Luminescence Laboratory). The same procedure was used for the pCAGA (provided by J.-M. Gauthier, Laboratoire GlaxoSmithKline), pGL2-E-cadherin (31), and pGL-ErbB3 (32) Firefly luciferase reporters.

Xenograft studies

Animal experiments were approved by the Vanderbilt Institutional Animal Care and Use Committee. Female ovariectomized athymic nude mice (Foxn1nu/nu; Harlan–Sprague-Dawley) were implanted subcutaneously with a 14-day release, 0.17-mg, 17β-estradiol pellet (Innovative Research of America). Twenty-four hours later, 5 × 106 MCF7 cells suspended in IMEM and Matrigel (BD Biosciences) at 1:1 ratio were injected subcutaneously into the right flank of each mouse. Approximately 4 weeks later, mice bearing tumors measuring ≥150 mm3 were randomized to treatment with vehicle (control), volasertib (10 mg/kg/day via orogastric gavage), fulvestrant (5 mg/week s.c.), or both drugs. Animal weight and tumor diameters (with calipers) were measured twice weekly and tumor volume was calculated with the formula: volume = width2 × length/2. After 6 weeks, tumors were harvested and snap-frozen in liquid nitrogen or fixed in 10% neutral-buffered formalin followed by embedding in paraffin for IHC analysis.

Results

PLK1 siRNA oligonucleotides inhibit ER transcriptional activity and cell growth

Initially, we transfected cells with ERE firefly-luciferase and Renilla-luciferase constructs. Transfection with ERα siRNA decreased ERE-firefly luciferase activity. Importantly, the Renilla reading was markedly decreased (93%), resulting in a greater firefly/Renilla ratio compared with control siRNA-transfected cells (Supplementary Table S1). In the Alamar Blue assay, ER siRNA...
decreased cell viability only by 62% (Supplementary Fig. S1B). These results suggested that RNAi oligonucleotides reducing ER expression had a nonspecific effect on Renilla expression in MCF7/LTED cells, thus skewing the results. For this reason, we could not use Renilla expression as a control in cells transfected with the siRNA pools. We next assessed whether LTED cell viability (Alamar Blue) and ER reporter activity can be measured consecutively. Firefly luciferase activity was similar in cells transfected with MERE-luc in the presence or absence of Alamar Blue dye (Supplementary Fig. S1A and S1C). Therefore, MCF7/LTED cells were next transfected with an ERE-luciferase construct and with siRNA pools targeting 720 kinases (schema in Supplementary Fig. S1A). Both cell viability (Alamar Blue) and ER reporter activity for each siRNA relative to nonsilencing controls (siCTL) were transformed to a Z-score; the median Z-score across three independent experiments was then calculated (Fig. 1A). Knockdown of 58 and 36 kinases was observed to significantly decrease cell viability and ER reporter activity, respectively (Fig. 1B and Supplementary Table S2). Of these, 10 kinases scored positive in both assays. Statistical analysis identified PLK1, RPS6KA2, and GSG2 as the top hits inhibiting both ER transcriptional activity and viability of MCF7/LTED cells (Table 1). We next validated the effect of these three genes using four independent siRNA oligonucleotides for each of them (Supplementary Fig. S2). Only in the case of PLK1, all four independent siRNA oligos decreased both ERE luciferase activity and cell viability. PLK1 downmodulation with siRNA resulted in inhibition of proliferation and focus formation in monolayer of both MCF7/LTED and HCC1428/LTED cells (Fig. 1C and D). Knockdown of PLK1 was validated by both qRT-PCR and immunoblot analysis. Interestingly, PLK1 siRNA also decreased ERα protein levels (Fig. 1E and F), suggesting that PLK1 has a role in ligand-independent ER transcriptional activity and ERα+ breast cancer cell growth via regulation of ERα expression.

To investigate the potential clinical significance of these findings, we correlated Ki-67 levels (a marker of cell proliferation) with PLK1 protein and mRNA expression in tumor biopsies from patients with an operable ERα+/HER2– breast cancer that had been treated with the AI letrozole for 10 to 21 days before surgery. In this setting, Ki-67 levels are used as a surrogate of the cell proliferation rate.
hormone independence because ER+ breast cancers that retain a high Ki-67 score after therapy are considered resistant to estrogen deprivation (with letrozole) and vice versa; tumors with a low posttreatment Ki-67 are considered highly hormone dependent and, thus, antiestrogen sensitive (11, 33). In tumor biopsies from patients treated with letrozole, PLK1 protein levels measured by RPPA positively correlated with the Ki-67 score (Fig. 2A). In addition, PLK1 mRNA transcript levels measured by RNA-seq correlated directly with Ki-67 mRNA expression (Fig. 2B and C).

Furthermore, both MCF7 and HCC1428 LTED cells displayed increased PLK1 expression compared with their parental cell lines (Fig. 2D). These results suggest that high PLK1 expression associates with and could be used as a marker of hormone-independent ER+ breast cancer.

PLK1 downmodulation decreases hormone-independent ER transcriptional activity

In MCF7/LTED cells, PLK1 siRNA decreased MERE-Luciferase activity (Fig. 3A) and expression of the ER-responsive genes progesterone receptor (PR), Trefoil factor 1 (TFF1), and growth regulation by estrogen in breast cancer 1 (GREB1; Fig. 3B). A more modest reduction in PR and TFF1 was observed in HCC1428/LTED cells transfected with PLK1 siRNA. To confirm that the effects of PLK1 siRNA were on ligand-independent ER transcription, we generated two luciferase reporters (Peak2 and Peak5) with ER-binding regions proximal to genes reported to be downregulated by ER siRNA or fulvestrant in the absence of estrogen (28). Notably, the effect of PLK1 siRNA on the ligand-independent reporters was similar to that of ER siRNA oligonucleotides (Fig. 3C). In addition, MCF7/LTED cells were transfected with both Peak5 firefly luciferase and Renilla luciferase plasmids 2 days after transfection with two independent PLK1 siRNAs to avoid the effects of RNAi on Renilla activity. We observed that both PLK1 siRNA oligonucleotides decreased estrogen-independent ER reporter activity (Supplementary Fig. S3) when measured as a ratio of firefly to Renilla luciferase. Conversely, overexpression of PLK1 in MCF7/LTED cells increased >4-fold Peak5-Luc reporter activity (Fig. 3D). To show that the effect of PLK1 downregulation on ER transcription was specific to ER and not secondary to an overall effect on chromatin segregation during mitosis, we examined other transcriptional reporters. PLK1 and ER siRNA did not affect the activity of the transcriptional reporters driven by SMAD, E-cadherin, and ERBB3 promoter binding regions in MCF7/LTED cells (Figs. 3E–G).

Table 1. Ranking of the top 10 hits on cell proliferation and ER transcription in the RNAi screen of MCF7/LTED cells

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cell proliferation rank</th>
<th>ER transcription rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADC2</td>
<td>22 (−1.38)</td>
<td>22 (−1.07)</td>
</tr>
<tr>
<td>CSNKIA1</td>
<td>34 (−1.21)</td>
<td>15 (−1.16)</td>
</tr>
<tr>
<td>FN3KRP</td>
<td>41 (−1.13)</td>
<td>20 (−1.10)</td>
</tr>
<tr>
<td>GSG2</td>
<td>15 (−1.51)</td>
<td>28 (−1.03)</td>
</tr>
<tr>
<td>NRBP1</td>
<td>12 (−1.15)</td>
<td>16 (−1.15)</td>
</tr>
<tr>
<td>PLK1</td>
<td>17 (−1.46)</td>
<td>5 (−1.32)</td>
</tr>
<tr>
<td>PRKCB1</td>
<td>42 (−1.13)</td>
<td>9 (−1.24)</td>
</tr>
<tr>
<td>RPS6KA2</td>
<td>1 (−2.73)</td>
<td>35 (−1.10)</td>
</tr>
<tr>
<td>STK35</td>
<td>23 (−1.36)</td>
<td>17 (−1.15)</td>
</tr>
<tr>
<td>TTK</td>
<td>9 (−1.61)</td>
<td>8 (−1.30)</td>
</tr>
</tbody>
</table>

Conversely, overexpression of PLK1 in MCF7/LTED cells increased >4-fold Peak5-Luc reporter activity (Fig. 3D). To show that the effect of PLK1 downregulation on ER transcription was specific to ER and not secondary to an overall effect on chromatin segregation during mitosis, we examined other transcriptional reporters. PLK1 and ER siRNA did not affect the activity of the transcriptional reporters driven by SMAD, E-cadherin, and ERBB3 promoter binding regions in MCF7/LTED cells (Figs. 3E–G).

PLK1 inhibitor decreases hormone-independent growth and ER transcriptional activity in LTED cells

We next used BI2536 and volasertib, small-molecule inhibitors derived from the dihydropteridinone class of compounds that target the ATP-binding pocket in the PLK1 kinase domain (34). Growth of MCF7/LTED and HCC1428/LTED cells was inhibited by low nmol/L concentrations of both BI2536 and volasertib in dose-dependent fashion (Figs. 4A and B), whereas parental MCF7 cells were only inhibited by concentrations in excess of 10 nmol/L (Supplementary Fig. S4A). In both LTED cell lines, treatment with volasertib abrogated hormone-independent ER reporter activity (Fig. 4C) and reduced ER protein levels (Fig. 4D and Supplementary Fig. S4B). Furthermore, volasertib increased expression of cleaved PARP, an apoptotic marker, and phosphorylated Histone H2AX, a marker of DNA damage and fragmentation (Fig. 4E and Supplementary Fig. S4B).

Volasertib enhances the antitumor effect of fulvestrant in vivo

We next investigated whether combined inhibition of PLK1 and ER with volasertib and the ER antagonist fulvestrant, respectively, would have a synergistic antitumor effect. Volasertib enhanced the antiproliferative effect of fulvestrant in MCF7 and MCF7/LTED cells (Fig. 5A and B and Supplementary Fig. S4C). The combination of fulvestrant and volasertib also increased
expression of cleaved PARP and phospho-Histone H2AX, whereas each drug alone did not (Fig. 5C). To examine the effect of the combination in vivo, we established MCF7 xenografts in ovariectomized nude mice. Mice with tumors measuring \( \geq 150 \) mm\(^3\) were treated with vehicle, volasertib, fulvestrant, or the combination of both inhibitors. After 6 weeks of treatment, the combination induced a statistically superior antitumor effect compared with fulvestrant or volasertib alone (Fig. 5D). During this period, the mice in all 4 treatment groups displayed stable body weight (data not shown) and no signs of toxicity. Treatment with volasertib reduced ER levels but the combination of volasertib and fulvestrant more potently reduced both ER and PR levels as measured by IHC of tumor sections (Figs. 5E–G).

**PLK1 inhibition decreases BCL-xL and JUNB expression in LTED cells**

It has been shown that PLK1 may have a tumor-suppressive role in cancer, including MCF7 breast cancer cells (21, 35, 36). On the basis of the findings from the RNAi screen, we sought to elucidate mechanistic differences between parental MCF7 and estrogen-independent MCF7/LTEDs in response to PLK1 loss. Using an Estrogen Receptor Signaling PCR Array with 84 ER-associated genes, a 24-hour treatment with each volasertib and PLK1 siRNA oligonucleotides altered (\( > 1.4 \)-fold) the expression of seven genes, including ER\( \alpha \), BCL2L1, the gene encoding BCL-xL, and JUNB in hormone-dependent parental MCF7 cells following treatment with each volasertib and PLK1 siRNA. In MCF7/LTED cells, however, volasertib and PLK1 siRNA decreased the expression of both BCL-xL and JUNB (Fig. 6A and B). HCC1428/LTED cells treated with volasertib also displayed a modest reduction in BCL-xL and JUNB, whereas PLK1 siRNA markedly decreased BCL-xL and JUNB transcript levels (Fig. 6C). BCL-xL and JUNB expression were approximately 5-fold and 17-fold higher, respectively, in MCF7/LTED cells compared with the parental MCF7 cells (Fig. 6D). We next confirmed that treatment with volasertib and downmodulation of PLK1 with two independent siRNAs decreased protein levels of ER, BCL-xL, and JUNB (Fig. 6E). mRNA and protein levels of BCAR1 (breast cancer antiestrogen resistance 1), which has been associated with poor prognosis and resistance to tamoxifen (37), were also decreased upon treatment with volasertib (Supplementary Fig. S5A and S5B). JUNB is a component of the AP-1 transcriptional complex that
drives the expression of genes involved in cellular growth and development (11). In addition, estrogen-activated ER is known to bind to AP-1 promoter sites in ER+ breast cancer cells (38). Therefore, we examined whether PLK1-mediated regulation of JUNB affected ER expression and transcriptional activity. MCF7/LTED cells transfected with JUNB siRNA exhibited reduced hormone-independent ER transcriptional activity (Fig. 6F and Supplementary Fig. S5C) and ER expression (Fig. 6F). Conversely, JUNB siRNA increased ER expression in the parental MCF7 cells (Fig. 6G). These observations suggest that PLK1 regulates ERα via transcriptional regulation of JUNB.

PLK1 transcription is driven by FOXM1, a member of the forkhead family of transcription factors (39). PLK1 and FOXM1 have an inter-regulatory relationship as part of a kinase-driven positive feedback loop that leads to the PLK1-mediated phosphorylation of FOXM1 and potentiation of its activity (29, 40). Consistent with this positive interaction, transfection of a vector encoding FOXM1 constitutively phosphorylated at the PLK1 site (FOXM1-EE) (29) increased ER expression and ER transcriptional activity (Supplementary Fig. S6A). Treatment with volasertib still reduced constitutive ER transcriptional reporter activity in the FOXM1-EE–expressing cells, suggesting that PLK1 mediates ER activity downstream of FOXM1 in LTED cells. Supporting an interaction between FOXM1 and PLK1 in hormone-independent primary tumors, FOXM1 transcript levels correlated positively with both Ki-67 and PLK1 mRNA levels in patients with ER+ breast cancer treated with letrozole (Supplementary Fig. S6B and S6C). Finally, we determined the effect of PLK1 downmodulation on the expression of other nuclear receptors involved in breast cancer pathogenesis, androgen receptor (AR), and ERβ. In ER+ breast cancers, AR expression is associated with lower risk of recurrence and increased overall survival (41, 42). ERβ has been shown to antagonize ERα by decreasing growth and ERα-mediated transcriptional activity (43). Furthermore, it was recently shown that ERβ can be regulated by activated AR to decrease ER+ breast cancer cell growth (44). We observed that PLK1 downmodulation augmented the expression of both AR and ERβ while decreasing the expression of the ERα target gene PR (Supplementary Fig. S6D).

These results suggest that, in breast cancer cells that adapt to estrogen deprivation, PLK1 positively regulates antiapoptotic genes (BCL-xl) and components of the AP-1 (JUNB)
transcription complex (Fig. 6H). In turn, this promotes ERα expression and transcriptional activity and is therapeutically targetable with PLK1 inhibitors in hormone-independent, ER-driven breast cancers.

Discussion

In this study, we identified PLK1 as a potential therapeutic target in hormone-independent ER⁺ breast cancer cells. Using a high-throughput RNAi screen approach to identify kinases that contribute to hormone-independent transcriptional activity and tumor cell viability, PLK1 was the top candidate gene associated with both of these phenotypes. In addition to ER transcriptional activity, PLK1 downmodulation decreased ER expression. Consistent with these results, treatment with the PLK1 inhibitor volasertib reduced LTED cell viability, ER levels, and ER transcriptional activity. Furthermore, volasertib enhanced the antitumor action of fulvestrant in MCF7 xenografts via enhanced ER downmodulation. Using an Estrogen Receptor Signaling PCR Array, we compared the effect of PLK1 inhibition on gene expression in parental and LTED MCF7 cells. Inhibition of PLK1 with volasertib and PLKsiRNA resulted in a reduction of BCL-xL and JUNB expression in LTED cells, while these genes were upregulated in parental MCF7 cells. Taken together, these findings suggest that PLK1 inhibition may provide therapeutic benefit to patients with endocrine-resistant ER⁺ breast cancer.

RNAi kinome screens have previously identified PLK1 as a gene causally associated with growth of basal-like breast cancer and tumor-initiating cells (35, 45). This finding may reflect a crucial role of PLK1 in mitosis of rapidly proliferating cancer cells. PLK1 was also recently shown to cooperate with estrogen-dependent
In this study, Wierer and colleagues showed that PLK1 phosphorylates the transcriptional regulator MLL2, which forms a complex with ER to drive transcription of tumor-suppressive genes. Those findings suggest that PLK1 drives proliferation via its role in interphase but it decreases growth via its interaction with ER. The findings in our report herein suggest that PLK1 has lost this negative cell cycle and transcriptional regulatory function in estrogen-independent breast cancer cells that still depend on unliganded ER function.

ER signaling (21). In this study, Bhola et al. showed that PLK1 phosphorylates the transcriptional regulator MLL2, which forms a complex with ER to drive transcription of tumor-suppressive genes. These findings suggest that PLK1 drives proliferation via its role in interphase but it decreases growth via its interaction with ER. The findings in our report herein suggest that PLK1 has lost this negative cell cycle and transcriptional regulatory function in estrogen-independent breast cancer cells that still depend on unliganded ER function.

PLKs exist in three different isoforms, PLK1, PLK2, and PLK3. Although PLK1 was shown to significantly affect both viability and ER transcription, PLK3 siRNA only decreased ER transcriptional activity in the siRNA screen (Supplementary Table S2). PLK3 is not aberrantly expressed in breast cancer and maintains
shown that ER can cooperate with AP-1 complexes at AP-I sites to decreased ER transcriptional activity and expression. It has been inhibition of PLK1 in LTE5 cell lines and knockdown of JUNB of the AP-1 transcription complex was also decreased upon independent growth of ER

hyperactivation of PI3K is causally associated with hormone-activation of c-Src and p85 PI3K (50). We have reported that complexes with ER and drives nongenomic signaling such as p130Cas has E2F and c-Jun

References


Disclosure of Potential Conflicts of Interest

E.Y. Allen has ownership interest (including patents) in Syapse. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N.E. Bhola, V.M. Jansen, S. Bafna, J.M. Gilnane, I. Meszoely, I. Mayer, V. Abramson, M. Sanders, C.L. Arteaga
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): N.E. Bhola, V.M. Jansen, S. Bafna, J.M. Gilnane, J.M. Balko, I. Mayer, F. Ye, E.V. Allen, C.L. Arteaga
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): N.E. Bhola, S. Bafna, M. Sanders, T.C. Dugger, C.L. Arteaga
Study supervision: C.L. Arteaga
Other (pathology): M.V. Estrada

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