The Prolyl Isomerase Pin1 Induces LC-3 Expression and Mediates Tamoxifen Resistance in Breast Cancer*

Received for publication, December 7, 2009, and in revised form, May 15, 2010. Published, JBC Papers in Press, May 17, 2010, DOI 10.1074/jbc.M109.092874

Gwang Mo Namgoong1, Prem Kanthal1, Hae-Guk Cho1, Sung-Chul Lim1, Yoon Kyeong Oh1, Bong Seok Kang1, Jeong-Hyun Shim*, Jin-Cheol Yoo1, and Hong Seok Choi1,2

From the 1BK21 Project Team, College of Pharmacy, and the Departments of 2Pathology and 8Radiation Oncology, School of Medicine, Chosun University, Gwangju 501-759, the 1Bio-Medical Research Institute, Kyungpook National University Hospital, Daegu 702-701, and the 2School of Dentistry, Chonbuk National University, Jeonju 561-756, South Korea

Endocrine therapies, which inhibit estrogen receptor signaling, are the most common and effective treatments for estrogen receptor-positive breast cancer. However, the utility of these agents is limited by the frequent development of resistance, and the precise mechanisms underlying endocrine therapy resistance remain incompletely understood. Here, we demonstrate that peptidyl-prolyl isomerase Pin1 is an important determinant of resistance to tamoxifen and show that Pin1 increases E2F-4- and Egr-1-driven expression of LC-3 as a result of an increased interaction with and phosphorylation of MEK1/2. In human tamoxifen-resistant breast cancer, our results show a significant correlation between Pin1 overexpression and high levels of LC-3. Promoter activity as well as expression levels of Pin1 were drastically higher in tamoxifen-resistant MCF7 cells than control MCF7 cells, as were levels of LC-3 mRNA and protein, an autophagy marker. Pin1+/− mouse embryonic fibroblasts showed lower 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced MEK1/2 phosphorylation than Pin1+/+ mouse embryonic fibroblasts. Silencing of Pin1 expression inhibited TPA-induced MEK1/2 phosphorylation in MCF7 cells. Moreover, PD98059, a specific inhibitor of MEK1/2, and juglone, a potent Pin1 inhibitor, significantly suppressed the TPA-induced expression of E2F-4 as well as Egr-1 transcription factors, which control LC-3 gene expression. Importantly, 4-hydroxytamoxifen, when used in combination with silencing of Pin1 or LC-3, increased cleaved poly(ADP-ribose) polymerase and DNAfragmentation to inhibit cologenic growth of MCF7 cells. We therefore link the Pin1-MEK pathway and LC-3-mediated tamoxifen resistance and show the therapeutic potential of Pin1 in the treatment of tamoxifen-resistant breast cancer.

Breast cancer is one of the most common malignancies in women and the second most common cause of female cancer-related deaths (1). However, deaths due to breast cancer have decreased because of the development of targeted therapies, including hormone therapy, in addition to conventional chemotherapy and surgical interventions (1). The majority of breast cancers in postmenopausal women express the estrogen receptors (ERs),3 and after surgery, they can be treated with hormone therapy alone, in the absence of more toxic chemotherapy, resulting in a relatively favorable prognosis (2). However, a significant fraction of these hormone-sensitive breast cancer patients will experience disease progression because of resistance to endocrine agents, such as tamoxifen, resulting in mortality (3). Tamoxifen is currently the most widely prescribed, orally active, selective ER modulator for the treatment of breast cancer (4). Although tamoxifen is an ER antagonist in breast tissue, it can also be a partial agonist. Antagonist activity enables the drug to block ER-mediated transcription and cancer cell growth in ER-positive breast cancer cells (5). However, tamoxifen resistance might occur when its agonistic activity overcomes its antagonistic effect (4). This variability could be related, in part, to the cellular milieu of ER co-activators and co-repressors (6). For example, increased levels of co-activators, such as SRC-3, enhance the estrogen agonist properties of tamoxifen, whereas decreased levels of co-repressors, such as SMRT (silencing mediator for retinoid and thyroid receptors) and nuclear receptor corepressor, correlate with acquired tamoxifen resistance (6).

Pin1 has two domains: a peptidyl-prolyl cis/trans-isomerase domain at its COOH terminus responsible for isomerization and a WW domain at the NH2 terminus, which functions as a binding element specific for Ser(P)/Thr-Pro motifs (7). Through these two domains, Pin1 binds to and isomerizes specific Ser(P)/Thr-Pro motifs and catalytically induces conformational changes after phosphorylation (7). Recently, Stanya et al. (8) showed that CDK2 (cyclin-dependent kinase 2)-mediated phosphorylation of SMRT, an ER co-repressor, creates a binding site for Pin1 peptidyl-prolyl cis/trans-isomerase, which in turn induces conformational changes to promote SMRT degradation. Moreover, this event mediates human epidermal growth factor receptor-2 (HER-2)-dependent SMRT protein degradation and resultant endocrine resistance (8). These findings help elucidate the molecular mechanism of ER regulation.

3 The abbreviations used are: ER, estrogen receptor; SMRT, silencing mediator for retinoid and thyroid receptors; MEF, mouse embryonic fibroblast; TPA, 12-O-tetradecanoylphorbol-13-acetate; PARP, poly(ADP-ribose) polymerase; DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MT2, 3-(4,5-dimethylthiazol-2-thiazoyl)-2,5-diphenyltetrazolium bromide; siRNA, small interfering RNA; MCV, murine stem cell virus; GFP, green fluorescent protein; RT-PCR, reverse transcription-PCR; GST, glutathione S-transferase; HA, hemagglutinin, 4-OH, 4-hydroxy.
Pin1 as a Therapeutic Target of Tamoxifen Resistance

and indicate that further investigation is needed for the role and therapeutic potential of Pin1 in the treatment of endocrine-resistant breast cancers.

Here, we have determined for the first time a significant correlation between Pin1 overexpression and high levels of autophagy-related protein LC-3 in human tamoxifen-resistant breast cancer. We found that Pin1 binds directly to MEK1/2 and regulates its activity, resulting in inducing the expression of E2F-4, Egr-1, and ultimately, LC-3 to affect tamoxifen resistance in breast cancer cells. Moreover, we show that 4-OH tamoxifen, when used in combination with silencing of Pin1 or LC-3, induces robust, caspase-dependent apoptosis of breast cancer cells. These results may have relevance to the development of tamoxifen resistance, as shown by the strong correlation, observed in tamoxifen-resistant breast cancer, between Pin1 overexpression and the presence of high levels of LC-3.

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—Dulbecco’s modified Eagle’s medium (DMEM), Eagle’s minimal essential medium, l-glutamine, gentamicin, and FBS were purchased from Invitrogen. Charcoal/dextran-treated FBS was obtained from HyClone (Logan, UT). 12-O-Tetradecanoylphorbol-13-acetate (TPA) and PD98059 were obtained from Calbiochem-Novabiochem. The Dual-Luciferase reporter assay kit was obtained from Promega (Madison, WI). Basal medium Eagle’s, juglone, 4-hydroxamoxifen, and 3-[4,5-dimethylthiazol-2-thiazoyl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich. The antibodies against phospho-MEK1/2, phospho-ERK1/2, and total antibodies against MEK1/2, ERK1/2, Beclin-1, Atg-5, Atg-12, and LC-3 were purchased from Cell Signaling Technology Inc. (Beverly, MA). The antibodies against Pin1, E2F-4, and Egr-1 were acquired from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Xpress antibody was obtained from Invitrogen. The anti-erbB2 antibody was purchased from Dako Cytomation (Glostrup, Denmark).

Tumor Samples—Breast cancer patients selected for immunohistochemical staining consisted of two groups: tamoxifen-resistant group (four patients, age range: 42–72) and non-tamoxifen-resistant group (eight patients, age range: 47–58). The eligibility for the tamoxifen-resistant group was mammary infiltrating duct carcinoma patient who had undergone mastectomy with adjuvant hormone therapy and subsequently had bone metastasis, and that for the non-tamoxifen-resistant group was mammary infiltrating duct carcinoma patient who had undergone mastectomy with adjuvant hormone therapy and subsequently had no local recurrence or metastasis within 5 years.

Immunohistochemical Staining—All tumors investigated in the study were tested for LC-3 and Pin1. Immunolocalization for LC-3 and Pin1 was performed using a Polink-2 horseradish peroxidase plus anti-rabbit diamobenzidine detection kit (Golden Bridge International, Inc., Mukilteo, WA) according to the supplier’s protocol. Slides were incubated for 1 h with anti-Pin1 antibody and overnight with anti-LC-3 antibody, respectively. An isotype-matched control antibody was also used. Positive control for LC-3 was capsaicin-treated WI38 cell, and those for Pin1 were mammary infiltrating duct carcinoma with strong nuclear staining in another study. Instead of the primary antibody, normal goat serum was used in negative control. Distinct nuclear staining was considered as positive immunoreactivity.

Cell Culture—MCF7 human breast cancer cells and Pin1+/+ and Pin1−/− mouse embryonic fibroblast (MEF) cells, which were kindly provided by Dr. Kun Ping Lu (Beth Israel Deaconess Medical Center, Harvard Medical School), were grown in DMEM supplemented with 10% fetal bovine serum. DMEM containing 10% charcoal/dextran-treated FBS and 3 µM 4-OH tamoxifen were used to culture the tamoxifen-resistant MCF7 breast cancer cells (TAMR-MCF7), which were provided by Dr. Keon Wook Kang (College of Pharmacy, Chosun University).

Construction of Mammalian Expression and Small Interfering RNA—The cDNA of the full sequence of Pin1, which was a gift from Dr. Kun Ping Lu, were subcloned to the pcDNA4/Xpress vector (Invitrogen). A segment encoding the full sequence of MEK1 was amplified by PCR and cloned in-frame into the BamHI/XbaI sites of the pCMV-HA (Clontech) or pGEX-5X-1 (GE Healthcare) vector to produce the plasmid pCMV-HA-MEK1 or pGEX-5X-1-MEK1, respectively. All MEK1 deletion mutants (pGEX-5X-1-D1, -D2, or D3) were generated from pGEX-5X-1-MEK1 by PCR and subcloned in-frame into the BamHI/XbaI sites of pGEX-5X-1 vector, respectively. The silencing of human Pin1 (accession number: NM_006221), human LC-3 (accession number: NM_022818), human E2F-4 (NM_001950), and human Egr-1 (NM_001964) was performed by transfecting the ON-TARGETplus siRNA SMART pool-specific or nonspecific control pool double-stranded RNA oligonucleotides (Dharmacon, Chicago, IL) using LipofectamineTM 2000 (Invitrogen).

Infection of GFP and Pin1 into B6 CI41 Cells—Pin1 was overexpressed stably in MCF7 cells using the MSCV-GFP retrovirus system. Human Pin1 was subcloned into the MSCV-GFP retroviral vector (Clontech Inc.), and phoenix cells (a packaging cell line) were transfected with the MSCV-GFP or MSCV-Pin1-GFP plasmids. The supernatants containing amphotropic, replication-incompetent retroviruses were collected and stored at −80 °C until needed. MCF7 cells (20% confluent) were multiply infected (eight times) with retrovirus particles.

Cell Viability by MTT Assay—Cells seeded on 96-well microplates at 10,000 cells/well were incubated with 4-OH tamoxifen for the indicated times. The respective medium was removed and then incubated with MTT solution (5 µg/ml) for 3 h. Absorbance was determined using a microplate reader.

Protein Extract and Immunoblotting—Cells were disrupted in immunoprecipitation assay buffer (50 mM Tris (pH 7.5), 5 mM EDTA, 150 mM NaCl, 1 mM dithiothreitol, 0.01% Nonidet P-40, 0.2 mM phenylmethylsulfonyl fluoride, and 1× protease inhibitor mixture) or radioimmune precipitation lysis buffer (50 mM Tris (pH 7.5), 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% Nonidet P-40, 0.1% SDS, 0.2 mM phenylmethylsulfonyl fluoride, and 1× protease inhibitor mixture) and subjected to the immunoblotting.

Reporter Gene Assays—Pin1 promoter luciferase activity was measured with the Dual-Luciferase® reporter assay system (Promega) according to the manufacturer’s instructions. Briefly, cells were co-transfected with a plasmid mixture con-
Ratifying the Pin1 promoter luciferase reporter gene (a gift from Dr. Kun Ping Lu) with the phRL-SV40 gene (Promega). At 48 h after transfection, firefly luciferase activity was measured using a GloMax®-Multi detection system (Promega). Subsequently, Renilla luciferase activity was measured to normalize the firefly luciferase data.

RNA Isolation and Semiquantitative RT-PCR—Total RNA was isolated from cells using the RNeasy® micro kit (Qiagen, Valencia, CA). Reverse transcription-PCR (RT-PCR) was performed using the AcesQuick RT-PCR system (Promega, WI) on an M MiniTM gradient thermal cycler (Bio-Rad) using the following PCR primers: human LC-3 (sense, 5'-AGCAGCAT-CCAACAAATC-3'; antisense, 5'-CTGTGTTCCGTCAC-CACAC-3'), human Pin1 (sense, 5'-AGCAGCGATGGTGC-GCAAAA-3'; antisense, 5'-GGCCAGAGCTCAAAGTG-3'), and β-actin (Promega), respectively. All data were normalized to β-actin as an internal control according to the manufacturer’s instructions.

In Vitro Binding Assay and GST Protein Expression—For expression of the Xpress epitope-tagged Pin1, the appropriate plasmids (pcDNA4/Xpress-Pin1) were translated in vitro with L-methionine using the TnT quick coupled transcription/translation system (Promega). For the GST pulldown assay, 5 μg of GST fusion protein of MEK1 and deletion mutants were collected on glutathione-Sepharose beads (GE Healthcare) and incubated for 4 h at 4 °C with Xpress-Pin1. The bound proteins were denatured in sample buffer and separated by 10–20% SDS-PAGE, and expression was detected by immunoblotting.

Detection of Apoptosis—An apoptosis assay was performed by terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end-labeling, and apoptosis was detected with an in situ cell death detection kit (Roche Applied Science) according to the manufacturer’s instructions. Briefly, cells were transfected with siRNA-sc, siRNA-Pin1, or siRNA-LC-3 and incubated for 48 h. The cells were then starved with serum-free DMEM for 24 h and treated with tamoxifen for an additional 24 h. Then, cells were stained with terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end-labeling solution and incubated at 37 °C for 2 h, washed twice with phosphate-buffered saline, and mounted with crystal mount reagent for 4 h in the dark. The amount of DNA fragmentation was detected by using an Axiovert 200 M fluorescence microscope and AxioVision software (Carl Zeiss Inc., Thornwood, NY).

Apoptosis Assessed by Flow Cytometry—The induction of early and late apoptosis was analyzed by flow cytometry using the FACSCalibur flow cytometer (BD Biosciences). TAMR-MCF7 cells (5 × 10⁵/dish), were grown in 6-cm dishes for 24 h in 10% FBS/DMEM. Then, cells were transfected with siRNA-sc or -Pin1, incubated for 48 h, and then treated with 4-OH tamoxifen. After additional incubation for 24 h, the medium was collected, and attached cells were harvested with 0.025% trypsin in 5 mM EDTA in phosphate-buffered saline. Cells were washed by centrifugation at 1,000 rpm for 5 min and processed for detection of early and late apoptosis using annexin V-fluorescein isothiocyanate and propidium iodide staining according to the manufacturer’s protocol.

RESULTS

Pin1 and LC-3 Levels Correlate in Tamoxifen-resistant Breast Cancer—To investigate the pathological relevance of the relationship between Pin1 and LC-3 expression in tamoxifen-resistant breast cancers, we analyzed these two proteins in eight human non-tamoxifen-resistant and four human tamoxifen-resistant breast cancer tissue samples. Immunohistochemical staining showed that there was a positive correlation between the levels of LC-3 and Pin1 in human tamoxifen-resistant breast cancer samples as tumors with high levels of LC-3 (in three of four samples) also had high levels of Pin1 protein levels (in all four samples) in tamoxifen-resistant cancers, whereas low levels of LC-3 (in seven of the eight samples) were paralleled by low levels of Pin1 (in seven of the eight samples) in non-tamoxifen-resistant cancers (p < 0.010, Fisher’s exact test; Fig. 1A).

To further examine whether Pin1 or LC-3 facilitates the development of antiestrogen resistance, we did a drug selection in which MCF7 cells were exposed to small, incremental increases of 4-OH tamoxifen. The stepwise drug selection was continued until the MCF7 cell population could sustain viability and proliferate when challenged with 3.0 μM 4-OH tamoxifen. The acquisition of 4-OH tamoxifen resistance in TAMR-MCF7 cells was verified using an MTT assay. 4-OH tamoxifen caused a concentration-dependent decrease in the cell viability of MCF7 cells but not TAMR-MCF7 up to 5 μM (Fig. 1B). To examine the effects on Pin1 promoter activity, MCF7 or TAMR-MCF7 cells were transfected with mixtures of the Pin1 promoter luciferase construct and phRL-SV-40 gene. The promoter activity of Pin1 was significantly increased in TAMR-MCF7 cells when compared with MCF7 cells (Fig. 1C, upper panels). Pin1 expression was higher in TAMR-MCF7 cells than in MCF7 cells in immunoblots (Fig. 1C, lower panels). These results suggested that Pin1 is likely to be involved in tamoxifen resistance of MCF7 cells. To determine whether autophagy proteins affect tamoxifen resistance, we next monitored the expression levels of autophagy proteins in TAMR-MCF7 cells. TAMR-MCF7 cells showed higher levels of LC-3 and, to a lesser extent, Beclin-1, ATG5, and ATG12 than control MCF7 cells (Fig. 1D, right lane). To further confirm whether overexpression of LC-3 in TAMR-MCF7 cells is affected by Pin1,
PCDNA4/Xpress-Pin1 were transfected into MCF7 cells, and the cells were incubated for 48 h. Pin1-overexpressing MCF7 cells showed increased levels of LC-3 (Fig. 1D, middle lane).

To examine the effect of Pin1 on the expression level of LC-3, which is an autophagy marker, we then analyzed mRNA levels of LC-3 followed by overexpression of Pin1 in
Pin1 as a Therapeutic Target of Tamoxifen Resistance

MCF7 cells or knockdown of Pin1 in TAMR-MCF7 cells, respectively. Semiquantitative RT-PCR results showed that LC-3 mRNA level was increased by up to 5-fold in Pin1-overexpressing MCF7 cells when compared with MCF7 cells, whereas the increased LC-3 mRNA level in TAMR-MCF7 cells significantly decreased with knockdown of Pin1 (Fig. 1E). Collectively, these data suggest that Pin1-mediated overexpression of LC-3 may be responsible for the 4-OH tamoxifen resistance in breast cancer.

**Pin1 Enhances MEK1/2 Phosphorylation Induced by TPA—** Activation of Ras/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling induced by TPA plays an important role in autophagy (9). Additionally, the inhibition of autophagy accelerates imatinib-induced cell death of imatinib-resistant cells (9). To determine whether MAPK signaling affects tamoxifen resistance, we monitored the activity of MAPK proteins in MCF7 and TAMR-MCF7 cells. TAMR-MCF7 cells showed a marked increase in MEK1/2 and ERK1/2 phosphorylation as well as LC-3 and Pin1 when compared with control MCF7 cells (Fig. 2A).

To confirm the interaction between Pin1 and MEK1, we next co-transfected HA-tagged MEK1 with Xpress-tagged Pin1 or Pin1 mutant affecting the WW domain (S16A) in HEK 293 cells. The cells lysates were immunoprecipitated using the normal IgG or anti-HA antibody, respectively, and blotted with the anti-Xpress antibody. The results showed that the exogenously expressed MEK1 only bound to the wild-type Pin1 but not to the Pin1 S16A mutant affecting the WW domain (Fig. 2B). To determine the region of MEK1 that was responsible for its interaction with Pin1, GST fusion protein of full-length MEK1 (MEK1-WT) or each of respective MEK1 deletion fragments (MEK1-D1, -D2, -D3) was incubated with *in vitro* translated Pin1, respectively, and the interaction was examined by GST pulldown assay. The results suggested that residues 68–330 of MEK1, which is included in the Ser/Thr kinase domain, were required for its interaction with Pin1 (Fig. 2C).

Next, we examined the time course analysis for the TPA-induced interaction of Pin1 and MEK1/2 in MCF7 cells. Reciprocal immunoprecipitation/immunoblotting showed that Pin1 was detectable in MEK1/2 immunoprecipitates from 5 to 15 min after TPA treatment (Fig. 2D). To assess whether MAPK signaling was regulated by Pin1, we exposed Pin1*+/+* and Pin1*−/−* MEF cells to TPA and immunoblotted with antibodies against phospho-MEK1/2 and phospho-ERK1/2. We found significantly decreased phosphorylation of MEK1/2 and ERK1/2 in Pin1*−/−* MEF cells when compared with Pin1*+/+* MEF cells (Fig. 2E). Next, to determine whether knockdown of Pin1 suppresses the phosphorylation of MEK1/2 and ERK1/2 induced by TPA, we transfected small interfering RNA (siRNA) for Pin1 or control siRNA in MCF7 cells. TPA induced less phosphorylation of MEK1/2 and ERK1/2 in Pin1 knockdown cells (Fig. 2F). Taken together, these data indicate that Pin1 enhanced the phosphorylation of MEK1/2 as well as ERK1/2 induced by TPA, resulting from its interaction with MEK1/2, and suggest that Pin1-enhanced phosphorylation of MEK1/2 may regulate a high level of LC-3.

**Pin1 Regulates TPA-induced ErbB2 Expression—** Resistance associated with the activated ErbB system in breast cancer cells is reversed by inhibiting MAPK or phosphatidylinositol 3-kinase-Akt signaling (10). In fact, targeting the ErbB2 pathway through blocking antibodies (Herceptin) has been suggested in the context of tamoxifen resistance based on the link between activation of growth factor signaling pathways and estrogen-independent tumor growth (11). As expected, TAMR-MCF7 cells showed a high level of ErbB2 when compared with control MCF7 cells, similarly to Pin1 (Fig. 3A). Given the role of Pin1 in activating MEK1/2 (Fig. 2), we further determined whether ablation or knockdown of Pin1 suppresses the ErbB2 expression induced by tumor promoters, such as TPA. Therefore, we exposed Pin1*+/+* and Pin1*−/−* MEF cells to TPA and performed immunoblotting with an antibody against ErbB2. Pin1*−/−* MEF showed significantly lower ErbB2 levels than Pin1*+/+* MEF cells treated with TPA in a dose-dependent analysis (Fig. 3B, upper panels) and time-dependent analysis (Fig. 3B, lower panels). To confirm the effect of Pin1 knockdown on the ErbB2 expression induced by TPA, we transfected siRNA-Pin1 or siRNA-sc (control) in MCF7 cells and then performed immunoblotting with antibodies against ErbB2 and Pin1. TPA-induced ErbB2 expression was significantly suppressed in siRNA-Pin1-transfected cells when compared with siRNA-sc-transfected cells (Fig. 3C). Similarly, pretreatment with PD98059, a MEK1/2 inhibitor, or juglone, a Pin1-specific inhibitor (12), dramatically decreased TPA-induced ErbB2 expression (Fig. 3D). Taken together, these results indicate that Pin1 regulates TPA-induced ErbB2 expression in MCF7 cells through enhancing MEK1/2 and ERK1/2 phosphorylation.

**Pin1 is Essential for TPA-induced LC-3 Expression via E2F-4 and Egr-1—** E2F-4 and Egr-1 regulate LC-3 transcription and may be novel targets for regulating autophagy in mammalian systems (13). We therefore examined whether Pin1 is necessary for E2F-4 and Egr-1 to induce LC-3. As expected, E2F-4 and Egr-1 levels were higher in TAMR-MCF7 cells than MCF7 cells, consistent with high LC-3 and Pin1 levels (Fig. 4A). Similarly, TPA could induce E2F-4 and Egr-1 expression in Pin1*+/+* MEF cells but not in Pin1*−/−* MEF cells (Fig. 4B).
Finally, the siRNA-Pin1, but not the siRNA-sc, blocked TPA-induced E2F-4 and Egr-1 expression (Fig. 4C). Juglone (Fig. 4D, left panel) and PD98059 (Fig. 4D, right panel) dose dependently decreased TPA-induced expression of E2F-4 and Egr-1.

Next, we determined the effect of knockdown of E2F-4 or Egr-1 on the TPA-induced LC-3, respectively. Interestingly, these results showed that both E2F-4 and Egr-1 are essential for the TPA-induced expression of LC-3 in MCF7 cells (Fig. 4E). Therefore, we further examined whether ablation of the E2F-4
or Egr-1 gene in Pin1-overexpressing MCF7 cells (MSCV-Pin1) affects the LC-3 levels induced by TPA. MSCV-Pin1 cells were transfected with siRNA-sc, -E2F-4, or -Egr-1, respectively, and then either treated or not treated with TPA. The results showed that TPA markedly induced the LC-3 expression in MSCV-Pin1 cells when compared with MSCV-GFP cells, whereas knockdown of E2F-4 or Egr-1 in MSCV-Pin1 cells suppressed TPA-induced LC-3 expression when compared with siRNA-sc-transfected MSCV-Pin1 cells (Fig. 4F). Taken together, these results strongly support our notion that the regulation of MEK1/2 by Pin1 is critical for regulating LC-3 expression via increases in transcriptional factors E2F-4 and Egr-1.

Pin1 Silencing Enhances Tamoxifen-induced Apoptotic Signaling in MCF7 and TAMR-MCF7 Cells—We next tested whether Pin1 and LC-3 knockdown would potentiating tamoxifen-induced cell death. 4-OH tamoxifen treatment reduced MCF7 cell viability by 20%, whereas Pin1 knockdown with siRNA produced almost complete sensitivity (Fig. 5A, open circles), as did LC-3 knockdown (Fig. 5A, black triangle) in MTT assays. To further examine whether ablation of the LC-3 gene in Pin1-overexpressing MCF7 cells affects tamoxifen sensitivity, we next transfected siRNA-sc or -LC-3 in MSCV-Pin1 MCF7 cells, respectively, and then cells were either treated or not treated with 4-OH tamoxifen. Pin1 overexpression caused 4-OH tamoxifen resistance in cell viability, whereas LC-3-silenced MSCV-Pin1 MCF7 cells resulted in significant decrease in cell viability (Fig. 5B).

Because 4-OH tamoxifen induces cell death by poly(ADP-ribose) polymerase (PARP) cleavage (14), we next tested the effects of knockdown of Pin1 or LC-3 on PARP cleavage. 4-OH tamoxifen induced dramatically higher cleavage of PARP after Pin1 knockdown (Fig. 5C) or LC-3 knockdown (Fig. 5D), providing a mechanism for the increased cell death. 4-OH tamoxifen-induced DNA fragmentation was also higher in Pin1 or LC-3 knockdowns, as measured in the terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end-labeling assay (Fig. 5E). These data indicate that 4-OH tamoxifen induces more cell death than PARP cleavage and DNA fragmentation if Pin1 or LC-3 expression is down-regulated. Furthermore, 4-OH tamoxifen induced apoptosis of control siRNA-transfected TAMR-MCF7 cells, which were characterized by a marked rate of total apoptosis that reached 15.7 and 36.9% at 5 and 10 μM 4-OH tamoxifen, respectively (Fig. 5F). Importantly, tamoxifen sensitivity in TAMR-MCF7 cells was highly increased by Pin1 silencing, and the rate of total apoptosis reached 40.3 and 92.4% at 5 and 10μM 4-OH tamoxifen, respectively (Fig. 5F).

The Inhibition of Pin1 or MEK1 Suppresses TPA-induced Neoplastic Cell Transformation—To assess whether inhibition of Pin1 or MEK1/2 suppresses Pin1-enhanced cell transformation induced by TPA, GFP-JB6 and Pin1-JB6 cells were treated with TPA in an absence or presence of juglone or PD98059 in soft agar. TPA treatment increased colony number and size more in Pin1-JB6 cells than in GFP-JB6 cells (Fig. 6, A and B). Juglone (Fig. 6A) and PD98059 (Fig. 6B) dose dependently blocked this increase in Pin1-JB6 cells.

Pin1 Silencing Increases Tamoxifen-induced Inhibition of Cologenic Growth—To determine the mechanism by which Pin1, E2F-4, or Egr-1 silencing regulates the expression of LC-3, resulting in increased tamoxifen sensitivity in TAMR-MCF7 cells, we next transfected siRNA-Pin1, -E2F-4, or -Egr-1 in TAMR cells, respectively. As expected, LC-3 expression levels were decreased by knockdown of Pin1, E2F-4, and Egr-1 in TAMR-MCF7 cells, respectively, when compared with control TAMR-MCF7 cells (Fig. 7A, right panel). In addition, Pin1 silencing decreased the activity of MEK1/2 and ERK1/2, as well as the expression levels of E2F-4, Egr-1, and LC-3 in TAMR-MCF7 cells, consistent with total MEK1/2, ERK1/2, and β-actin levels (Fig. 7A).

We next tested whether knockdown of Pin1, LC-3, E2F-4, and Egr-1 would increase tamoxifen sensitivity in TAMR-MCF7 cells. 4-OH tamoxifen treatment reduced the viability of
FIGURE 4. Pin1 enhances expression of E2F-4 and Egr-1 induced by TPA. A, increased expression of E2F-4 and Egr-1 in TAMR-MCF7 cells. B, Pin1+/+ and Pin1−/− MEF cells were serum-starved for 24 h, treated with 10 ng/ml TPA, harvested, lysed, and immunoblotted. C, MCF7 cells were transfected with either siRNA-sc or siRNA-Pin1. After 48 h, cells were serum-starved, treated with TPA for 12 h, and then harvested and lysed. The lysates were resolved by SDS-PAGE and immunoblotted. D, MCF7 cells were serum-starved for 24 h, treated with either juglone (left panels) or PD98059 (right panels), and incubated for 2 h. Following TPA treatment for 12 h, cells were harvested in ice-cold phosphate-buffered saline and lysed. The lysates were resolved by SDS-PAGE and immunoblotted. E and F, MCF7 cells (E) or MSCV-Pin1 MCF7 cells (F) were transfected with siRNA-sc, -E2F-4, or -Egr-1, respectively. After 48 h, cells were serum-starved, treated or not treated with 10 ng/ml TPA for 12 h, harvested, lysed, and immunoblotted. Corresponding signal intensities of each protein were densitometrically determined and normalized in each lane and are given below each lane.
Pin1 as a Therapeutic Target of Tamoxifen Resistance

FIGURE 5. Silencing of Pin1 and LC-3 increases tamoxifen-induced apoptosis. A, MCF7 cells were transfected with siRNA-sc, Pin1, or -LC-3, respectively. At 48 h after incubation, cells were serum-starved for 24 h and treated with 4-OH tamoxifen for 24 h. Cell viability was measured by an MTT assay and plotted. The inset on the right side indicates expression levels of Pin1 and LC-3 following transfection of siRNA-LC-3. Columns, mean of triplicate samples; bars, S.E. *, p < 0.05, when compared with control cells. B, MSCV-Pin1 MCF7 cells were transfected with either siRNA-sc or siRNA-LC-3. After 48 h, cells were treated or not treated with 15 μM 4-OH tamoxifen for 24 h. Cell viability was measured by an MTT assay. The inset on the right side indicates expression levels of Pin1 and LC-3 following transfection of siRNA-LC-3. Columns, mean of triplicate samples; bars, S.E. *, p < 0.05, when compared with control cells. C and D, MCF7 cells were transfected with either siRNA-Pin1 (C) or siRNA-LC-3 (D) and then incubated for 48 h. Following 24 h of serum starvation, cells were treated with 4-OH tamoxifen (4-OHT) for 24 h, lysed, and immunoblotted. Corresponding signal intensities of each protein were densitometrically determined and normalized to β-actin in each lane and are given below each lane. E, MCF7 cells were transfected with siRNA-sc, siRNA-Pin1, or siRNA-LC-3 and then incubated for 48 h. Following 24 h of serum starvation, cells were treated with 4-OH tamoxifen for 24 h. DNA fragmentation induced by 4-OH tamoxifen was detected. FITC, fluorescein isothiocyanate. F, TAMR-MCF7 cells were transfected with siRNA-sc or -Pin1 and then incubated for 48 h. Cells were treated with 4-OH tamoxifen for 24 h. The induction of early and late apoptosis rate induced by 4-OH tamoxifen was analyzed by flow cytometry. Columns, mean of triplicate samples; bars, S.E. *, p < 0.05, when compared with control cells.

FIGURE 6. Juglone (JUG) and PD98059 (PD) inhibit Pin1-enhanced neoplastic cell transformation promoted by TPA. A and B, GFP-JB6 and Pin1-JB6 cells were exposed to TPA with/without treatment with juglone (A) or PD98059 (B) in soft agar. The average colony number was calculated, and the colonies from three separate experiments were photographed. Columns, mean of triplicate samples; bars, S.D. *, p < 0.05, when compared with control cells.

DISCUSSION

The phosphorylation of proteins on serine or threonine residues that immediately precede proline residues (Ser/Thr-Pro) is an important signaling mechanism for cell cycle regulation, transcription, cell differentiation, and proliferation (7, 15). The prolyl isomerase Pin1 binds to and isomerizes the peptidyl-prolyl bond in specific phosphorylated Ser/Thr-Pro motifs to induce conformational changes in its target proteins (7). These conformational changes can have profound effects on the function of Pin1 substrates, modulating their activity, phosphorylation status, protein-protein interaction, subcellular localization, and stability (16). Interestingly, it was reported that Pin1 regulates SMRT and SRC-3 as downstream effectors of HER-2 signaling (8, 17), which is often increased in endocrine-resistant breast tumors and contributes to activate proliferation and/or survival and hormone resistance (6, 18). Here, we show that there was a positive correlation between the levels of LC-3 and Pin1 in human tamoxifen-resistant breast cancer samples (Fig. 1A). Although the small numbers and unmatched sample groups are inevitable in our study, Fisher’s exact tests revealed that there was a significant association between expression of LC-3 and Pin1 (p < 0.010). Recently, quantitative immunohistochemical analysis of human melanomas showed a strong correlation between the levels of B-Raf protein and LC-3, suggesting that high oncogenic B-Raf levels trigger autophagy, which may have a role in tumor progression (19). In the context of B-Raf signaling, the oncogenic activity of B-Raf was increased in cells overexpressing wild-type Pin1, whereas their transforming activity was reduced in cells overexpressing a dominant negative Pin1 (20). These reports supported our hypothesis that high levels of Pin1 may regulate LC-3 expression, resulting in tumor progression as well as tamoxifen resistance in breast cancer. In this study, we further found that the expression level and promoter activity of Pin1 were significantly increased in tamoxifen-resistant MCF7 breast cancer cells established by long...
FIGURE 7. **Silencing of Pin1 and LC-3 increases tamoxifen sensitivity to inhibit the tumorigenicity of MCF7 cells.** A and B, TAMR-MCF7 cells was transfected with siRNA-sc, -Pin1, -E2F-4, or -Egr-1, respectively. A, after 48 h, cells were harvested and lysed and then immunoblotted. Corresponding signal intensities of each protein were densitometrically determined and normalized to β-actin in each lane and are given below each lane. P-MEK1/2, phospho-MEK1/2; P-ERK1/2, phospho-ERK1/2. B, cells were treated or not treated with 15 μM 4-OH tamoxifen for 72 h. Cell viability was measured by an MTT assay. The inset in the bottom indicates expression levels of LC-3 following transfection of siRNA-LC-3. Columns, mean of triplicate samples; bars, S.D. *, p < 0.05, when compared with control cells.

C, MSCV-GFP or MSCV-Pin1 cells were treated with 4-OH tamoxifen (4-OHT) in soft agar, and average colony numbers were measured. Columns, mean of triplicate samples; bars, S.D. *, p < 0.05, when compared with control cells.

D, MCF7 cells were transfected with siRNA-sc, -Pin1, or -LC-3, respectively, and subjected to soft agar assays as described above in the absence or presence of 5 μM 4-OH tamoxifen. Columns, mean of triplicate samples; bars, S.D. *, p < 0.05, when compared with control cells.

E, TAMR-MCF7 cells were treated with 5 μM 4-OH tamoxifen with/without juglone in soft agar, and average colony numbers were measured. Columns, mean of triplicate samples; bars, S.D. *, p < 0.05, when compared with control cells.
Pin1 as a Therapeutic Target of Tamoxifen Resistance

term exposure to tamoxifen (Fig. 1C). Autophagy is a key mechanism of cell survival in ER-positive breast cancer cells, resulting in the development of tamoxifen resistance (21). Also, antiestrogen resistance could be reduced by targeting autophagyosome function, which is regulated by LC-3, Beclin-1, Atg-5, and Atg-12 (21–23), suggested that high Pin1 expression in tamoxifen-resistant MCF7 cells may enhance autophagy through increased expression of autophagy-related proteins, such as LC-3, to produce tamoxifen resistance. As expected, LC-3 levels were higher in tamoxifen-resistant MCF7 cells, and Pin1 overexpression produces the same expression patterns as tamoxifen-resistant cells, suggesting that Pin1 regulates tamoxifen resistance via enhanced LC-3 expression (Fig. 3D).

Overexpression of many growth factor receptors, as well as growth factors, confers varying degrees of estrogen-independent growth on ER-positive breast cancer cells (24). Transfection of constitutively active MEK1 or c-Raf into MCF7 cells, which results in hyperactivation of ERK1/2, causes loss of ER-mediated gene expression, characterized by acquisition of antiestrogen resistance (24, 25). In addition, activation of MAPK signaling, circumventing the reliance upon ERα signaling, causes tamoxifen resistance by reducing CDK10 expression (26). Interestingly, the induction of an autophagy marker, LC-3, a mammalian homolog of yeast Atg8 (Aut7-Apg8), by TPA is mediated by the Ras-MAPK pathway (9, 27). A recent study shows that depletion of ERK partially inhibited autophagy, whereas specific inhibition of MEK completely inhibited autophagy (28). As shown in this study, TPA induced the interaction of Pin1 with MEK and resulted in expression levels of LC-3 mRNA and protein (Fig. 2D). Thus, our findings propose that the Pin1-MEK-ERK-pathway regulation of LC-3 may induce autophagy and tamoxifen resistance in MCF7 cells.

LC-3 expression is directly regulated by the E2F-4 and Egr-1 transcription factors, which have putative binding sites at the LC-3 promoter, and Egr-1 also indirectly regulates LC-3 conversion through regulation of Atg4B (13). We have shown here that phosphorylation of MEK1/2 and ERK1/2 is markedly increased in tamoxifen-resistant MCF7 cells when compared with MCF7 cells (Fig. 2A). Furthermore, TPA dramatically induces the interaction of Pin1 with MEK1/2 in MCF7 cells (Fig. 2D) and enhances the phosphorylation of MEK1/2 and ERK1/2 in Pin1+/+ MEF or siRNA-control-transfected MCF7 cells, but the TPA-induced phosphorylation of MEK1/2 and ERK1/2 is suppressed in Pin1−/− MEF or siRNA-Pin1-transfected cells (Fig. 2, E and F). Interestingly, E2F-4 and Egr-1 are also highly expressed in tamoxifen-resistant MCF7 cells and in response to TPA in MCF7 cells (Fig. 4, A and C). TPA-induced E2F-4 and Egr-1 expression is suppressed by Pin1 knockdown, juglone, or PD98059 in MCF7 cells, resulting in down-regulation of LC-3 expression (Fig. 4, C and D). These results indicated that Pin1 interacts with MEK1/2 to play a pivotal role in LC-3 expression through regulating E2F-4 and Egr-1 transcriptional factors.

Overexpression of ErbB2 is a predictor for tamoxifen resistance in ER+ disease, and ErbB2+ and ER+ breast cancer forms a subgroup with poor prognosis in premenopausal breast cancer (29). The induction of autophagy is closely related to the cell survival triggered by ErbB2 gene-amplified human breast cancer cells in response to the anti-ErbB2 monoclonal antibody, trastuzumab (30). Knockdown of autophagy genes in combination with tamoxifen in tamoxifen-resistant ErbB2-overexpressing MCF7 cells, reduced cell viability with increased mitochondrial-mediated apoptosis (29). Interestingly, a majority of breast cancers overexpressed Pin1 (54%), and Pin1 overexpression was more prevalent in the HER-2-overexpressing tumors (62.5%) than in HER-2-negative breast cancers (31). Therefore, we hypothesized that Pin1-enhanced phosphorylation of MEK1/2, which increases LC-3 expression, may also regulate ErbB2 expression, resulting in tamoxifen resistance in MCF7 cells. ErbB2 levels are higher in tamoxifen-resistant MCF7 cells and also in response to TPA in MCF7 cells (Fig. 3, A and C). TPA dramatically induces ErbB2 expression in Pin1+/+ MEF or siRNA-control-transfected MCF7 cells, whereas the TPA-induced phosphorylation of MEK1/2 and ERK1/2 was suppressed in Pin1−/− MEF or siRNA-Pin1-transfected cells (Fig. 3, B and C). Furthermore, the TPA-induced expression of ErbB2 is almost totally inhibited by PD98059 or juglone (Fig. 3D), suggesting that the TPA-induced expression of ErbB2 may be mediated by an interaction between Pin1 and MEK1/2 in breast cancer.

We show here that knockdown of Pin1 and LC-3 expression with siRNA enhances apoptotic pathways after tamoxifen treatment, as measured by the MTT assay (Fig. 5A), PARP cleavage, and DNA fragmentation (Fig. 5, C and D). Tamoxifen markedly increased LC-3 expression, but not PARP cleavage, in siRNA-sc-transfected MCF7 cells, indicating that LC-3 may play an important role in terms of cancer cell survival. Our findings are consistent with earlier reports showing that autophagy inhibition activates the mitochondrial apoptotic pathway and increases apoptosis, at least in part through caspase-9 (32, 33). In addition, overexpression of Pin1 in JB6 Cl41 cells enhanced TPA-induced cell transformation (Fig. 6, A and B). We also confirmed that the Pin1 inhibitor, juglone, as well as the MEK1/2 inhibitor, PD98059, dramatically suppressed TPA-induced cell transformation in Pin1-overexpressing cells, indicating a role for MEK1/2 (Fig. 6, A and B). Overexpression of Pin1 attenuated tamoxifen-suppressed tumorigenicity in MCF7 cells, whereas loss of Pin1 and LC-3 increased tamoxifen activity, indicating that Pin1 and LC-3 have an important role in tamoxifen resistance in breast cancer cells (Fig. 7, D and E).

In summary, our data indicate that Pin1 induces the expression of LC-3 via regulation of E2F-4 and Egr-1 and facilitates the progression of breast cancer to tamoxifen resistance. Our findings also indicate that autophagy inhibition targeting Pin1 and LC-3 might be advantageous in a combination therapy setting to sensitize breast cancer to tamoxifen, which is in complete agreement with two recent studies (29, 34) in which autophagy was shown to reduce the efficacy of chemotherapy and tamoxifen therapy, respectively, in ER+ breast cancer cells. Further, to our knowledge, our work is the first to show that Pin1 inhibition can sensitize tamoxifen-resistant cells and indicates that this approach may be a viable strategy to sensitize therapy-resistant cancers.
REFERENCES

1. Berry, D. A., Cronin, K. A., Plevritis, S. K., Fryback, D. G., Clarke, L., Zelen, M., Mandelblatt, J. S., Yakovlev, A. Y., Habbema, J. D., and Feuer, E. J. (2005) N. Engl. J. Med. 353, 1784–1792
2. Joslyn, S. A. (2002) Breast Cancer Res. Treat. 73, 45–59
3. Davood, S., and Cristofanilli, M. (2007) Ann. Oncol. 18, 1289–1291
4. Riggs, B. L., and Hartmann, L. C. (2003) N. Engl. J. Med. 348, 618–629
5. Oesterreich, S., Zhang, Q., Hopp, T., Fuqua, S. A., Michaelis, M., Zhao, H. H., Davie, J. R., Osborne, C. K., and Lee, A. V. (2000) Mol. Endocrinol. 14, 369–381
6. Osborne, C. K., Shou, J., Massarweh, S., and Schiff, R. (2005) Clin. Cancer Res. 11, 865s–870s
7. Lu, K. P., Liou, Y. C., and Zhou, X. Z. (2002) Cancer Sci. 93, 49–61
8. Mishima, Y., Terui, Y., Mishima, Y., Taniyama, A., Kuniyoshi, R., Takizawa, T., Kimura, S., Ozawa, K., and Hatake, K. (2008) Cancer Sci. 99, 2200–2208
10. Ghayad, S. E., Vendrell, J. A., Larbi, S. B., Dumontet, C., Bieche, I., and Dumontet, C., Bieche, I., and Schiff, R. (2004) Clin. Cancer Res. 10, 5215–5225
11. Clarke, R., Liu, M. C., Bouker, K. B., Gu, Z., Lee, R. Y., Zhu, Y., Skaar, T. C.,ジューキ, D., Dhir, R., Landreneau, R. J., Schuchert, M. J., Yousem, S. A., Nakahira, K., Pilewski, J. M., Lee, J. S., Zhang, Y., Ryter, S. W., and Choi, A. M. (2008) PLoS One 3, e3316
12. Ghayad, S. E., Vendrell, J. A., Larbi, S. B., Dumontet, C., Bieche, I., and Cohen, P. A. (2010) Int. J. Cancer 126, 545–562
13. Clarke, R., Liu, M. C., Bouker, K. B., Gu, Z., Lee, R. Y., Zhu, Y., Skaar, T. C., Gomez, B., O’Brien, K., Wang, Y., and Hilakivi-Clarke, L. A. (2003) Oncogene 22, 7316–7339
14. Mizushima, N., Yamamoto, A., Matsui, M., Yoshimori, T., and Ohsumi, Y. (2000) Autophagy 5, 400–403
15. Riggs, B. L., and Hartmann, L. C. (2003) J. Natl. Cancer Inst. 95, 926–935
17. Yi, P., Wu, R. C., Sandquist, J., Wong, J., Tsai, S. Y., Tsai, M. I., Means, A. R., and O’Malley, B. W. (2005) Mol. Cell. Biol. 25, 9687–9699
18. Shou, J., Massarweh, S., Osborne, C. K., Wakeling, A. E., Ali, S., Weiss, H., and Schiff, R. (2004) J. Natl. Cancer Inst. 96, 926–935
19. Maddodi, N., Huang, W., Havighurst, T., Kim, K., Longley, B. J., and Setaluri, V. (2010) J. Invest. Dermatol. 130, 1657–1667
20. Ritt, D. A., Monson, D. M., Specht, S. I., and Morrison, D. K. (2010) Mol. Cell. Biol. 30, 806–819
21. Samaddar, J. S., Gaddy, V. T., Duplantier, J., Thandavan, S. P., Shah, M., Smith, M. J., Browning, D., Rawson, J., Smith, S. B., Barrett, J. T., and Schoenlein, P. V. (2008) Mol. Cancer Ther. 7, 2977–2987
22. Lim, S. E., Negroponte, N., Yang, P., Liu, W., Das, G. M., Thomas, T., and Thomas, T. J. (2008) Cancer Res. 68, 7855–7863
23. Schoenlein, P. V., Periyasamy-Thanadan, S., Samaddar, J. S., Jackson, W. H., and Barrett, J. T. (2009) Autophagy 5, 400–403
24. El-Ashry, D., Miller, D. L., Kharbanda, S., Lippman, M. E., and Kern, F. G. (1997) Oncogene 15, 423–435
25. Wang, J., Whiteman, M. W., Lian, H., Wang, G., Singh, A., Huang, D., and Denmark, T. (2009) J. Biol. Chem. 284, 21412–21424
26. Oem, E., Turner, N. C., Elliott, R., Syed, N., Gareau, O., Gasco, M., Tutt, A. N., Crook, T., Lord, C. J., and Ashworth, A. (2008) Cancer Cell 13, 91–104
27. El-Ashry, D. (2001) J. Cell Sci. 114, 355–365
28. Medina, M., Valentin, D., Pello, G., and Wróbel, M. (2005) Mol. Cancer Res. 3, 259–266
29. Vazquez-Martin, A., Oliveras-Ferraros, C., and Menendez, J. A. (2009) PLoS One 4, e6251
30. Lam, P. B., Burga, L. N., Wu, B. P., Hofstatter, E. W., Lu, K. P., and Wulf, G. M. (2008) Mol. Cancer 7, 91
31. Gonzalez-Polo, R. A., Boya, P., Pauleau, A. L., Jalil, A., Larochette, N., Souquère, S., Eskelinen, E. L., Pierron, G., Saftig, P., and Kroemer, G. (2005) J. Cell Biol. 168, 3091–3102
32. Boya, P., Gonzalez-Polo, R. A., Casares, N., Perfettini, J. L., Dessen, P., Larochette, N., M étivier, D., Meley, D., Souquère, S., Yoshimori, T., Pier- ron, G., Codogno, P., and Kroemer, G. (2005) Mol. Cell. Biol. 25, 1025–1040
33. Abedin, M. J., Wang, D., McDonnell, M. A., Lehmann, U., and Kelekar, A. (2007) Cell Death Differ 14, 500–510

Pin1 as a Therapeutic Target of Tamoxifen Resistance

BREAST CANCER RESEARCH AND TREATMENT