Prolyl Isomerase PIN1 Negatively Regulates SGK1 Stability to Mediate Tamoxifen Resistance in Breast Cancer Cells

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Abstract. Background/Aim: Endocrine therapies that inhibit oestrogen receptor (ER)-α signaling are the most common and effective treatment for ER-α-positive breast cancer. The present study aimed to elucidate the mechanisms by which down-regulation of serum- and glucocorticoid-inducible protein kinase-1 (SGK1) expression confers tamoxifen resistance in breast cancer. Materials and Methods: SGK1 expression and the cytotoxic effects of combinatorial 4-hydroxy-tamoxifen (4-OHT) treatment with SGK1 overexpression were investigated by immunoblotting, bromodeoxyuridine incorporation, and soft agar assay. Results: We showed that PIN1 down-regulates SGK1 expression through interaction with and ubiquitination of SGK1. PIN1 silencing in MCF7 cells increased SGK1 expression. In tamoxifen-resistant human breast cancer, immunohistochemical staining analysis showed an inverse correlation between SGK1 expression and severity of tamoxifen resistance. Importantly, 4-OHT in combination with overexpression of SGK1 increased cleavage of poly-(ADP-ribose) polymerase and DNA fragmentation to inhibit clonogenic growth of tamoxifen-resistant MCF7 (TAMR-MCF7) cells. Conclusion: We suggest that PIN1-mediated SGK1 ubiquitination is a major regulator of tamoxifen-resistant breast cancer cell growth and survival.

Approximately 70% of breast tumours express oestrogen receptor α (ERα), and the majority are dependent on oestrogen signaling (1). ERα acts as a hormone-dependent nuclear transcription factor, binding of oestrogen to ERα causes a conformational change within the receptor that allows dimerization and binding to oestrogen-responsive elements located in the promoter region of ERα target genes (2). Since most ERα-positive breast carcinomas are fully-dependent on oestrogen signaling, they can be effectively treated with anti-oestrogens, such as tamoxifen, or aromatase inhibitors that prevent oestrogen synthesis (3). Tamoxifen binds to ERα, blocking the interaction between oestrogen and its receptor (4). This inhibition of ERα leads to cell-cycle arrest at the G1 cell-cycle checkpoint, limiting cellular proliferation (5). Despite its widespread use, the efficacy of tamoxifen is limited by the development of drug resistance (4). All patients with metastatic disease and 40% of patients with early-stage breast cancer treated with adjuvant tamoxifen eventually experience relapse with tamoxifen-resistant disease (6). Several mechanisms have been proposed to explain resistance to endocrine therapy, including down-regulation of ER expression and function (7, 8), increased cellular responses to the partial agonist activities of tamoxifen (9), overexpression of human epidermal growth factor receptor 2 (10), or the transcriptional co-activator SRC1 (11), and stabilization of the interaction between ERα and SRC1 by cyclin D1 (12) and cyclin A-dependent kinase 2 (13). Although these studies are highly informative, the underlying molecular mechanism by which reduction of serum- and glucocorticoid-inducible protein kinase-1 (SGK1) expression during chemo-endocrine treatment occasionally causes tamoxifen resistance remains unclear.

SGK1 was previously cloned as a serum- and glucocorticoid-inducible transcript expressed in rat mammary tumour cells (14). The expression of SGK1, but not of SGK2 or SGK3, is acutely regulated by glucocorticoids and serum (15).
SGK1 is an important regulator of diverse cellular processes including metabolism, proliferation, channel conductance, cell volume, cell survival, and differentiation (16-18). SGK1 is controlled in a phosphoinositide-3-kinase-dependent manner in response to insulin or growth factor signalling (19). Activated SGK1 can phosphorylate glycogen synthase kinase-3β (GSK-3β), v-raf murine sarcoma viral oncogene homolog B (B-RAF), forkhead-like protein-1, neural precursor cell expressed developmentally down-regulated protein-4, p27, and stress-activated protein kinase kinase-1 (19-22). Interestingly, SGK1 negatively regulates B-RAF kinase activity by phosphorylating only a single RAC-alpha serine/threonine-protein kinase (AKT) consensus site, Ser364. SGK1-mediated phosphorylation and inhibition of B-RAF might play a role in the cross-talk between the SGK1 signalling pathway and the RAF-extracellular signal-regulated kinase (ERK) pathway (20). In fatty livers, SGK1 has been identified as a probable negative regulator of hairy and enhancer of split-5 (HES5) gene expression and regulates contextual fear memory formation (23). In addition, SGK1 inhibits the neurogenic locus notch homolog protein-1 (NOTCH1) signaling pathway via phosphorylation of the ubiquitin ligase, F-BOX/WD repeat domain-containing-7 (FBXW7), suggesting that SGK1 might represent a potential molecular target for improving the efficacy of anticancer drugs.

PIN1 is a peptidyl-prolyl isomerase that binds and isomerizes specific phosphorylated serine or threonine residues that precede proline (pSer/Thr-Pro) in certain proteins (24). PIN1-induced conformational changes have profound effects on the function of many substrates by modulating their activity levels, phosphorylation status, protein–protein interactions, sub-cellular localization, and protein stability (25). PIN1 is highly overexpressed in many types of human cancers, and high PIN1 levels correlate with poor prognosis in breast cancer (26, 27). Recently, our group reported that PIN1 facilitated the progression of tamoxifen resistance in breast cancer (28, 29). However, it is not known whether PIN1 regulates the stability of SGK1, thus causing tamoxifen resistance.

Materials and Methods

**Cell culture.** MCF7, HEK293, and PIN1+/+ and PIN1−/− MEF cells, kindly provided by Dr. Kun Ping Lu (Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA), were maintained in Dulbecco’s modified Eagle’s medium with...
10% foetal bovine serum (FBS). To establish the tamoxifen-resistant TAMR-MCF7 cell line, stepwise drug selection was continued until the MCF7 cell population survived and proliferated in the presence of 3 μM tamoxifen. Established TAMR-MCF7 cells were maintained in Dulbecco’s modified Eagle’s medium with 10% charcoal/dextran-treated FBS and 3 μM tamoxifen.

Antibodies and reagents. Antibodies against phospho-RAF1 (P-RAF1; Ser338), phospho-ERK1/2, and ERK1/2 were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). Anti-PIN1, -SGK1, and -RAF1 were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-Xpress and anti-GAL4 antibodies were from Invitrogen (Carlsbad, CA, USA) and Abcam (Cambridge, MA, USA) respectively. Anti-pSer/Thr-Pro antibody.
was from Millipore (Billerica, MA, USA). 4-Hydroxy-tamoxifen (4-OHT), MG132, and cycloheximide were from Sigma-Aldrich (St. Louis, MO, USA).

Construction of mammalian expression vectors and small interfering RNA. The cDNA of PIN1 wild-type, -WW domain, and -peptidylprolyl isomerase domain (PIN1-WT, -WW, and -PPIase respectively), which were a gift from Dr. Kun Ping Lu, were subcloned into pcDNA4-Xpress (Invitrogen, Carlsbad, CA, USA) and pGEX-5X-1 (GE Healthcare Biosciences, Pittsburgh, PA, USA). pcDNA4-Xpress-PIN1-S16A was a gift from Dr. Jeong-Hyun Shim (Chunbuk National University). Human PIN1 (accession number: NM_006221) was silenced by transfecting cells with the ON-TARGETplus siRNA SMART pool-specific double-stranded RNA

Figure 3. Peptidyl-prolyl cis-trans isomerase NIMA-interacting-1 (PIN1) induces ubiquitination of serum- and glucocorticoid-inducible protein kinase-1 (SGK1). A: A mock plasmid and Xpress-PIN1 were transfected into MCF7 cells. After 48 h, the cells were treated with cycloheximide (CHX, 100 μg/ml) for different periods, harvested, and immunoblotted. B: siRNA-control and siRNA-PIN1 were transfected into MCF7 cells. After 48 h, the cells were treated with CHX (100 μg/ml) for different periods, harvested, and immunoblotted. C: MCF7 cells were starved, treated with CHX (100 μg/ml) for 12 h, and then exposed to 10% fetal bovine serum for the indicated times. SGK1 and PIN1 proteins were immunoblotted. D: Mock and pcDNA4/Xpress-PIN1 plasmids were transfected into MCF7 cells. After 48 h, cells were treated with a proteasome inhibitor, MG132 (20 μM) for 24 h, harvested, and immunoblotted. E: pBIND/GAL4-SGK1, pcDNA4/Xpress-PIN1-WT and -PIN1-S16A were co-transfected along with pcDNA/HA-ubiquitin into MCF7 cells. After 48 h, ubiquitinated proteins from whole-cell lysates were immunoprecipitated, and immunoblotting analysis was performed with antibody to GAL4. Whole-cell lysates were immunoblotted with anti-GAL4 and anti-Xpress antibodies against GAL4-SGK1 and Xpress-PIN1 respectively. F: siRNA-control and siRNA-PIN1 were co-transfected with pcDNA/HA-ubiquitin into MCF7 cells. After 48 h, cells were starved for 24 h, exposed to 10% FBS for 4 h, and harvested. Whole-cell lysates were subjected to anti-HA immunoprecipitation followed by anti-SGK1 immunoblotting.
oligonucleotides (Dharmacon, Chicago, IL, USA) using LipofectamineTM 2000 (Invitrogen).

Protein immunoblotting and immunoprecipitation. For immunoblotting, cells grown to 70-80% confluence were harvested in radioimmunoprecipitation assay lysis buffer, disrupted by sonication, and centrifuged at 15,000 × g for 10 min. Protein samples were then subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis and immunoblotting. For immunoprecipitation, cells were harvested in immunoprecipitation buffer and lysed. Equal amounts of protein were subjected to immunoprecipitation followed by immunoblotting analysis.

Glutathione-S-transferase (GST) pull-down assays. For GST pull-down assays, cells were transfected with GAL4-SGK1. Whole-cell lysates were prepared 48 h after transfection and incubated with glutathione-sepharose beads (GE Healthcare Biosciences) containing either GST-PIN1 or GST at 4°C for 4 h. The precipitated proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and expression was detected by immunoblotting.

RNA isolation and real-time polymerase chain reaction (PCR). Total RNA was isolated from cells using the RNeasy Micro KitTM (Qiagen, Valencia, CA, USA). To obtain cDNA, total RNA (1 μg) was reverse-transcribed using an oligo(dT) primer to obtain cDNA. The cDNA was amplified using a high capacity cDNA synthesis kit (Bioneer, Daejon, Korea) in a thermal cycler (Bio-rad, Hercules, CA, USA). Real-time PCR was performed with StepOneTM (Applied Biosystems, Foster City, CA, USA) using a SYBR® Green premix according to the manufacturer’s instructions (Applied Biosystems). The following primer sequences were used: human SGK1: sense: 5'-AGGGCAGTTTTGGAAAGGTT-3', antisense: 5'-CTGTAAAACTTTGACTGCATAGAACA-3'; human PIN1: sense: 5'-AGCAGCAGTTGGTGCAAAAAA-3', antisense: 5'-GGCCAGAGACTCAAGTCCCT-3; and β-actin (Promega Biosciences, San Luis Obispo, CA, USA).

Tumor samples. Patients with breast cancer were selected for immunohistochemical staining and consisted of two groups as follows: the tamoxifen-resistant group (n=9, age range=42-72 years) and the non-tamoxifen-resistant group (n=20, age range=47-58 years). The tamoxifen-resistant group included patients with mammary infiltrating ductal carcinoma who had undergone mastectomy with adjuvant hormone therapy and subsequently had tamoxifen-resistant breast cancer. Data from cell viability, real-time PCR, protein immunoblotting and immunoprecipitation with anti-SGK1, anti-phospho-RAF1, and anti-RAF1. Immunolocalization for each protein was performed using a Polink-2 horseradish peroxidase plus anti-rabbit 3,3'-diaminobenzidine detection kit (Golden Bridge International, Inc., Mukilteo, WA, USA) according to the manufacturer’s protocol. Slides were incubated for 1 h with anti-PIN1 and overnight with anti-SGK1, anti-phospho-RAF1, and anti-RAF1. Instead of the primary antibody, normal goat serum was used in the negative control. Distinct nuclear staining was considered positive immunoreactivity.

Cell proliferation assay (5-bromo-2'-deoxyuridine, BrdU, incorporation). Cells were seeded (5x10^4 cells/well) in 96-well plates in 100 μl of 5% FBS-DMEM. After 24 h, they were treated with 1, 3, and 5 μM of 4-OHT. After 48 h, cells were labelled with 10 μl/well BrdU labelling solution, and then incubated for 4 h at 37°C in an atmosphere with 5% CO2. Cell proliferation was estimated by measuring absorbance at 370 nm. Significant differences were evaluated using the Student’s t-test (*, p<0.05), significant decrease of cell viability in 4-OHT-treated cells compared with DMSO-treated control cells.

Apoptosis analysis. Apoptosis was detected by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labelling (TUNEL) with an in situ Cell Death Detection Kit (Roche Applied Science, Indianapolis, IN, USA) according to the manufacturer’s instructions. For this analysis cells were transfected with the pBIND-GAL4-SGK1 vector (Promega, Madison, USA). After 48 h, the cells were starved, treated with 4-OHT for 24 h, stained with TUNEL solution, incubated at 37°C for 2 h, washed with phosphate-buffered saline, and mounted with crystal mount reagent for 4 h in the dark. The amount of DNA fragmentation was detected using an Axiovert-200M fluorescence microscope (Carl Zeiss Inc., Thornwood, NY, USA). The increase of DNA fragmentation in 4-OHT-treated cells was evaluated compared with DMSO-treated control cells.

Anchorage-independent cell transformation assay. Briefly, cells (8x10^5) were exposed to 5 μM 4-OHT in 1 ml of 0.3% basal medium of Eagle’s agar containing 10% FBS. The cultures were maintained at 37°C in a 5% CO2 incubator for 10-15 days, and the cell colonies were scored using an Axiovert-200M fluorescence microscope.

Statistical analysis. Fisher’s exact test with two-sided values of probability was used to analyse the correlation between PIN1, SGK1, and phospho-RAF1 in tumours from patients with tamoxifen-resistant breast cancer. Data from cell viability, real-time PCR, and soft agar assays were statistically analysed using unpaired t-tests, and p-values less than 0.05 were considered significant.

Results

PIN1 down-regulates the expression of SGK1 protein. To explore the effects of PIN1 on SGK1 protein expression, PIN1+/+ and PIN1−/− MEFs were cultured under normal culture conditions and immunoblotting was performed with antibodies against SGK1 and phosphorylated SGK-1 (P-SGK1). Increased levels of SGK1 as well as P-SGK1 were observed in PIN1−/− MEFs compared to PIN1+/+ MEFs (Figure 1A). To ensure that the increased SGK1 levels in PIN1−/− MEFs were due to loss of PIN1, PIN1−/− MEFs were rescued by enforced expression of PIN1. The results showed that SGK1 and P-SGK1 levels were down-regulated in rescued PIN1−/− MEFs (Figure 1A). To further confirm whether PIN1 expression regulates SGK1 levels, pcDNA4/Xpress and siRNA-PIN1 were transfected into MCF7 cells. PIN1 overexpression resulted in a decrease of SGK1 level in MCF7 cells, whereas PIN1 silencing increased the level of SGK1 (Figure 1B).
Next, different amounts of pcDNA4/Xpress-PIN1 were transfected into MCF7 cells and the cells were incubated for 48 h. The results showed that SGK1 protein levels were gradually down-regulated by increased PIN1 expression (Figure 1C). As previous evidence indicates that the PIN1 WW domain, specifically Ser16, regulates its ability to function as a pSer/Thr-Pro-binding module (30), we asked whether the PIN1 mutant, PIN1-S16A, has a different effect on SGK1 protein expression. The results showed that SGK1 levels decreased in PIN1-WT-transfected MCF7 cells, but not in PIN1-S16A-transfected MCF7 cells (Figure 1D). Given the close correlation between PIN1 and SGK1 protein expression, we analysed SGK1 mRNA levels after overexpression and knockdown of PIN1 in MCF7 cells. Real-time PCR results showed that SGK1 mRNA levels were not affected by overexpression or by knockdown of PIN1 in MCF7 cells (Figure 1E), suggesting that PIN1 may affect the stability of SGK1.

**PIN1 association with SGK1 depends on SGK1 phosphorylation at Ser/Thr-Pro motif.** To examine whether PIN1 physically interacts with SGK1, HEK293 cells were co-transfected with pcDNA/Xpress-PIN1 and pBIND/GAL4-SGK1. Reciprocal immunoprecipitation/immunoblotting using anti-Xpress and anti-GAL4 antibodies showed that exogenously expressed PIN1 interacted with GAL4-SGK1 in vitro (Figure 2A). To determine which region of PIN1 was responsible for its interaction with SGK1, we then performed GST pull-down assays with GST-PIN1-WT, -WW, and -
PPIase recombinant proteins. The results showed that the SGK1-binding site on PIN1 encompasses amino acids 1–44 in the WW domain (Figure 2B). Given that the Ser16 motif of PIN1 is a critical factor for SGK1 expression (Figure 1D), we examined whether PIN1-S16A affects its interaction with SGK1. Immunoprecipitation/immunoblotting analyses revealed that SGK1 binds to PIN1-WT, but not its S16A mutant in vitro (Figure 2C). Since PIN1 is known to target Ser/Thr-Pro motifs (30), we examined whether SGK1 could be phosphorylated at a serine or threonine within a Ser/Thr-Pro motif. Indeed, FBS stimulation induced the phosphorylation of SGK1 at a Ser/Thr-Pro motif, suggesting that the phospho-Ser/Thr-Pro motif in SGK1 may be necessary for PIN1 binding (Figure 2D).

**PIN1 overexpression leads to reduction of SGK1 levels through ubiquitination.** To examine whether SGK1 is appropriately degraded in the presence of PIN1, Xpress-PIN1 was transfected into MCF7 cells, followed by treatment with cycloheximide for different time periods. The results showed that the overexpression of PIN1 enhanced the degradation of SGK1 by cycloheximide treatment in MCF7 cells (Figure 3A). To further examine the effect of PIN1 on SGK1 stability, siRNA-PIN1 was transfected into MCF7 cells, followed by time-dependent treatment with cycloheximide. In contrast to PIN1 overexpression in MCF7 cells, PIN1 silencing attenuated SGK1 degradation by cycloheximide in MCF7 cells (Figure 3B). Next, cycloheximide-treated MCF7 cells were serum-starved and endogenous PIN1 expression was induced by the addition of 10% FBS. In contrast to PIN1 expression, which was increased, SGK1 levels decreased on stimulation with FBS (Figure 3C). We then asked whether the ubiquitin-mediated proteolytic pathway is responsible for SGK1 degradation by comparing protein stability in the absence and presence of a proteasome inhibitor, MG132, with/without transfection of Xpress-PIN1. MG132 strikingly inhibited PIN1-mediated SGK1 degradation, suggesting involvement of the ubiquitin-mediated pathway (Figure 3D). To examine whether PIN1 mediates SGK1...
ubiquitination *in vitro*, PIN1-WT or PIN1-S16A was co-transfected with GAL4-SGK1 and HA-ubiquitin in MCF7 cells. SGK1 was ubiquitinated in the presence of PIN1-WT, but not PIN1-S16A (Figure 3E). Consistent with this, PIN1 silencing in MCF7 cells reduced the ubiquitination of SGK1 induced by FBS and increased its stability (Figure 3F).

*Increased RAF1 activity in TAMR-MCF7 cells is associated with a decrease of SGK1 and increase of PIN1 levels.* To investigate the pathological relevance of the relationship between SGK1 and PIN1 in tamoxifen-resistant breast cancer, we analyzed these proteins in human non-tamoxifen-resistant and human tamoxifen-resistant breast cancer tissue samples. Immunohistochemical staining showed overexpression of PIN1 to be associated with decreased SGK1 expression in human tamoxifen-resistant breast cancer samples (*p* < 0.01, Fisher's exact test; Figure 4A). Comparison of PIN1, SGK1, and P-RAF1 (Ser338) levels in MCF7 and TAMR-MCF7 cells showed decreased SGK1 expression, and increased RAF1 phosphorylation (Ser338) and PIN1 expression in TAMR-MCF7 cells (Figure 4B). Consistent with this result, SGK1 overexpression in TAMR-MCF7 cells reduced the phosphorylation of RAF1 without changing PIN1 levels (Figure 4C), suggesting that the SGK1 signaling pathway may inhibit phosphorylation of RAF1 at Ser338. To further examine whether phosphorylation of RAF1 is dependent on PIN1-regulation of SGK1 levels, siRNA-PIN1 was transfected into TAMR-MCF7 cells. The result showed that PIN1 silencing in TAMR-MCF7 cells increased the level of SGK1 and reduced RAF1 phosphorylation (Figure 4D).

*SGK1 overexpression enhances tamoxifen-induced apoptotic signalling.* Since PIN1 down-regulates SGK1 expression, we assessed whether SGK1 overexpression could potentiate tamoxifen sensitivity in TAMR-MCF7 cells. Treatment with 4-OHT reduced viability of TAMR-MCF7 cells by 23.5%, whereas SGK1 overexpression increased sensitivity to 4-OHT by 52.2% (Figure 5A). We examined the effects of SGK1 overexpression on 4-OHT-induced cell death by poly-ADP ribose polymerase cleavage. Tamoxifen induced higher poly-ADP ribose polymerase cleavage and DNA fragmentation, as measured in a TUNEL assay, after SGK1 overexpression in TAMR-MCF7 cells (Figure 5B and C). Next, we examined whether SGK1 overexpression affects the tamoxifen-induced inhibition of clonogenic growth of TAMR-MCF7 cells. The results showed that SGK1 overexpression in TAMR-MCF7 cells significantly increased their sensitivity to 4-OHT (Figure 5D).

**Discussion**

The phosphorylation of proteins on serine or threonine residues that immediately precede proline resides (Ser/Thr-Pro) is an important signalling mechanism for cell-cycle regulation, transcription, cell differentiation, and proliferation (31, 32). 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 (31). These conformational changes can have profound effects on the function of PIN1 substrates, modulating their activity, phosphorylation status, protein–protein interactions, sub-cellular localization, and stability (33). Herein, we propose a role for PIN1 as a negative regulator of SGK1.

The PIN1 WW domain, specifically Ser16, regulates its ability to function as a pSer/Thr-Pro-binding module (30). Our study indicates that SGK1 interacts with the WW domain of PIN1 and its interaction is dependent on the phosphorylation of SGK1. Our data further suggest that the interaction between SGK1 and PIN1 causes a decrease in the steady-state levels of SGK1. Although SGK1 is unstable in WT MEFs and other cells expressing PIN1, it is quite stable in PIN1−/− MEFs and was further stabilized by the proteasome inhibitor MG132. Furthermore, SGK1 protein levels were reduced by stimulation with FBS, subsequent to an increase in PIN1 level. It was recently reported that SGK1 is regulated primarily by transcriptional induction following various environmental stimuli, as well as by post-translational phosphorylation, ubiquitination, and proteasomal degradation (17). Therefore, the steady-state levels of SGK1 determine its overall kinase activity and the rapid turnover of SGK1 *via* proteasomal degradation plays an important role in limiting SGK1 activity following stress-induced transcription (34). Previous studies have indicated that PIN1 can modulate polyubiquitination and subsequent protein degradation of substrates with a short lifetime, such as Myc, p53, β-catenin, and CDK10 (35). The regulation of SGK1 stability is further supported by our findings that SGK1 is polyubiquitinated after overexpression of PIN1. Although we cannot conclude that this ubiquitination is a direct consequence of the binding of SGK1 to PIN1, our results with mutant PIN1 strongly support this conclusion. When PIN1 was mutated at Ser16, it was unable to bind to SGK1 and promote its polyubiquitination. In addition, PIN1 knockdown suppressed SGK1 polyubiquitination induced by FBS. Taken together, these results suggest that PIN1 may promote ubiquitination-mediated SGK1 degradation through its direct interaction with SGK1.

Epidermal growth factor receptor (*e.g.* HER2) and its downstream RAF1/mitogen-activated protein kinase signalling pathways, which were barely detected in control oestrogen-treated tumours, increased slightly with tamoxifen treatment and were markedly increased when tumours became tamoxifen-resistant (36). Increased RAF1/mitogen-activated protein kinase phosphorylation or activation is associated with early relapse after adjuvant tamoxifen therapy, suggesting that nuclear RAF1 phosphorylation
(Ser338) is a candidate for identifying patients with ER-α-positive breast cancer at risk of relapse when treated with tamoxifen alone (37). This hypothesis contradicts previous findings that increased RAF1 expression makes patients with ER-α-positive breast cancer more responsive to chemotherapeutic agents, similar to the effect of paclitaxel on human breast cancer cells (38). Interestingly, it was reported that an active SGK1 mutant effectively inhibits B-RAF activation in response to serum or RAS by Ser364 phosphorylation. However, the major unanswered question is whether RAF1 is regulated by SGK1 during acquisition of tamoxifen resistance in breast cancer. To answer this question, we investigated the pathological relevance of the relationship between PIN1, SGK1, and P-RAF1 (Ser338) in tamoxifen-resistant breast cancer. We found that SGK1 expression was decreased in TAMR-MCF7 cells and tamoxifen-resistant breast cancer cells, whereas PIN1 expression was increased. However, SGK1 overexpression in TAMR-MCF7 cells reduced the RAF1 phosphorylation. In addition, PIN1 silencing in TAMR-MCF7 cells increased SGK1 expression as well as reducing RAF1 phosphorylation levels, indicating that PIN1-induced SGK1 ubiquitination may mediate increased RAF1 Ser338 phosphorylation, causing tamoxifen resistance in breast cancer.

In the present study, we demonstrated that PIN1 interacts with SGK1 in a phosphorylation-dependent manner and promotes ubiquitin-proteasome degradation of SGK1. In addition, SGK1 down-regulation is associated with RAF-1 phosphorylation in tamoxifen-resistant breast cancer. Based on the above findings, we developed a strategy of overexpressing SGK1 in order to sensitize breast cancer cells to tamoxifen. The results showed that following SGK1 overexpression, TAMR-MCF7 cells were sensitized to tamoxifen. In conclusion, this newly-identified mechanism provides the scientific basis for a viable strategy to sensitize therapy-resistant breast cancer.

Conflicts of Interest

The Authors declare no conflicts of interest.

Acknowledgements

This work was supported by research funds from Chosun University (2011).

References


