약학석사 학위청구논문

Polo-like Kinase 1: 타목시펜 저항성 유방암 치료의 신규 약물타겟

Polo-like Kinase 1: a novel target for tamoxifen-resistant breast cancer therapy

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초록

Polo-like Kinase 1: 타목시펜 저항성 유방암 치료의 신규 약물타겟

유방암은 여성에게서 가장 많이 발견되는 암 중 하나로 주로 폐경기 여성에게서 진단된다. Luminal subtype로 분류되는 에스트로겐 수용체 (estrogen receptor, ER) 양성 유방암의 대표적인 치료제는 타목시펜 (tamoxifen) 이다. 그러나 유방암 환자는 선천적 또는 타목시펜을 장기간 투여 시 타목시펜에 대한 항암제 저항성을 가지게 되는데, 현재까지 명확한 치료법이 발견되지 않은 실정이다. 최근 문헌들에 의하면 많은 항암제 저항성 암종에서 Plk1 (Polo-like kinase 1) 이 높은 발현을 나타낸다고 밝혀졌다. Plk1은 세포증식 및 분열을 조절하는 핵심적인 kinase 이다. 본 논문에서는 대조 유방암 세포 (MCF-7) 에 비하여 타목시펜 저항성 유방암 세포 (TAMR-MCF-7) 에서 Plk1이 과발현 됨을 확인하였다. 본 논문에서는 대표적 Plk1 억제제인 BI 2536 (Plk1 활성 억제제) 을 사용하여 세포실험과 이종이식 동물실험에서 암 진행(progression) 억제를 최초로 규명하였다. 최근 문헌들에 의하면 암 세포의 항암제 저항성 획득은 E-cadherin의 억제, N-cadherin, vimentin, snail 등의 발현증가로 대별되는 epithelial-mesenchymal transition (EMT)

현상과 관련이 있으며, 이는 암의 침식 (invasion), 이동 (migration), 전이 (metastasis) 등을 유도하는 것으로 알려져 있다. 세포 이동능 평가 (transwell migration assay) 시험에서 Plk1 저해제인 BI 2536을 타목시펜 저항성 유방암 세포에 처치 하였을 때, 세포 이동능이 현저하게 감소하였으며, 세포침식인자인 vimentin의 감소를 확인하였다. N-cadherin 및 snail은 대표적인 상피-중간엽 이행 유도인자로 알려져 있다. BI 2536을 타목시펜 저항성 유방암세포에 처치 후 N-cadherin, E-cadherin, snail 단백질 양을 정량한 결과, Plk1 억제로 인하여 N-cadherin, snail 단백질이 감소하고 E-cadherin이 부분적으로 복원되는 것을 관찰하였다. 결론적으로 타목시펜 저항성 유방암 세포에서 Plk1 저해제는 암의 침식, 이동을 억제하고 E-cadherin 복원을 유도하여 상피-중간엽 이행을 역전 시켰다. 상기결과를 통하여 본 연구에서는 Plk1이 타목시펜 저항성 유방암 치료의 신규 약물타겟으로 작동함을 제시한다.

주요어 : Tamoxifen-resistant breast cancer (TAMR-MCF-7), Polo-like kinase (Plk1), Epithelial-mesenchymal transition (EMT), E-cadherin, N-cadherin, Snail, Vimentin, Estrogen receptor (ER), BI (Boehringer Ingelheim)

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List of Abbreviations

ATP	Adenosine triphosphate
BI	Boehringer Ingelheim
CD	Cluster of differentiation
EMT	Epithelial mesenchymal transition
ER	Estrogen receptor
FBS	Fetal bovine serum
mRNA	Messenger RNA
MTT	3-(4,5-dimethylthiazole-2yl)-2,5-diphenyltetrazolium bromide
PBS	Phosphate-buffered saline
Plk1	Polo-like kinase 1
PR	Progesterone receptor
SDS	Sodium dodecylsulfate
SERM	Selective estrogen receptor modulator
TAM	Tamoxifen
TAMR	Tamoxifen-resistant
TNBC	Triple-negative breast cancer
TIC	Tumor initiating cell
MTT	3-(4,5-dimethylthiazole-2yl)-2,5-diphenyltetrazolium bromide

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I. Introduction

One of the most occurring cancers in women is breast cancer. Breast cancer is mostly diagnosed from women in post-menopause (Desantis et al., 2013). Approximately 70-75% of breast cancers are luminal subtypes, which express estrogen receptor (ER) -α. This type of breast cancers depend its growth and survival on estrogen signaling. For many years, endocrine-therapy has been used as a standard strategy to treat luminal subtype breast cancers (Jordan et al., 2007). Tamoxifen, known as a selective estrogen receptor modulator (SERM), selectively blocks estrogen signaling via targeting the estrogen receptor in luminal subtype breast cancer cells (Suleiman et al., 2006). Unfortunately, approximately one-third of patients acquire tamoxifen-resistance at the beginning of the treatment and later, patients who showed initial response acquire the resistance as well (Murphy et al., 2011). Understanding the mechanism of resistance acquisition may uncover potential therapeutic targets for tamoxifen-resistant breast cancer.

Polo-like kinase 1 (Plk1), a serine/threonine protein kinase, is a regulator of completion of cell cycle (Barr et al., 2004; Takaki et al., 2008). Plk1 inhibition has been reported to induce mitotic arrest. Consequently, Plk1 inhibition leads decrease in cell growth and increase in apoptosis, suggesting a promising target for cancer therapy (Driscoll et al., 2014). Several reports suggest that Plk1 is a possible target for the treatment of doxorubicin-resistant prostate cancer (Feng et al., 2012; Nakouzi et al.,

2014). Moreover, recent reports revealed that Plk1 inhibition enhances the efficacy of androgen signaling inhibition in castration-resistant prostate cancer (Zhang et al., 2014). However, potential role of Plk1 in tamoxifen-resistant breast cancer has not been covered. We previously generated tamoxifen-resistant breast cancer (TAMR-MCF-7) cell line from ER-positive MCF-7 cells after long-term treatment of 4-hydroxytamoxifen (Choi et al., 2007; Kim et al., 2009). Here, we observed for the first time that Plk1 was up-regulated in TAMR-MCF-7 cells compared to the parental MCF-7 cells, and Plk1 inhibition potently suppressed cell proliferation and tumor growth of TAMR-MCF-7 cells. Tamoxifen-resistance breast cancer cells acquire motile and invasive properties (Min et al., 2012). We further revealed that Plk1 inhibition partially recovered epithelial-mesenchymal transition (EMT) phenotypes of TAMR-MCF-7 cells and efficiently inhibited the cell migration.

II. Material and Methods

1. Reagent and antibodies

Antibodies of Plk1, cdc25c, anti-rabbit IgG HRP-linked, anti-mouse IgG HRP-linked were purchased from Cell Signaling (MA, USA). Antibodies of cyclin B1, vimentin, bovine anti-goat IgG-HRP were purchased from Santa Cruz Biotechnology (CA, USA). Antibodies of N-cadherin, E-cadherin were purchased from BD Biosciences (CA, USA). Antibody of snail was purchased from abcam (MA, USA) and anti-beta-actin antibody was purchased from Sigma (St. Louis, MO). Hematoxylin and eosin were purchased from Sigma (St. Louis, MO), and BI 2536 was purchased from Medchem express (NJ, USA).

2. Cell Culture

MCF-7 cells were cultured in DMEM (Thermo scientific, IL, USA) containing 10% FBS (Hyclone, UT, USA) and 1% penicillin/streptomycin solution (Thermo scientific, IL, USA). TAMR-MCF-7 cells were generated according to previously reported methods (Phuong et al., 2011; Knowden et al., 2003; Choi et al., 2007) and cultured in DMEM containing 10% charcoal stripped FBS (Gemini bioproduct, CA, USA), 1% penicillin/streptomycin solution (Thermo scientific, IL, USA) and 3 μM of 4-hydroxytamoxifen (Sigma-aldrich, MO, USA).

3. Immunoblot analysis

Cells were lysed in lysis buffer (137mM NaCl, 20 mM Tris-Cl pH 7.5, 1% Triton X-100, 10% glycerol, 2 mM EDTA, 1 mM sodium orthovanadate, 25 mM β-glycerolphosphate, 2 mM sodium inorganic pyrophosphate, 1 mM phenylmethylsulfonylfluoride, and 1 μg/ml leupeptin). Cell lysates were transferred electrophoretically to 8-12% gradient sodium dodecylsulfate-polyacrylamide (SDS) gel with multiple gel casters (Hoefer, Inc., CA, USA). Then, it was transferred to nitrocellulose paper with transfer buffer (25mM Tris, 192mM glycine, 20% v/v methanol, pH 8.3), immunoblotted with primary and secondary antibodies and detected with LAS-3000 (Fujifilm, OH, USA), enhanced by HRP-substrate luminol reagent and HRP-substrate peroxide solution (Millipore, MA, USA).

4. Human samples and immunohistochemistry

Human tumor samples of tamoxifen-responsive and tamoxifen-nonrepsonsive patients were received from Dr. Lim SC (Chosun University, Gwangju). Blocks for all the samples were consecutively cut in 4 μm sections and mounted on poly-l-lysine coated glass slides. Xylene was used to remove the paraffin from the sections, and the samples were rehydrated. Antigen retrieval was performed by boiling sections for 5 min in 1 μM sodium citrate buffer (pH 6.0) in a microwave oven. Endogenous peroxidase activity

was blocked using 3% hydrogen peroxide in methanol for 10 min, followed by three times washing with PBS. Sections were then incubated overnight with anti-aromatase antibody (1:200, Abcam, Cambridge, UK) at 4℃. After washing with PBS, sections were incubated with HRP-conjugated anti-rabbit IgG for 30 min and washed with PBS. The color was developed by incubation with DAB solution. Finally, sections were counterstained with hematoxylin, dehydrated, mounted, and observed.

5. Cell proliferation

Cells were seeded 5 x 10^3 cells/ml each well in 96 well-plates and incubated for 6 h before BI 2536 treatment. After 2 h MTT reagent incubation, DMSO was treated. Then cell proliferation was assessed using 570 nm absorbance was measure with Berthold Tristar LB 941 (Berthold Technologies, Bad Wild, Germany)

6. Invasion assay

Cell invasion was measured via transwell migration assay. The number of cell invaded (seeded 2x10^4) was measured after 24 h incubation. Filters were removed, formalin-fixed for 20 min, incubated in methanol for 1min, stained with hematoxylin for 10 min and 4 min with eosin. With 400X magnification, migrated cells to the lower filter side were analyzed.

7. Xenograft assay

Six week old BALB/c athymic nude mice (Raon Bio Inc., Seoul, Korea) were inoculated subcutaneously with 4×10^6 TAMR-MCF-7 cells. When tumors reached ~200 mm^3 (about 14 days), the mice were randomly allocated to control and BI 2536 treated groups. BI 2536 (Medchem express, NJ, USA), 10 mg/kg and 20 mg/kg) were intraperitoneally injected twice a week. Tumor volumes were measured as described previously (Ahn et al., 2010). Animal care was maintained in accordance with Seoul National University institutional guidelines.

8. GEO dataset cluster analysis

Gene expression in breast cancer patients (n= 49) was analyzed from database (http:www.oncomine.com)

9. Statistical analysis

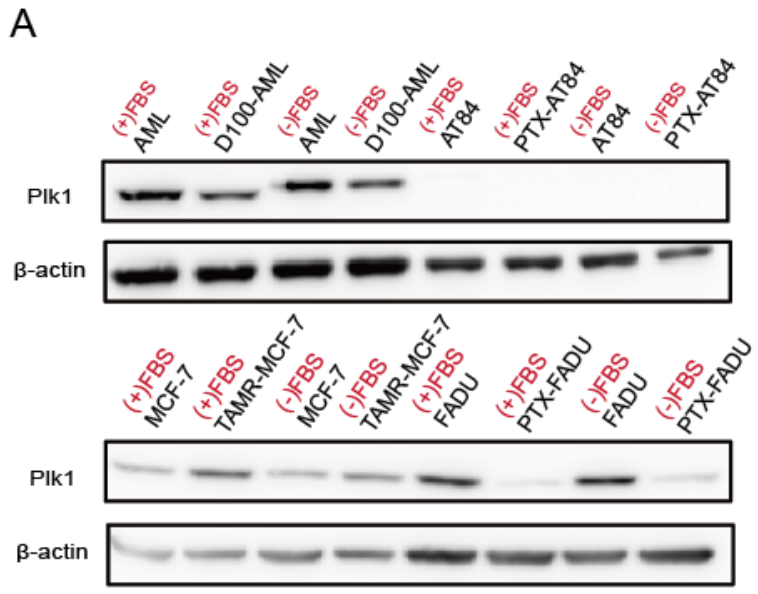
Immunoblot analysis was quantified by using LAS-3000mini (Fujifilm, Tokyo, Japan). Density of band area was measured after excluding background. The group differences were examined through student's t-test. Standard of statistical significance was set at either $P < 0.05$ or $P < 0.01$.

III. Results

1. Plk1 up-regulation in tamoxifen-resistant breast cancer cells

Previously, Plk1 overexpression in androgen−insensitive prostate cancer cells and gemcitabine-resistant pancreatic cancer cell has been reported (Deeraksa et al., 2013; Song et al., 2013). We first assessed Plk1 expression levels in four sets of chemo-resistant cancer cell lines (doxorubicin-resistant acute myeloid leukemia, paclitaxel-resistant oral squamouse cancer, paclitaxel-resistant hypopharyngeal cancer and tamoxifen-resistant breast cancer) compared to their parent cells. Western blot analyses showed that Plk1 was only overexpressed at either serum-containing condition or serum-free condition in TAM-resistant MCF-7 cells compared to control MCF-7 cells (Fig. 1A). To confirm the result, tumor tissues were obtained from two groups of patients, which differed in terms of the occurrence of relapse after TAM therapy. Four cases included in “TAM-responsive group” experienced no recurrence for at least 6 years of follow-up after mastectomy with adjuvant TAM therapy. The other four cases in “TAM-non responsive group” relapsed within 3 to 4 years after mastectomy with adjuvant TAM therapy. Immunohistochemistry with Plk1 antibody showed that Plk1-positive cells were only detected in tumor tissues from TAM-non responsive patients (Fig. 1B). We further analyzed Plk1 gene expression in apocrine breast cancer, basal-like subtype invasive breast cancer, and luminal-like subtype invasive breast cancer from 49 patients through GEO analysis. The data showed Plk1

expression was increased in basal-like subtype invasive subtype invasive breast cancer compared to apocrine breast cancer and luminal-like subtype invasive breast cancer, suggesting Plk1 expression is elevated in non-luminal like breast cancer compared to luminal-like subtype breast cancer (Fig. 1C). We then checked expression of Plk1 downstream target proteins, cdc25c (M-phase cycle protein) and cyclin B1 (G2/M-phase protein) (Fig. 1D) (Barr et al., 2004; Lee et al., 1995). In comparison to MCF-7 cells, significant increases in protein expressions of Plk1 (1.50 fold), cdc25c (1.58 fold), and cyclin B1 (5.43old) were seen in TAMR-MCF-7 cells (Fig. 1E). These results demonstrate that Plk1 up-regulation is closely related with the acquisition of TAM-resistance in ER-positive breast cancer.


A
(+)FBS AML
(+)FBS D100-AML
(-)FBS AML
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Plk1
β-actin
(+)FBS MCF-7
(+)FBS TAMR-MCF-7
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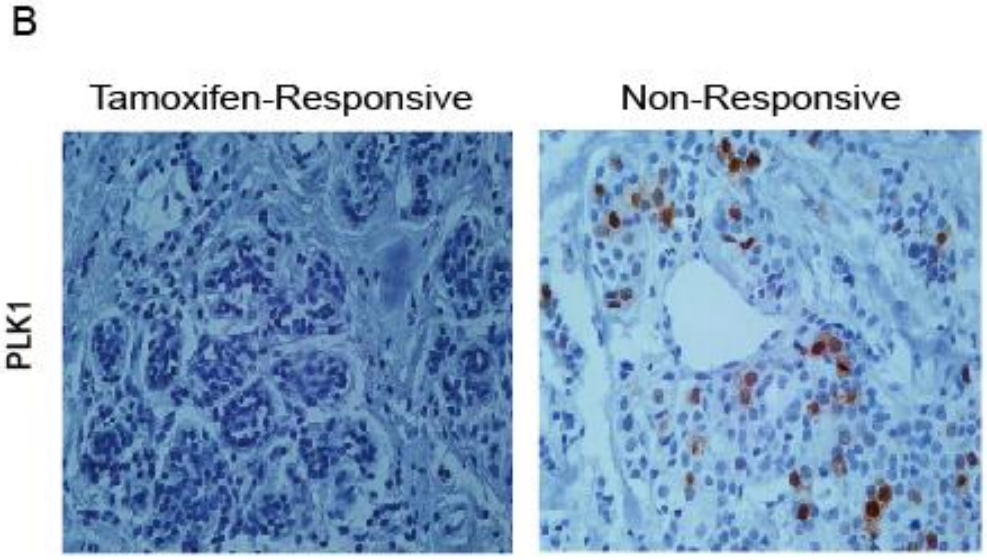
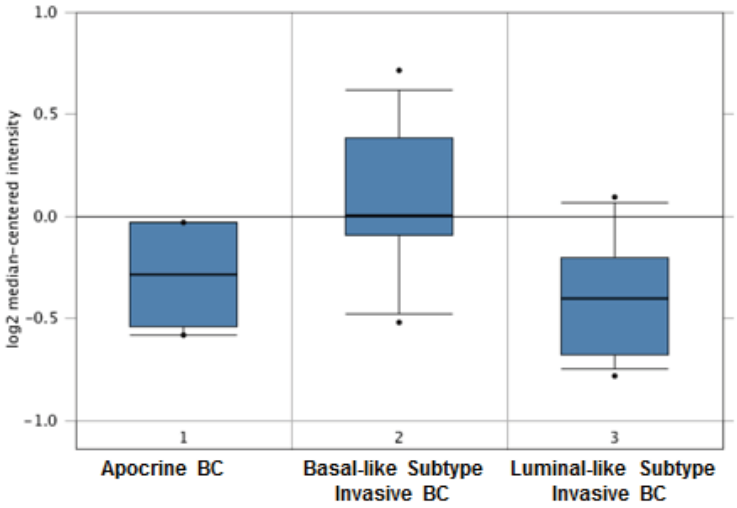
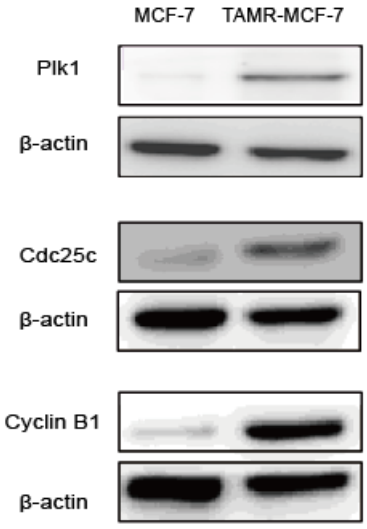
Plk1
β-actin


B
Tamoxifen-Responsive
Non-Responsive
PLK1

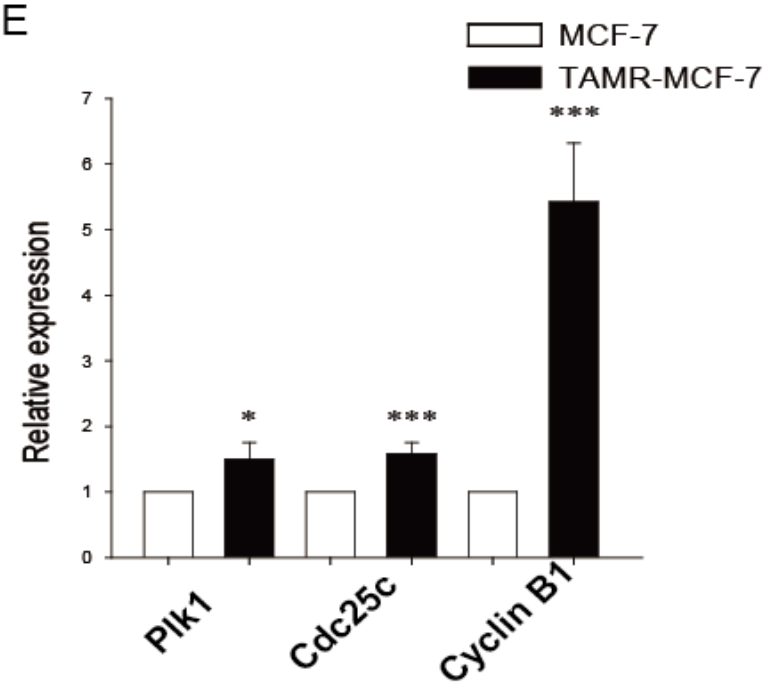
C


1.0
0.5
0.0
-0.5
-1.0
log2 median-centered intensity
1
2
3
Apocrine BC
Basal-like Subtype Invasive BC
Luminal-like Subtype Invasive BC

D


MCF-7
TAMR-MCF-7
Plk1
β-actin
Cdc25c
β-actin
Cyclin B1
β-actin

E


MCF-7
TAMR-MCF-7
Relative expression
0
1
2
3
4
5
6
7
*
***
***
Plk1
Cdc25c
Cyclin B1

1. **Figure 1. Plk1 up-regulation in tamoxifen-resistant breast cancer cells**

(A) Protein expression of Plk1 was measured in docetaxel-resistant acute myeloid leukemia, paclitaxel-resistant oral squamouse cancer, tamoxifen-resistant breast cancer and paclitaxel-resistant hypopharyngeal cancer compared to their parent cells via western blot analysis. Data were confirmed by two repeated experiments.

(B) Immunohistochemistry was performed in order to compare Plk1 expression in human sample of tamoxifen-responsive patients and tamoxifen-non responsive patients.

(C) Plk1 gene expression was measured through GEO analysis in apocrine breast cancer, basal-like subtype invasive breast cancer, and luminal-like subtype invasive breast cancer in patients (n= 49).

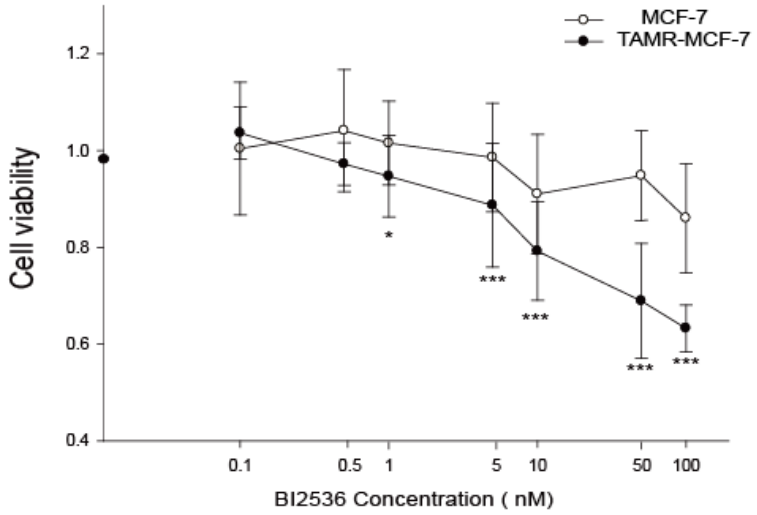
(D) Plk1, cdc25c and cyclin B1 expression was compared in tamoxifen-resistant MCF7 and the parent cell.

(E) Relative Plk1, cdc25c and cyclin B1 levels (normalized by beta-actin) from data (C) were determined by scanning densitometry. Plk1, cdc25c and cyclin B1 were normalized against the expressions in MCF-7. (mean±SD with 3 different samples experiments).

2. Effect of Plk1 inhibitor on cell proliferation TAMR-MCF-7 cells

We then tested if TAMR-MCF-7 cells are more sensitive to cell proliferation inhibition by BI 2536, an ATP-competitive Plk1 activity inhibitor. Both MCF-7 and TAMR-MCF-7 cells were cultured in 5% FBS-containing condition. BI 2536 was treated (0.1, 0.5, 1, 5, 10, 50 and 100 nM) for 24 h. Significant cell proliferation inhibition was found at 1, 5, 10, 50 and 100 nM BI 2536 treatment samples in TAMR-MCF-7 cells. Overall, inhibitory effect of BI 2536 on cell proliferation is more sensitive in tamoxifen-resistant MCF7 than the parent MCF-7 (Fig. 2A). Plk1 inhibition induces decrease in cdc25c expression and increase in cyclin B1 expression (Gumireddy et al., 2005). We analyzed protein expressions of cdc25c (M-phase cycle protein), and cyclin B1 (G2/M-phase protein) by Western blot analyses after incubation with 1 nM BI 2536 for 24 or 48 h, respectively. BI 2536 treatment reduced cdc25c protein expression, and increased in cyclin B1 expression, suggesting Plk1 inhibition induced G2/M arrest (Fig. 2B). These findings demonstrate that Plk1 inhibition reduced cell growth presumably through G2/M arrest.

A


MCF-7
TAMR-MCF-7
Cell viability
1.2
1.0
0.8
0.6
0.4
0.1
0.5
1
5
10
50
100
BI2536 Concentration (nM)
*
***
***
***
***

B

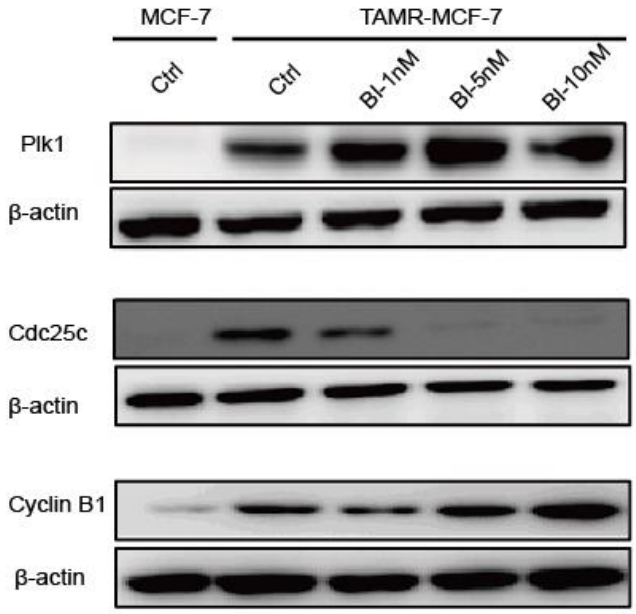

MCF-7
TAMR-MCF-7
Ctrl
Ctrl
BI-1nM
BI-5nM
BI-10nM
Plk1
β-actin
Cdc25c
β-actin
Cyclin B1
β-actin

Figure 2. Effect of Plk1 inhibitor on cell proliferation TAMR-MCF-7 cells

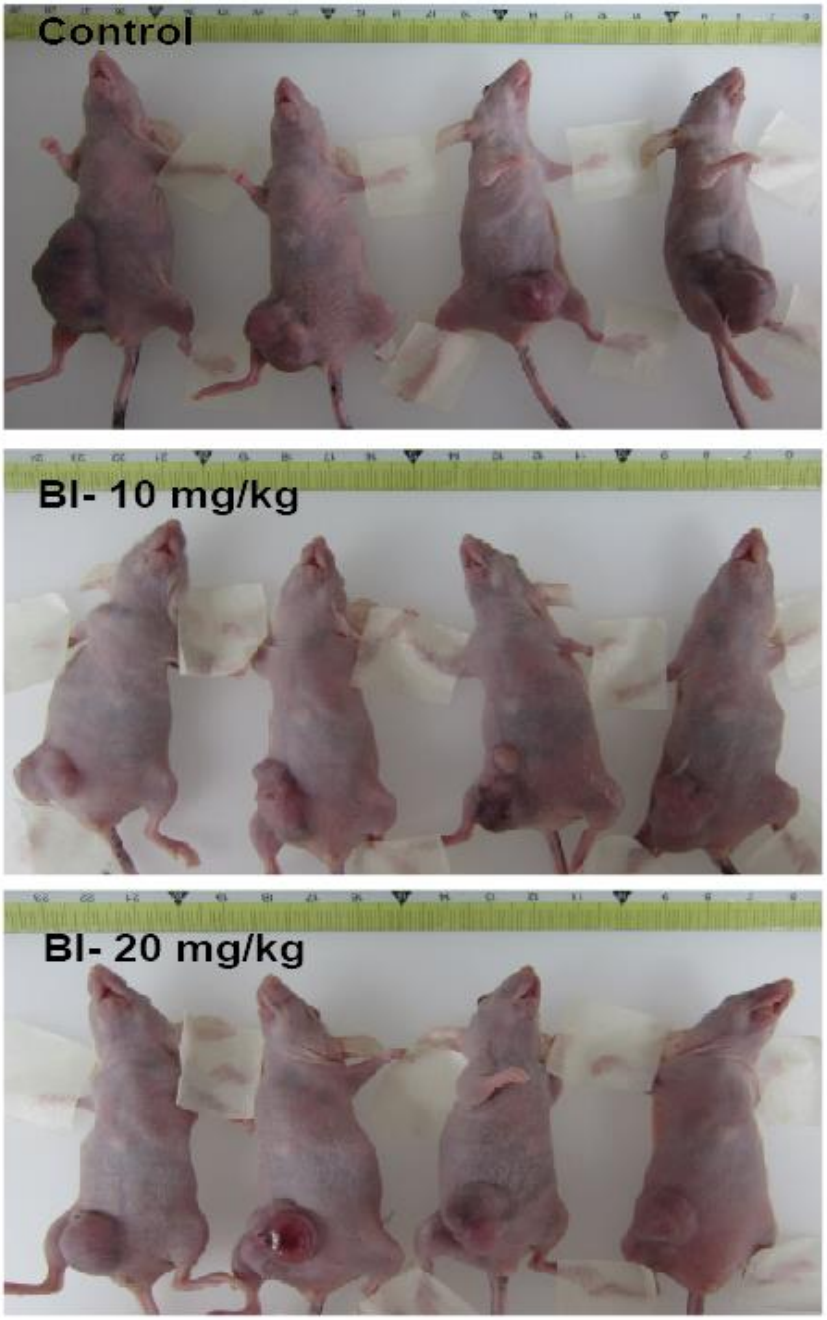
(A) Effect of BI 2536 on cell proliferation of both MCF-7 and TAMR-MCF-7 cells in 5% FBS-containing media. Cell proliferation was determined by MTT assay. BI 2536 was treated (0.1, 0.5, 1, 5, 10, 50 and 100 nM) for 24 h. Results from 8 samples represent mean±SD.

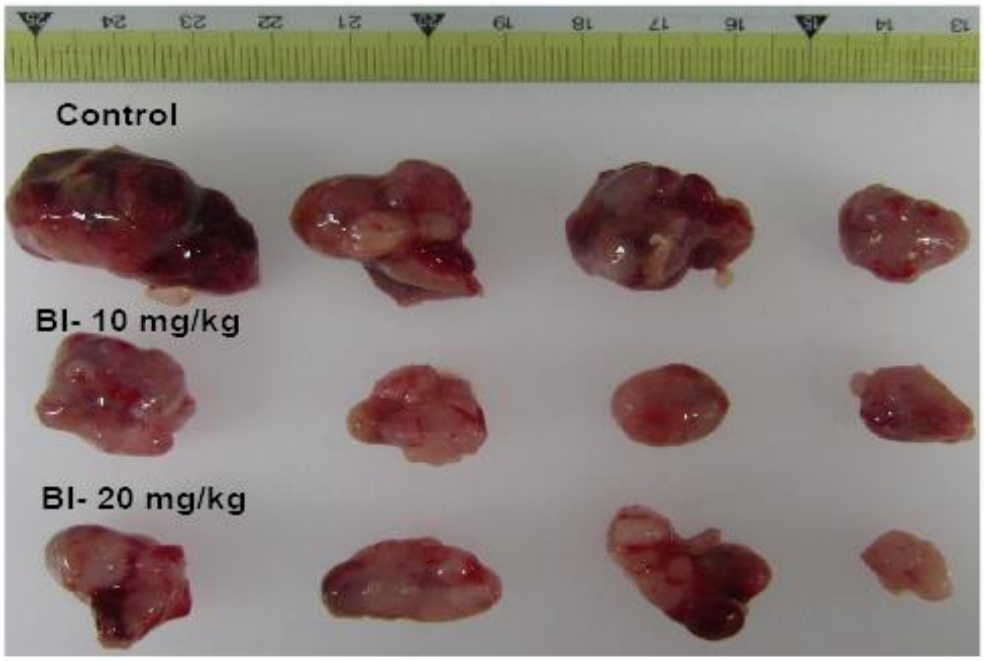
(B) Plk1 inhibition-mediated down-regulation of cdc25c and up-regulation of cyclin B1 in TAMR-MCF-7. BI 2536 (1, 5 and 10 nM) was treated for either 24 h or 48 h.

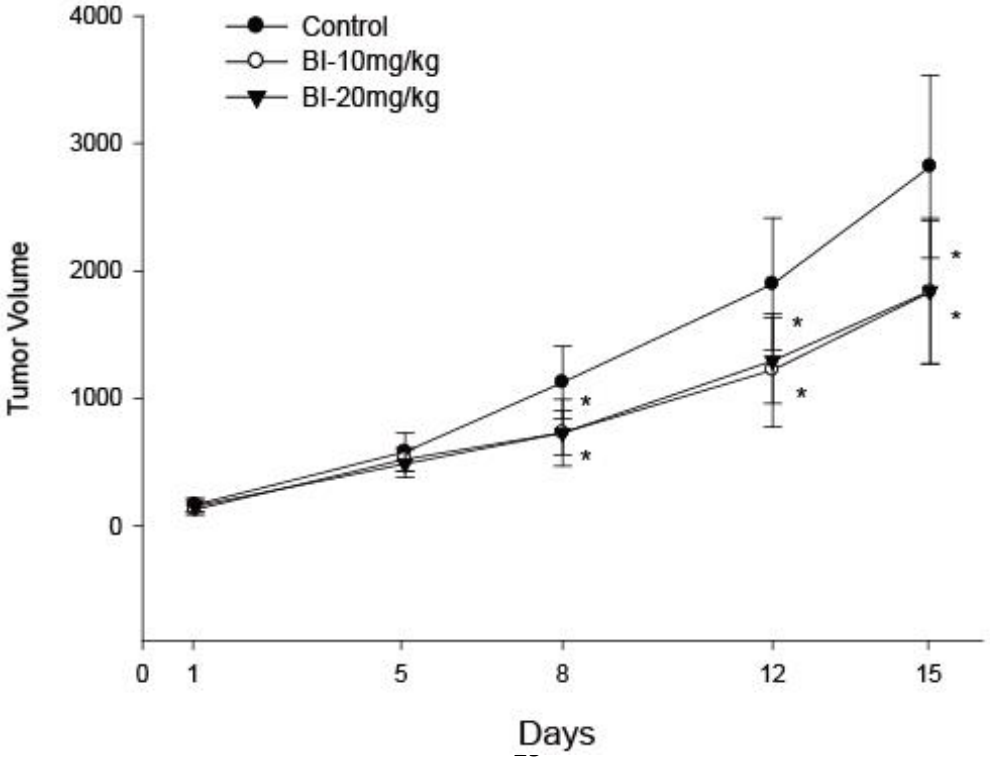
3. Effect of Plk1 inhibitor on tumor growth in TAMR-MCF-7-implanted xenograft

To determine the *in vivo* effect of BI 2536, athymic nude mice bearing TAMR-MCF-7 were established. 10 or 20 mg/kg BI 2536 was intraperitoneally injected twice a week (Maire et al., 2012). Tumor volume and mouse body weight were measured on the same day of BI 2536 treatment. Significant tumor growth inhibition was found at both the treatment groups (10 and 20 mg/kg). Either BI 2536 10 or 20 mg/kg showed significant tumor growth inhibition effect from 8 days of the injection. After 19 days of the injection, mice were sacrificed. Tumor volume was gradually decreased in both 10 and 20 mg/kg BI 2536-treated groups compared to the control group (Fig. 3A). BI 2536 did not affect any body weight loss (Fig. 3B). Tumor weight was also significantly reduced by BI 2536 treatment (Fig. 3C).

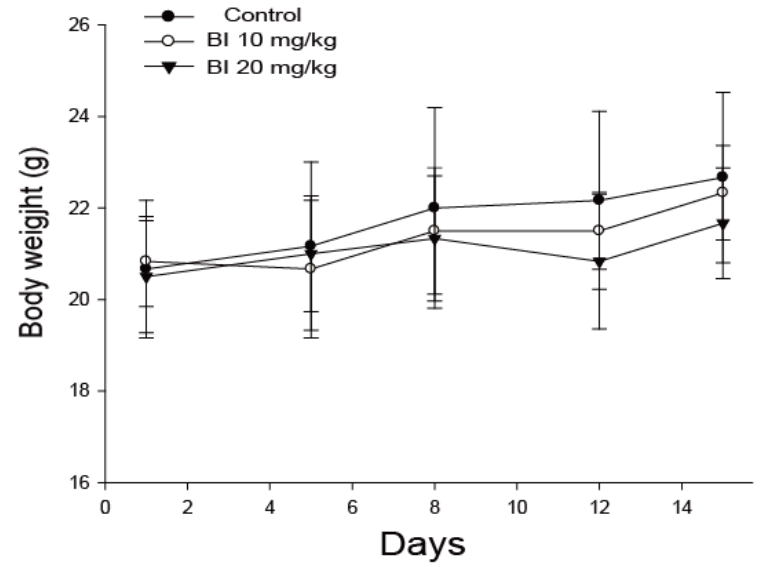
A




Control
BI- 10 mg/kg
BI- 20 mg/kg


Control
BI-10mg/kg
BI-20mg/kg
Tumor Volume
4000
3000
2000
1000
0
0
1
5
8
12
15
Days
*
*
*
*
*

B


Control
BI 10 mg/kg
BI 20 mg/kg
Body weight (g)
26
24
22
20
18
16
0
2
4
6
8
10
12
14
Days

C

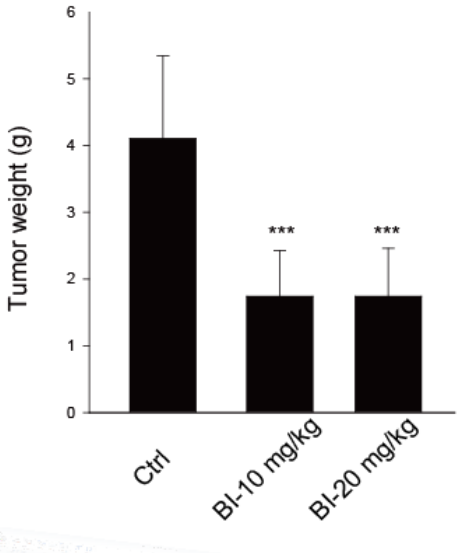

Tumor weight (g)
6
5
4
3
2
1
0
***
***
Ctrl
BI-10 mg/kg
BI-20 mg/kg

Figure 3. Effect of Plk1 inhibitor on tumor growth in TAMR-MCF-7-implanted xenografts

(A) TAMR-MCF-7 cells ($4x10^6$/mouse) were flank-inoculated. After 20 days, BI 2536 was administrated alone either at 10mg/kg or 20mg/kg intraperitoneally to mice (n = 5). Tumor volume was measured with calipers.

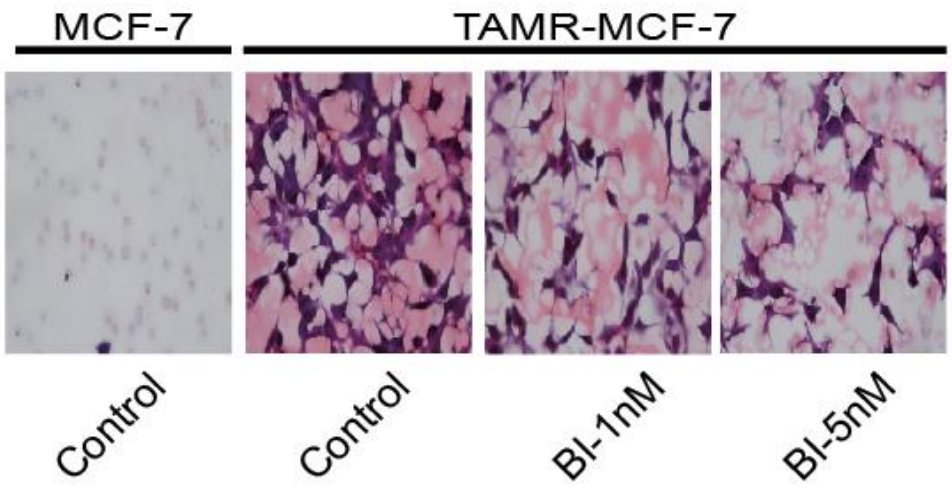
(B) Body weight was measured every time when BI 2536 was treated.

(C) Tumor weight was measured at the day of sacrifice (19 days).

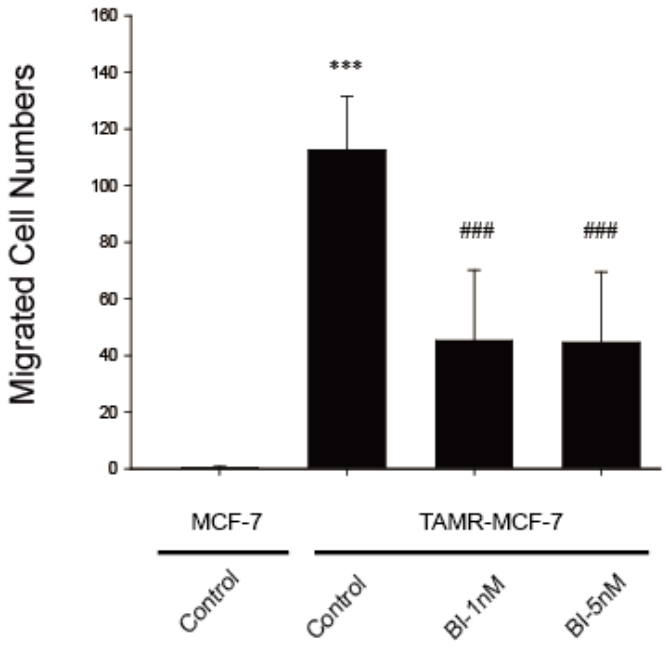
4. Migration inhibition and epithelial-mesenchymal transition rescue by Plk1 inhibition in TAMR-MCF-7 cells

Tamoxifen-resistant breast cancer cells acquire motile and invasive properties (Min et al., 2012). Our previous study demonstrated that chemo-resistance and higher invasion phenotypes are associated with epithelial mesenchymal transition (EMT) induction in TAMR-MCF-7 cells (Kim et al., 2009). Suppression of E-cadherin and up-regulation of vimentin, N-cadherin and snail are representative markers for EMT phenotype change in epithelial cells (Theiry et al., 2009). It has been also shown that metastasis of breast cancer into the brain is prevented by Plk1 inhibition (Qian et al., 2011). When we compared cell migration of MCF-7 and TAMR-MCF-7 cells, only TAMR-MCF-7 cells showed higher invasiveness in transwell migration analysis (Fig. 1A and 1B). The enhanced cell migration was potently inhibited by 1 or 5 nM BI 2536. Moreover, incubation of TAMR-MCF-7 cells with the Plk1 inhibitor for 48 h caused the decreased protein expression of vimentin, N-cadherin and snail in TAMR-MCF-7 cells (Fig. 4C). Loss of E-cadherin in TAMR-MCF-7 cells was partially recovered by 48 h incubation of 1 nM BI 2536 (Fig. 4D). The data implies that Plk1 inhibition efficiently suppresses cancer cell migration as well as tumor growth in TAM-resistant breast cancer cells.

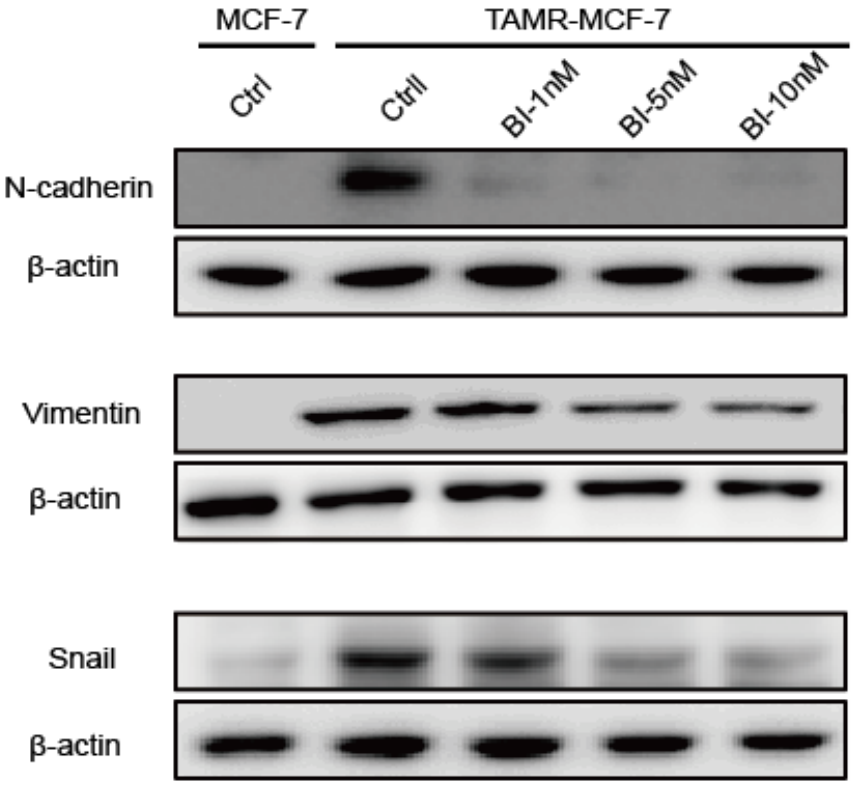
A


MCF-7
TAMR-MCF-7
Control
Control
BI-1nM
BI-5nM

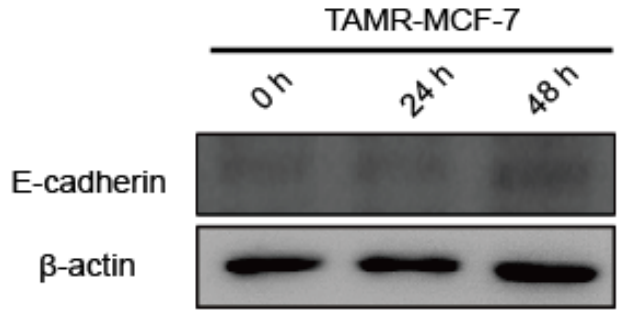
B


Migrated Cell Numbers
160
140
120
100
80
60
40
20
0
***
###
###
MCF-7
TAMR-MCF-7
Control
Control
BI-1nM
BI-5nM

C


MCF-7
TAMR-MCF-7
Ctrl
Ctrl
BI-1nM
BI-5nM
BI-10nM
N-cadherin
β-actin
Vimentin
β-actin
Snail
β-actin

D


TAMR-MCF-7
0 h
24 h
48 h
E-cadherin
β-actin

5. **Figure 4. Migration inhibition and epithelial-mesenchymal transition rescue by Plk1 inhibition**

(A) Transwell migration assay was performed to measure cell migration. BI 2536 either 1 nM or 5 nM was treated for 24 h in TAMR-MCF-7. Hematoxylin and eosin were used to stain cells. Images were taken from microscopic view.

(B) TAMR-MCF-7 cell migration was significantly increased compared to the parent MCF-7. TAMR-MCF-7 cell migration was significantly reduced in both BI 2536 1 nM and 5 nM treatment. Data represents mean±SD of eight field images ($P<0.005$).

(C) Plk1 inhibition-mediated downregulation of vimentin, N-cadherin and snail in TAMR-MCF-7. BI 2536 (1, 5 and 10 nM) was treated for 48 h

(D) Plk1 inhibition induced rescue in E-cadherin. BI 2536 was 1 nM treated in either 24 h or 48 h.

IV. Discussion

Approximately 70% of breast cancers are luminal subtypes. These breast cancer subtypes are estrogen receptor (ER) positive (Jordan et al., 2007). Tamoxifen, a selective estrogen receptor modulator (SERM) has been used for several decades to treat these types of breast cancer patients (Suleiman et al., 2006). Unfortunately, most of ER-positive breast cancer patients with five years of tamoxifen treatment acquire resistance (Schiff et al., 2003). Therefore, understanding the mechanism of tamoxifen-resistance in breast cancer is crucial.

Previous studies have reported that Plk1 is overexpressed in chemo-resistant prostate and pancreatic cancer cells and the chemo-resistances were overcome by BI 2536, a dihydroteridinone Plk1 inhibitor (Deeraksa et al., 2013; Song et al., 2013). The present study found for the first time that Plk1, along with M-phase cell cycle proteins, were up-regulated during tamoxifen-resistance acquisition in ER-positive MCF-7 cells. Our results showed nanomolar ranges of BI 2536 resulted in a marked inhibition of cell proliferation of TAMR-MCF-7 cells, whereas parent MCF-7 cells marginally respond to BI 2536. The results demonstrate that the up-regulated Plk1 in TAMR-MCF-7 cells is critical for the exaggerated cell growth of the cell type.

TAMR-MCF-7 cells show higher motile and invasive properties compared to MCF-7 cells (Fig. 4A). During the invasion of epithelial types of cancer cells to the adjacent tissues, a process involving the loss of cell-cell adhesions and the acquisition of migratory capabilities is essential. Epithelial-mesenchymal transition (EMT) is involved

in the efficient invasion and motility of cancer cells, because migration of single cancer cells is elicited by using either mesenchymal or amoeboid phenotypes (Friedl et al., 2011). The invasive and metastatic phenotype is associated with down-regulation of E-cadherin expression and the E-cadherin-EMT pathway is a crucial signaling in breast cancers, as it regulates tumor progression, invasion, migration and metastasis (Onder et al., 2008; Wheelock et al., 2008). Our study showed that Plk1 inhibition suppressed the expression of typical mesenchymal markers (vimentin, N-cadherin and snail) and partially rescued E-cadherin (Fig. 4C; D). Although the precise signaling pathway is not clarified in this study, Plk1 seems to be required for the EMT progress in TAMR-MCF-7 cells.

Triple-negative breast cancer (TNBC) is an ER/PR (Progesterone receptor)/Her2 negative breast cancer. Such breast cancers characterized as poor prognosis and high rate of relapse, similar to tamoxifen-resistant MCF-7 cells that our lab generated (Metzger-Filho et al., 2012; Liedtke et al., 2008). Chemo-resistant breast cancers display EMT, invasive properties, high fraction of CD 44+/CD 24- cells and stem cell-like phenotype (Dean et al., 2005; Turley et al., 2008; Wicha et al., 2006). These breast cancer cells are known as tumor-initiating cells (TICs). TICs are resistant to chemotherapy and cause reoccurrence, because of their self-renewal properties (Wicha et al., 2008; Charafe-Jauffret et al., 2009; Zhou et al., 2009; Sajithlal et al., 2010). CD 44+/CD 24- cells lack ER-α expression. A previous study reported that pharmacological inhibition of Plk1 suppressed cell proliferation of TICs formed by the exposure of

chemotherapeutic agents (taxol, doxorubicin, 5-fluorouracil) in SUM-149, a TNBC cell line (Hu et al., 2012). We presume long-term treatment of tamoxifen in MCF-7 cells may increase the number of CD 44+/CD 24- subpopulation. TICs reside in TAMR-MCF-7 cells may express elevated Plk1 level. Cell proliferation inhibition by Plk1 blocking was more prominent in TAMR-MCF-7 cells than the parent MCF-7 cells (Fig. 2B) because TICs cell death occurred via BI 2536 treatment. Possibly, BI 2536 induced partial E-cadherin rescue and cell proliferation inhibition occurred more sensitively in TAMR-MCF-7 than the parent cell because the BI 2536 specifically targeted TICs. Overall, we suggest here that Plk1 is novel therapeutic target for tamoxifen-resistant breast cancer therapy.

V. Reference

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Abstract

Polo-like Kinase 1: a novel target for tamoxifen-resistant breast cancer therapy

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One of the most occurring cancers in women is breast cancer. Breast cancer is mostly diagnosed from women in post-menopause. Polo-like kinase 1 (Plk1), a serine/threonine protein kinase, is a regulator of completion of cell cycle. In our study, we used MCF-7 and Tamoxifen-resistant MCF-7 (TAMR-MCF-7) cell lines. Here we report that Plk1 overexpression mediates tamoxifen-resistance in MCF-7. BI 2536 is a dihydroteridinone derivative compound that inhibits the activity of Plk1 in an ATP-competitive manner. Our *in vitro* studies revealed that cell proliferation inhibition through BI 2536 was more

sensitive in TAMR-MCF-7 cells than the parent MCF-7 cells. By performing TAMR-MCF-7 derived xenograft model, we identified BI 2536 inhibited tumor growth *in vivo*. Moreover, we observed that BI 2536 suppressed N-cadherin, Snail, Vimentin and recued loss of E-cadherin. These intriguing findings suggest Plk1 inhibition rescued epithelial-mesenchymal transition (EMT) in TAMR-MCF-7 cells. Our results propose that Plk1 represents a novel target for tamoxifen-resistant breast cancer therapy.

Key words: Tamoxifen-resistant breast cancer (TAMR-MCF-7), Polo-like kinase (Plk1), Epithelial-mesenchymal transition (EMT), E-cadherin, N-cadherin, Snail Vimentin, Estrogen receptor (ER)

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