



# Increased Expression of FosB through Reactive Oxygen Species Accumulation Functions as Pro-Apoptotic Protein in Piperlongumine Treated MCF7 Breast Cancer Cells

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**Piperlongumine (PL), a natural alkaloid compound isolated from long pepper (*Piper longum*), can selectively kill cancer cells, but not normal cells, by accumulation of reactive oxygen species (ROS). The objective of this study was to investigate functional roles of expression of SETDB1 and FosB during PL treatment in MCF7 breast cancer cells, PL downregulates SETDB1 expression, and decreased SETDB1 expression enhanced caspase 9 dependent-PARP cleavage during PL-induced cell death. PL treatment generated ROS, ROS inhibitor NAC (N-acetyl cysteine) recovered SETDB1 expression decreased by PL. Decreased SETDB1 expression induced transcriptional activity of FosB during PL treatment, PARP cleavage and positive annexin V level were increased during PL treatment with FosB overexpression whereas PARP cleavage and positive annexin V level were decreased during PL treatment with siFosB transfection, implying that FosB might be a pro-apoptotic protein for induction of cell death in PL-treated MCF7 breast cancer cells. PL induced cell death in A549 lung cancer cells, but molecular changes involved in the induction of these cell deaths might be different. These results suggest that SETDB1 mediated FosB expression may induce cell death in PL-treated MCF7 breast cancer cells.**

**Keywords:** breast cancer, cell death, FosB, piperlongumine, reactive oxygen species, SETDB1

## INTRODUCTION

Piperlongumine (PL) is a natural alkaloid compound isolated from long pepper (*Piper longum*). PL can enhance the accumulation of reactive oxygen species (ROS) and induce cell death in colon, bladder, breast, pancreatic, osteosarcoma, and lung cancers. This compound can selectively kill cancer cells, but not normal cells (Raj et al., 2011). Many genes are regulated during induction of cell death by PL treatment. PL treatment can decrease expression level of protein regulator of cytokinesis 1 (PRC1) in gastric carcinomas. PRC1 is known to play key roles in microtubule organization in eukaryotes (Zhang et al., 2017). PL can activate CHOP mediated up-regulation of DR5 which potentiates TRAIL-induced cell death in breast cancer (Jin et al., 2014). It is known to down-regulate the expression of HER family receptors in breast cancer cells (Jin et al., 2017). PL can also inhibit transforming growth factor (TGF)- $\beta$ -induced epithelial-to-mesenchymal transition by modulating the expression of E-cadherin, Snail1, and Twist1

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(Park et al., 2017).

SETDB1, a histone methyltransferase, is involved in tri-methylation of histone H3 (H3K9me3) in variety of cancers (Yang et al., 2002). SETDB1 is abnormally expressed in various human cancers. It contributes to tumor growth and metastasis (Xiao et al., 2018). Dysregulation of SETDB1 expression is involved in melanoma, lung cancer, ovarian cancer, breast cancer, implying that SETDB1 might contribute to tumor growth and metastasis (Regina et al., 2016). Under hypoxic conditions, H3K9me3 by SETDB1 can repress expression of ATM and p53-Associated KZNF Protein/ZNF420 (APAK), a negative regulator of p53, in oxygen-dependent but HIF-1-independent manner (Olcina et al., 2016). Increased H3K9me3 by SETDB1 is altered in stress response such as early multi-drug tolerance (Al Emran et al., 2018). SETDB1 can repress SNAIL1-mediated gene reprogramming during TGF- $\beta$ -induced EMT, indicating balancing function of SETDB1 (Du et al., 2018). SETDB1 expression can be decreased by various anticancer drugs such as doxorubicin and taxol in A549 human lung cancer cells. Its decreased expression is tightly connected with increased FosB gene expression (Lee and Kim, 2013). FosB belongs to Fos family that can dimerize with proteins of the JUN family, thereby forming transcription factor complex AP-1 (Barrett et al., 2017).  $\Delta$ FosB, a truncated variant of FosB, has been well-studied in the development of addiction-related neuroplasticity or increased sensitivity to behavioral effects of drug abuse (Gajewski et al., 2016). However, functional roles of full length FosB are not well known yet.

Our previous studies have suggested that increased FosB expression can induce cell migration and transforming activity in A549 cells, suggesting that functional role of FosB expression may be connected with resistance to cell death during treatment with anticancer drugs (Na et al., 2016). However, this phenomenon is only studied in A549 lung cancer cells after treatment with anticancer drugs.

In this study, we treated MCF7 breast cancer cells with natural alkaloid compound PL to investigate functional roles of SETDB1 and FosB expression. Results showed that change of SETDB1 and FosB expression might be required for the induction of cell death in PL-treated MCF7 breast cancer cells.

## MATERIALS AND METHODS

### Cell culture and reagents

Breast cancer cell line MCF7 was obtained from American Type Culture Collection (USA). MCF7 cells were cultured in DMEM medium (Welgene, Korea) containing 10% fetal bovine serum (Invitrogen, USA). A549 human lung cancer cell line was purchased from the Korean Cell Line Bank. Cells were cultured in RPMI 1640 containing 10% fetal bovine serum in a humidified incubator at 37°C. PL, N-acetyl cysteine (NAC), and dimethyl thiozoyl-2',5'-diphenyl-2-H-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (USA).

### Apoptosis analysis

Apoptosis was analyzed using annexin V-FITC (Biovision, USA) and propidium iodide (PI) staining according to the manufacturer's instructions. Briefly, MCF7 cells were treated

with 10  $\mu$ M of PL for 24 h. Cells were suspended in 1 $\times$  binding buffer. Cells were then incubated with 5  $\mu$ l of Annexin V for 10 min at room temperature in a dark room. PL treated cells were then observed with a confocal microscope at magnification of 20 $\times$ . For fluorescence-activated cell sorting (FACS) analysis, MCF7 cells were trypsinized and stained with annexin V/PI staining for 10 min at room temperature followed by analysis with a FACSCalibur (Becton-Dickinson, USA).

### MTT assay

Cell viability was determined using MTT colorimetric assay to detect viable cells that could convert water-soluble MTT to an insoluble purple formazan. MTT (Sigma-Aldrich) was dissolved in phosphate-buffered saline (PBS) after filtration with a 0.2  $\mu$ m filter. After transfection with FosB or siFosB in 60 mm dishes, cells were seeded into 96-well plates at density of 2,000 cells/well followed by PL treatment on the next day. Cells were incubated with MTT solution for 2 h at 37°C. The MTT solution was removed and replaced with 100  $\mu$ l of dimethylsulfoxide to dissolve formazan crystals. Absorbance was measured at wavelength of 570 nm using a microplate reader (Bio-Rad, USA). This experiment was performed in triplicate.

### RNA isolation and reverse transcriptase polymerase chain reaction (RT-PCR) analysis

Total RNA was isolated from cells using TRIzol reagent (Invitrogen). cDNA was synthesized with random hexamer from 1  $\mu$ g of total RNA using M-MLV reverse transcriptase (Ezzyomics, Korea). RT-PCR was then performed using cDNA as template with the following gene-specific primers: FosB (sense; 5'-TACTCC ACACCAGGCATGAG-3' and antisense; 5'-CTTCGTAGGGGATCTGCAG-3'; 373 bp), SETDB1 (5'-TTA-ACACAGGCCCTGAATTTCT-3' and 5'-TACCCCTGTGGGTAG-ACACTCT-3'; 441 bp) and  $\beta$ -actin (5'-GGATTCTATGTGGGC-GACGA-3' and 5'-CGCTCGGTGAGGATCTTCATG-3'; 438 bp).

### FosB plasmid, siRNA and transfection

FosB plasmid was obtained from Origene (USA) and subcloned into pcDNA3 expression vector. pcDNA3-FosB plasmid was transfected into cells using Lipofectamine 2000 (Invitrogen). SETDB1, FosB, and control siRNAs were purchased from Bioneer (Korea). Transient siRNA transfections were performed using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions.

### Western blot analysis

Cells were lysed in lysis buffer supplemented with proteasome inhibitors. Protein concentrations were measured using the Bradford method. Cell lysates were separated via SDS-PAGE and transferred to nitro cellulose membranes followed by immunoblotting with specific primary and horseradish peroxidase-conjugated secondary antibodies. Antibodies specific for SETDB1 (12317) and FosB (11959) were obtained from Abcam (USA). Antibodies for cleaved PARP (9541) and caspase 9 (9502) were obtained from Cell Signaling Technology (USA) and antibody for  $\beta$ -actin (A5316) was obtained from Sigma (USA).

### Immunostaining

Cells were washed with cold PBS followed by fixation in 4% paraformaldehyde solution for 10 min at room temperature. After washing with PBS three times, cells were permeabilized with 0.2% Triton X-100 solution for 10 min followed by washing with PBS three times. Blocking was performed using 3% bovine serum albumin in PBS for 1 h followed by incubation with primary antibodies SETDB1 (1:300), FosB (1:300), annexin V (1:100), and caspase 9 (1:50) in 0.03% Triton X-100 at room temperature for 2 h. Cells were then washed with PBS three times followed by incubation with secondary antibodies Alexa 488 goat anti-mouse IgG (1:500; Abcam) and rhodamine goat anti-mouse IgG (1:500; Cell Signaling Technology) in 0.03% Triton X-100. Cells were washed with PBS three times and then stained with DAPI for counter staining. Cells were then washed with PBS three times and mounted with anti-fade reagent.

### Measurement of ROS production

Accumulation of ROS was assessed by staining with fluorescent probe 2'-7'-dichlorofluorescein diacetate (H<sub>2</sub>DCFDA) (Thermo Fisher Scientific, USA). Cells were pre-treated with NAC for 1 h followed by treatment with PL for 6 h. After treatment, cells were washed with PBS twice and incubated with 10  $\mu$ M of H<sub>2</sub>DCFDA for 30 min in a 5% CO<sub>2</sub> incubator. ROS was measured with a FACSCalibur. Images of ROS were acquired for five randomly chosen areas using a confocal microscope (Olympus, Japan).

### Luciferase assay

Luciferase vectors for pGL3-FosBp-luc were prepared as

described previously (Na and Kim, 2018). MCF7 cells were transfected with luciferase plasmids using Lipofectamine 2000 for 24 h followed by treated with PL for 12 h. Luciferase activity was measured using a Renilla luciferase assay kit (Promega, USA). Luciferase activities were read on a Lumina Glomax 96 microplate luminometer (Promega).

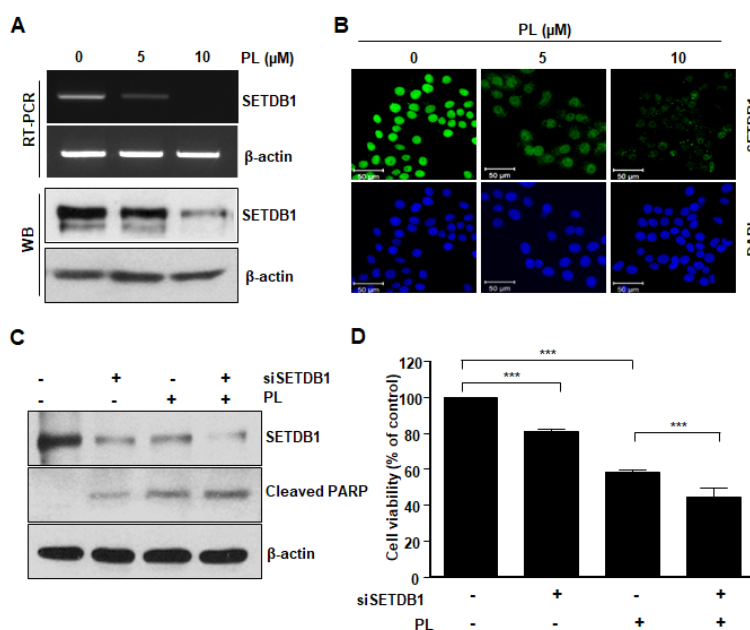
### Statistical analysis

All statistical analyses were performed with GraphPad Prism 8 software (GraphPad Software, USA). All values for experiments are presented as mean  $\pm$  SD. All multiple comparisons were performed using one-way ANOVA followed by Tukey's post hoc test.  $P < 0.05$  was considered statistically significant. Statistical significance was indicated by \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; and ns, not significant ( $P > 0.05$ ).

## RESULTS

### PL downregulates SETDB1 expression

We investigated whether treatment of PL could change expression levels of SETDB1 in MCF7 breast cancer cells. MCF7 cells were treated with indicated concentration of PL for 24 h. PL decreased SETDB1 mRNA and protein expression in a dose-dependent manner (Fig. 1A). Immunostaining experiment showed that SETDB1 level was gradually decreased by PL treatment (Fig. 1B). To examine the functional significance of decreased expression SETDB1 by PL treatment, we performed transfection of MCF7 cells with siSETDB1 together with PL treatment and examined PARP cleavage using Western blot analysis. PL treatment or siSETDB1 transfection increased PARP cleavage. Cleaved PARP was additively in-



**Fig. 1. PL treatment downregulates SETDB1 expression.** (A) MCF7 cells were treated with PL at indicated concentration for 24 h. mRNA and protein levels were estimated by RT-PCR and western blot, respectively. (B) MCF7 cells were immuno-stained with specific antibodies to SETDB1 (green). DAPI was used as a counter staining for nucleus. Scale bars = 50  $\mu$ m. (C) MCF7 cells were transiently transfected with SETDB1 siRNA for 16 h followed by treatment with 10  $\mu$ M PL for 24 h. Protein levels of SETDB1 and PARP cleavage were examined by western blot analyses. (D) Cell viability was assessed by MTT assay. Statistical significance is indicated as \*\*\* $P < 0.001$ .

creased after siSETDB1 transfection along with PL treatment (Fig. 1C). MTT assay showed that viability of MCF7 cells was decreased in the group with siSETDB1 transfection along with PL treatment compared to that in PL treated group (Fig. 1D). These data suggest that decreased SETDB1 expression is important for induction of cell death in PL-treated MCF7 breast cancer cells.

### SETDB1 expression is regulated by ROS production during PL treatment

To examine a putative relationship of ROS production with decreased SETDB1 expression, we pretreated cells with 10 mM of antioxidant NAC for 1 h followed by PL treatment for 6 h. Fluorescent intensity of H<sub>2</sub>DCFDA was significantly increased by PL treatment, but completely restored after combinatory treatment with PL and NAC (Fig. 2A). Immunostaining experiment showed that SETDB1 expression decreased by PL was recovered by treatment with NAC and PL, indicating that accumulated ROS could affect SETDB1 expression (Fig. 2B). Moreover, recovery effect of NAC on SETDB1 expression was confirmed using RT-PCR and western blot (Fig. 2C). Interestingly, PARP cleavage induced by PL was reduced by NAC treatment, suggesting that decreased expression of SETDB1 via ROS accumulation was required for the induction of cell death.

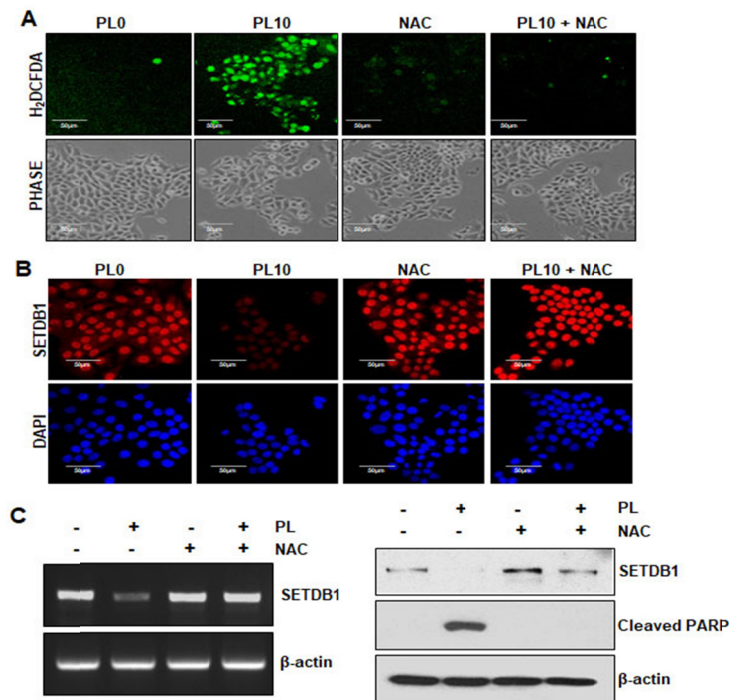
### SETDB1 mediated FosB expression is regulated by ROS production during PL treatment

SETDB1 directly regulates FosB expression after treatment

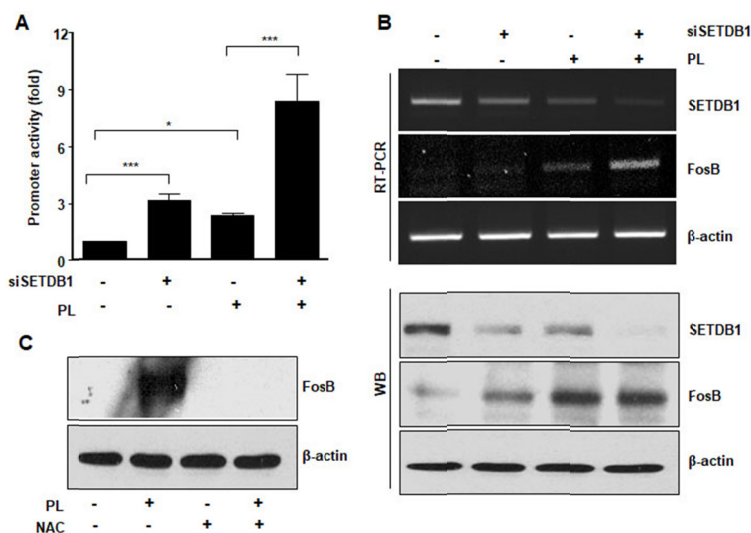
with various anticancer drugs (Na and Kim, 2018). We performed luciferase assay after transfecting PL-treated MCF7 breast cancer cells with FosB promoter construct. After siSETDB1 transfection or PL treatment, FosB promoter activity in MCF7 cells was increased 3.2-fold or 2.8-fold, respectively. However, combination of siSETDB1 transfection and PL treatment dramatically increased FosB promoter activity up to 9-fold (Fig. 3A). Western blot and RT-PCR analyses showed that FosB expression was increased by transfection of siSETDB1 or the combinatory treatment of PL and siSETDB1, indicating that FosB expression was regulated by SETDB1 (Fig. 3B). In addition, FosB expression increased by PL treatment was restored in MCF7 cells by NAC treatment. These results suggest that SETDB1 mediated FosB expression is regulated by ROS accumulation in PL-treated MCF7 breast cancer cells (Fig. 3C).

### FosB overexpression shows synergistic effect for PL-induced cell death

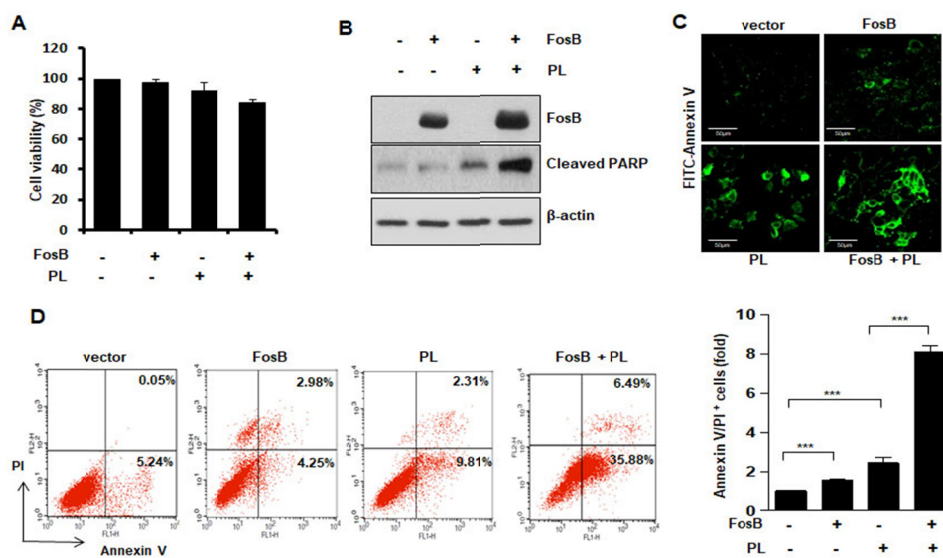
To investigate functional role of FosB during PL-induced cell death in MCF7 breast cancer cells, we performed extensive cell death assays in PL-treated MCF7 cells along with transfection of FosB plasmid. MTT assay showed that the combination of PL and FosB slightly decreased cell viability compared to PL treatment only (Fig. 4A). Western blot analysis showed that FosB overexpression slightly induced PARP cleavage. Moreover, PARP cleavage was significantly increased in PL treated MCF7 cells after FosB overexpression, suggesting that FosB expression might be function to mediate PL-treated cell



**Fig. 2. Decreased SETDB1 expression is associated with ROS.** (A) MCF7 cells were treated with 10 mM NAC for 1 h followed by treatment with 10 μM of PL for 6 h. ROS were stained with H<sub>2</sub>DCFDA and observed under a fluorescence microscope. Scale bars = 50 μm. (B) MCF7 cells were immune-stained with SETDB1 antibody (red). DAPI was used for nuclear staining. Scale bars = 50 μm. (C) mRNA and protein levels were analyzed after treatment with NAC or PL. Left, RT-PCR; right, Western blot.



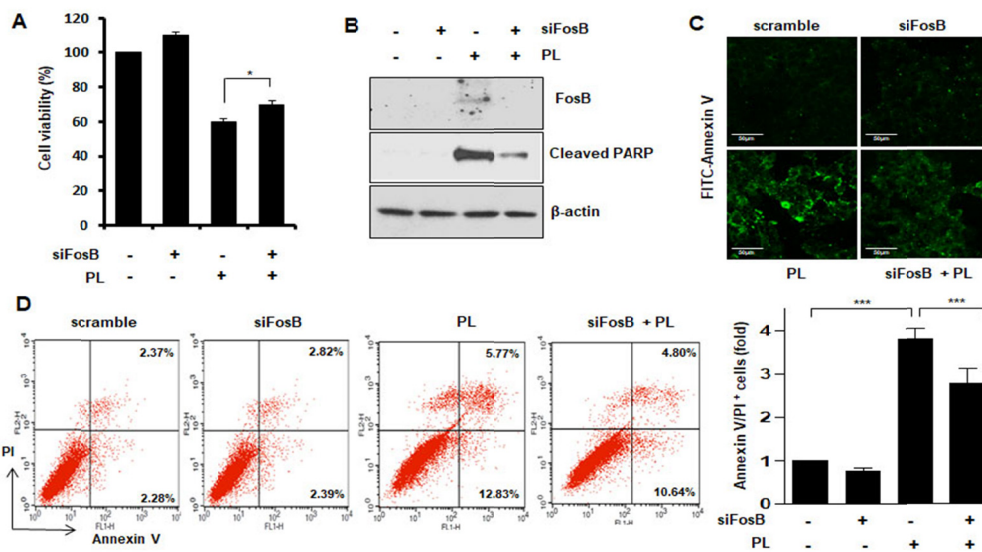
**Fig. 3. SETDB1 mediated FosB expression is regulated by ROS during PL treatment.** (A) Luciferase assay for FosB promoter was performed after siSETDB1 transfection and PL treatment. FosB promoter activity was increased in the presence of siSETDB1. It was increased after siSETDB1 transfection along with PL treatment. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . (B) MCF7 cells were transiently transfected with siSETDB1 for 16 h followed by treatment with 10  $\mu$ M PL for 24 h. mRNA and protein expression levels were analyzed by RT-PCR and western blot, respectively. (C) MCF7 cells were treated with 10 mM NAC for 1 h followed by treatment with 10  $\mu$ M PL for 24 h. Protein level of FosB was measured by Western blot.



**Fig. 4. FosB expression triggers death of PL-treated MCF7 cells.** (A) MCF7 cells were transfected with FosB plasmid for 16 h followed by treatment with 10  $\mu$ M PL for 24 h. MTT assay was performed to evaluate death of MCF7 cells. (B) PARP cleavage was analyzed by Western blot after FosB transfection along with PL treatment. (C) Annexin V positive cells were observed under a confocal microscope after FosB transfection along with PL treatment. Scale bars = 50  $\mu$ m. (D) Annexin V/PI positive cells were sorted by flow cytometry. Negative stained cells for both annexin V and PI are viable cells (lower left). Cells stained positive for annexin V and negative for PI mean early apoptotic stage (lower right). Positively stained cells for both annexin V and PI are at the end stage of apoptosis (upper right). In the graph, percentages of dead or dying cells were significantly increased after FosB transfection along with PL treatment (\*\*\* $P < 0.001$ ).

death in MCF7 human breast cancer cells (Fig. 4B). On the other hand, the group with combination of PL treatment and FosB transfection showed strong positive annexin V stain-

ing (Fig. 4C). We also measured annexin V/PI positive cells using flow cytometry. FosB overexpression slightly increased annexin V/PI positive cells compare to empty vector trans-



**Fig. 5. PL-induced cell death is decreased by siFosB transfection.** (A) MCF7 cells were transfected with siFosB followed by PL treatment. MTT assay was performed to evaluate death of MCF7 cells ( $*P < 0.05$ ). (B) PARP cleavage was analyzed by Western blot after siFosB transfection along with PL treatment. (C) Annexin V positive cells were observed under a confocal microscope after siFosB transfection along with PL treatment. Scale bars = 50  $\mu$ m. (D) Annexin V/PI positive cells were sorted by flow cytometry after siFosB transfection along with PL treatment. In the graph, percentages of cell death were significantly decreased after siFosB transfection along with PL treatment ( $***P < 0.001$ ).

fection, indicating that FosB expression affect cell death in PL-induced cell death of MCF7 cells. Moreover, Annexin V/PI positive cells were increased in the group with a combination of PL treatment and FosB overexpression compared to that in the group with PL treatment along (Fig. 4D). We performed western blot analysis and immunostaining to examine caspases responsible for PARP cleavage. MCF-7 cells are caspase-3 deficient with a partial deletion in the *CASP-3* gene (Wang et al., 2016). As a result, caspase 9 was increased by FosB overexpression, PL treatment, or combination of PL and FosB transfection, suggesting that caspase 9 might be a key molecule for PARP cleavage during PL-treated MCF7 cells (Supplementary Fig. S1A).

#### PL-induced cell death is decreased by siFosB transfection

We also examined effect of siFosB transfection during PL-induced cell death of MCF7 cells. MTT assay showed that siFosB transfection increased cell viability, and then the combination of PL and siFosB significantly recovered the cell death by PL treatment only (Fig. 5A). Western blot analysis showed that combination of PL treatment and siFosB transfection decreased PARP cleavage compare to PL treatment only (Fig. 5B). Immunostaining of positive annexin V was decreased at the group with combination of PL treatment and siFosB transfection whereas PL showed strong positive annexin V staining (Fig. 5C). On the cell sorting experiment for annexin V/PI positive cells using flow cytometry, annexin V/PI positive cells were decreased in the group with a combination of PL treatment and siFosB transfection compared to that in the group with PL treatment along (Fig. 5D). On the other hand, Western blot analysis and immunostaining showed that active caspase 9 by PL treatment was decreased by siFosB

transfection, suggesting that activity of caspase 9 is tightly regulated by FosB during PL induced cell death in MCF7 cells (Supplementary Fig. S1B). Therefore, these results suggest that the existence of FosB is necessary for cell death induction by PL in MCF7 breast cancer cells.

## DISCUSSION

Our previous studies have suggested that SETDB1 expression is decreased by DZNep and doxorubicin (Lee and Kim, 2013; Noh et al., 2014). Such decrease of SETDB1 expression is associated with increased FosB gene expression in A549 human cancer cells (Na et al., 2016). In addition, SETDB1 expression is negatively regulated by p53 tumor suppressor during paclitaxel induced-cell death (Noh et al., 2014). Although expression of SETDB1 and FosB is known to be regulated during chemotherapy in human cancer, the molecular mechanism involved in such regulation remains unclear.

In this study, we investigated the functional role of expression of SETDB1 and FosB in MCF7 breast cancer cells during PL treatment. PL induced cell death through ROS accumulation in MCF7 breast cancer cells. It regulated the linked expression of SETDB1 and FosB. Although the regulation of SETDB1 and FosB by anticancer drugs was similar, our data showed that increased FosB expression was associated with increased PARP cleavage and increased annexin V staining in PL-treated MCF7 breast cancer cells (Fig. 4). The present data suggest that decreased SETDB1 expression and the increased FosB expression might be functionally linked to the induction of death in MCF7 breast cancer cells caused by PL treatment.

SETDB1 is known to have oncogenic role in many human cancers, including nasopharyngeal carcinoma (Huang et al.,

2018), hepatocellular carcinoma (Wong et al., 2016), non-small cell lung cancer (Sun et al., 2015), and breast cancer (Ryu et al., 2018). Overexpression of SETDB1 promotes metastasis by regulating SMAD7 in TNBC (Ryu et al., 2018). SETDB1 overexpression accelerates tumorigenesis through activation of AKT that functions as an oncogene in ovarian cancer cells (Guo et al., 2019). Silenced SETDB1 can inhibit proliferation, migration, and repress tumor growth by miR-381-3p related regulation (Wu et al., 2018). Therefore, it seems that SETDB1 expression is decreased for inducing cell death during anticancer drug treatment.

FosB expression functionally induced death of MCF7 cells after PL treatment. This was unexpected considering that FosB overexpression was associated with invasion and proliferation of A549 human lung cancer cells (Na et al., 2016). However, FosB expression could be induced by antimicrobial peptide, tilapia piscidin-4 (TP4), via mitochondrial damage-triggered  $Ca^{2+}$  dysregulation, leading to cell death of breast cancers (Ting et al., 2016). Moreover, FosB transcriptionally activates protocadherin- $\beta$ 13 (*PCDHB13*), which is functionally associated with disruption of microtubules, suggesting that elevated expression of FosB and *PCDHB13* induced cell death of NSCLC (Ting et al., 2019). Although the functional role of FosB expression is confusing according to therapeutic drugs, several interpretations may therefore be possible to explain the present study.

A549 and MCF7 cells exhibit significantly different sensitivities to doxorubicin which has growth inhibitory effect (Ho et al., 2013; Rogalska et al., 2014). ERK pathway is involved in cell proliferation, differentiation, and migration (Dhillon et al., 2007). Doxorubicin regulates expression SETDB1 and FosB through ERK activation while increased FosB expression is correlated with transforming activity of A549 cells (Na and Kim, 2018). However, PL induced FosB expression via accumulation of ROS in MCF7 breast cancer cells (Fig. 2). PL can inhibit GSTP1 antioxidant enzyme and show anticancer effects by ROS signaling (Liu et al., 2013). We also determined whether molecular changes for cell death were necessary for the induction of cell death at the same concentration of PL in A549 human lung cancer cells. PL treatment decreased SETDB1 expression. However, such decrease in the expression of SETDB1 in A549 cells was recovered by NAC (Supplementary Fig. S2A). On the other hand, FosB expression was not induced in PL-treated A549 cells, but cleaved PARP in PL-treated A549 cells, suggesting that FosB might not be involved in cell death of A549 cells. Furthermore, the amount of cleaved PARP showed no change in the group with FosB overexpression or siFosB transfection followed by PL treatment (Supplementary Figs. S2B and S2C). Moreover, caspase 9 activation by PL was not detected in A549 cells, whereas activation of caspase 9 was shown in MCF7 cells, indicating that there might be discrepancy in molecular signaling pathway for PL-induced cell death between MCF7 and A549 cells. Although activation of caspase 9 might be necessary for PL-induced death of MCF7 cells, it might not be necessary for PL-induced death of A549 cells. Different in sensitivities or signaling pathways of A549 or MCF7 cancer cells after treatment with PL or doxorubicin might be because these cancer cells are derived from different tissues. Therefore, functional

differences of FosB expression during PL treatment might be due to different mechanisms leading to death of MCF7 cells and A549 cells.

FosB gene has been ranked in the top ten early response genes with changed expression in human cancer tissues during treatment with anti-cancer drugs (Lopez-Knowles et al., 2015). Early response genes are activated at transcription level in the first round of response to stimuli before synthesis of any new proteins. AP-1 transcription factor that regulates genes is responsible for cell proliferation and apoptosis.  $\Delta$ FosB overexpression can increase the expression of MMP-9 to induce cellular viability and decrease cell apoptosis in MCF-7 breast cancer cells (Li et al., 2016). FosB siRNA completely abrogated effects of stress on tumor metastasis, indicating that FosB gene expression could have anti-apoptotic function (Shahzad et al., 2010). However, AP-1 subunits show variable expression patterns under hypoxia condition, suggesting that combination of AP-1 hetero dimers might play a role in the decisive phenomenon of cell to choose between proliferation and apoptosis (Yadav et al., 2017). Although increased FosB expression is a common phenomenon during treatment with various anticancer drugs, early response genes by drug sensitivity in different types of cells or different signalling pathway might have functional importance.

Cancer cells cope with homeostatic balances for survival or cell death in response to anticancer drugs (Kim, 2018). Our current data showed that FosB expression might function toward pro-apoptotic mechanism. The oncogenic function of Fos family in human cancers has been extensively studied. However, it was limited to functional studies on c-Fos oncogene (Tkach et al., 2003; Tulchinsky, 2000). Regarding functional differences between Fos family members, FosB might have different functional meaning compared to c-Fos (Tang et al., 2016). Although knowledge on FosB is still incomplete, FosB protein might be specialized to have functions in both survival and death of cancer cells depending on different stimuli. More functional studies on FosB will be needed using various molecular approaches and different types of cancer cells.

*Note: Supplementary information is available on the Molecules and Cells website ([www.molcells.org](http://www.molcells.org)).*

#### Disclosure

The authors have no potential conflicts of interest to disclose.

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