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# Transcriptional repression of cyclin-dependent kinase inhibitor p21 gene by phospholipase D1 and D2

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**Abstract Phospholipase D (PLD) is known to stimulate cell cycle progression and to transform murine fibroblast cells into tumorigenic forms, although the precise mechanisms are not elucidated. In this report, we demonstrated that both PLD1 and PLD2 repressed expression of cyclin-dependent kinase inhibitor p21 gene in an additive manner. The phospholipase activity of PLDs was important for the effect. PLD1 repressed the p21 promoter by decreasing the level of p53, whereas PLD2 via a p53-independent pathway through modulating Sp1 activity. Taken together, we suggest that PLD isozymes stimulate cell growth by repressing expression of p21 gene, which may ultimately lead to carcinogenesis.**


*Key words:* Carcinogenesis; Cyclin-dependent kinase inhibitor; Cell cycle; p21; p53; Phospholipase D; Sp1

## 1. Introduction

Phospholipase D (PLD) catalyzes the hydrolysis of phosphatidylcholine to produce phosphatidic acid and choline [1]. The resulting phosphatidic acid is generally recognized as the main signaling product of PLD and functions as an effector in multiple physiological processes. To date, two distinct isoforms of mammalian PLD have been cloned and characterized [2,3]. PLD1 has a low basal activity and is up-regulated by small G proteins (ARF, Rho and Ral), protein kinase C (PKC), and phosphatidylinositol 4,5-bisphosphate ($PIP_2$) in vitro. In contrast, PLD2 has a high basal activity, requires $PIP_2$, and is up-regulated by ARF and PKC [4,5]. The PLD pathway is thought to play a critical role in regulating cell responses that contribute to mitotic signaling and transformation [6–10]. Because of the important cellular functions of PLD and its products, the enzymatic activity of PLD is tightly regulated by a variety of hormones, growth factors, cytokines, and other agonists involved in cellular signaling [11,12].

PLD activity is increased in response to treatment of mammalian cells with a variety of mitogenic signals. In addition, it has been reported that PLD activity is significantly elevated in human breast cancer [13], human renal cancer [14], human gastric cancer tissue [15] and experimental colon cancer [16] as well as in cells transformed by several transforming oncogenes including v-Src [17], H-Ras [18], v-Raf [6] and v-Fps [19]. PLD1 and PLD2 could cooperate with either c-Src or epidermal growth factor receptor to transform rat fibroblasts [7,8]. Overexpression of either PLD1 or PLD2 inhibits expression of p21, which enables to overcome a cell cycle block induced by high-intensity Raf signaling [18]. In addition, overexpression of PLD isozymes transforms murine fibroblast cells into tumorigenic forms [9]. Taken together, these reports indicate that the abnormally elevated PLD activity may cause uncontrolled cell growth, which ultimately leads to carcinogenesis. Despite these studies, the molecular mechanisms by which PLD regulates cellular transformation are largely unknown.

In this study, we investigated whether PLD isozymes modulate expression of p21, which is a universal inhibitor of cyclin-dependent kinase (CDK) and DNA replication that induces cell cycle arrest at the $G_1$-S checkpoint. In addition, we analyzed the p21 promoter in detail to identify PLD-responsive elements, which may provide a possible mechanism(s) by which PLD modulates cell cycle control leading to cell growth stimulation.

## 2. Materials and methods

### 2.1. Plasmids

PLD-expressing constructs containing cDNA for either PLD1 or PLD2 in pcDNA3.1 (Invitrogen) were described previously [20,21]. pcDNA PLD1-K898R and pcDNA PLD2-K758R, encoding a catalytic mutant of PLD isozyme 1 and 2, respectively, were also kindly provided by Dr. Sung Ho Ryu (POSTECH, Pohang, Korea). Both luciferase and CAT constructs containing a series of 5′ deletion mutants of the p21 promoter were described by Datto et al. [22] and El-Deiry et al. [23], respectively. pTG13 that contains 13 copies of p53 binding site was described previously [24]. pGL2-2×Sp1 that contains two copies of Sp1 binding site in pGL2-basic (Promega) was also described previously [25].

### 2.2. Transfection and luciferase assay

HeLa and Hep3B cell lines used in this study were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. Cells were seeded at $2\times10^5$ cells per 60 mm diameter plate and transfected the next day with a calcium phosphate-DNA precipitate containing target and effect plasmid DNAs as previously described [26]. Empty pcDNA3.1 vector was supplemented to equalize the amount of DNA in the reaction mixture. To control the variation for transfection efficiency, 1 μg of pCH110 (Pharmacia) containing the *Escherichia coli lacZ* gene under the control of SV40 promoter was cotransfected. After 48 h, the level of expression from the target was analyzed by either CAT assay [26] or luciferase assay [27] depending

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*Abbreviations:* CDK, cyclin-dependent kinase; $PIP_2$, phosphatidylinositol 4,5-bisphosphate; PLD, phospholipase D; PKC, protein kinase C

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on the reporter construct used and values obtained were normalized to the β-galactosidase activity measured in the corresponding cell extracts.

*2.3. Western blotting analysis*

Cells were lysed in buffer (50 mM Tris–HCl, pH 8, 150 mM NaCl, 0.1% sodium dodecyl sulfate (SDS), 1% NP-40) supplemented with protease inhibitors. Protein concentration of cell extracts was measured using the bovine serum albumin (BSA) protein assay kit (Bio-Rad). 10 μg of cell extracts was separated by SDS–polyacrylamide gel electrophoresis (PAGE) and transferred onto a nitrocellulose membrane (Hybond PVDF; Amersham). Western blotting was performed with either anti-human p53 monoclonal antibody (Santa Cruz), anti-human p21 rabbit polyclonal IgG (Santa Cruz), anti-human actin monoclonal IgG (Santa Cruz) antibody, anti-human PLD rabbit polyclonal antibody and subsequently detected by chemiluminescent ECL kit (Amersham) as recommended by the manufacturer. A polyclonal antibody that recognizes both PLD1 and PLD2 was generated as previously described [28].

## 3. Results

Recent studies have demonstrated that overexpression of PLD isozymes stimulates cell growth and induces neoplastic transformation of murine fibroblast cells [9]. This phenomenon might result from their repressive effect on p21 expression because p21 is a universal inhibitor of cyclin–CDK complexes that induces cell cycle arrest at the $G_1$-S checkpoint [29]. To test this possibility, we determined whether expression of PLD1 or PLD2 affects the level of p21 protein. As shown in Fig. 1, human cervical carcinoma HeLa cells transiently transfected with either PLD1 (lane 2) or PLD2 (lane 3) clearly showed the decrease of p21 protein level compared to the control cells (lane 1) approximately 2- and 5-fold, respectively, whereas the level of actin was not affected by the expression of PLDs. It is likely that the higher expression level of PLD2 compared to PLD1 might be responsible for the stronger repression of p21 in PLD2-expressing cells. In addition, the level of p53 protein was decreased up to 50% by expression of PLD1 (Fig. 1, lane 2), suggesting that PLD1 might repress the expression of p21 gene by down-regulating the level of its upstream activator, p53. However, the p53 level was not altered in the cells expressing PLD2 (Fig. 1, lane 3). Interestingly, the catalytically inactive PLD1-K898R did not decrease the protein level of p21 and p53 (Fig. 1, lane 4), indicating that the phospholipase activity of PLD1 might be important not only for the repression of p21 but also for the decrease of p53.

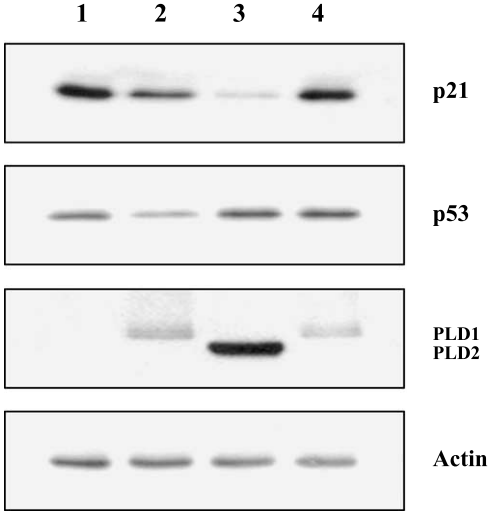


Fig. 1. Repression of p21 gene expression by PLD isozymes. HeLa cells were transfected with an empty vector (lane 1), PLD1- (lane 2), PLD2- (lane 3), or PLD1-K898R- (lane 4) expression plasmid and the protein levels of p53, p21, PLD and actin were measured by Western blotting.

Next, to examine whether PLD1 and PLD2 repress the expression of p21 at the transcription initiation level, we investigated the effects of PLD isozymes on the promoter activity of p21, using the p21 luciferase reporter (p21P) that contains luciferase gene under the control of full-length p21 promoter [22]. Initially, p21P and PLD constructs were co-transfected into HeLa cells and luciferase assay was performed. Both PLD1 and PLD2 specifically repressed the p21 promoter activity in a dose-dependent manner, up to approximately 3- and 7-fold, respectively (Fig. 2a). To investigate whether the phospholipase activity of PLD1 and PLD2 is necessary for the repression of p21, we employed catalytically inactive mutants, PLD1-K898R and PLD2-K758R. Both mutants showed significantly impaired ability to repress the p21 promoter activity (Fig. 2b), indicating that the phospholipase activity of PLD isozymes is responsible for the majority of the effect.

Next we investigated whether the repression of p21 promoter by PLD1 and PLD2 requires decrease of p53 that is an important activator for the p21 transcription. For this purpose, p21P Δ2.3, which is driven by the truncation of p21 promoter and thus is not responsive to p53 [22] was tested. Consistently to the result of Western blot, luciferase activity from p21P Δ2.3 was not significantly decreased by PLD1 (Fig. 3a), suggesting that PLD1 might repress p21 gene through down-regulation of p53. In contrast, PLD2 repressed the truncated promoter of p21P Δ2.3 approximately 6-fold. In addition, the effect of PLD1 and PLD2 on the promoter activity of p21 was additive (Fig. 3a). Taken together, it is possible to speculate that PLD1 and PLD2 repress the promoter activity of p21 through different pathways. To provide more discrete evidences for the difference in p53 dependency, the differential roles of PLD1 and PLD2 were examined in human hepatocarcinoma Hep3B cells in which a functional p53 protein is absent. Expression of PLD1 did not repress the promoter activity of p21 in Hep3B cells (Fig. 3b), confirming that PLD1, in contrast to PLD2, is dependent on p53 for the repression of p21 gene. To further demonstrate that the p21 promoter is repressed by PLD1 via a p53-dependent pathway, a luciferase construct pTG13 that contains 13 copies of p53 binding site [24] was employed. As expected the luciferase activity was significantly repressed by PLD1 but not by PLD2 (Fig. 3c). Based on the above results, we conclude that PLD1 represses the p21 promoter by down-regulating its upstream activator p53 whereas PLD2 does via a p53-independent pathway.

We next tried to determine regions of the p21 promoter responsible for p53-independent repression by PLD2. To this end, CAT and luciferase constructs containing a series of 5′ deletion mutants of the p21 promoter (Fig. 4a) were employed. According to the CAT assay shown in Fig. 4b, the activity from all constructs tested here (p21A to p21G) was repressed by PLD2 although the repression fold was slightly different. Therefore, the PLD2-responsive region must be present at the downstream of −246. Therefore, lucif-

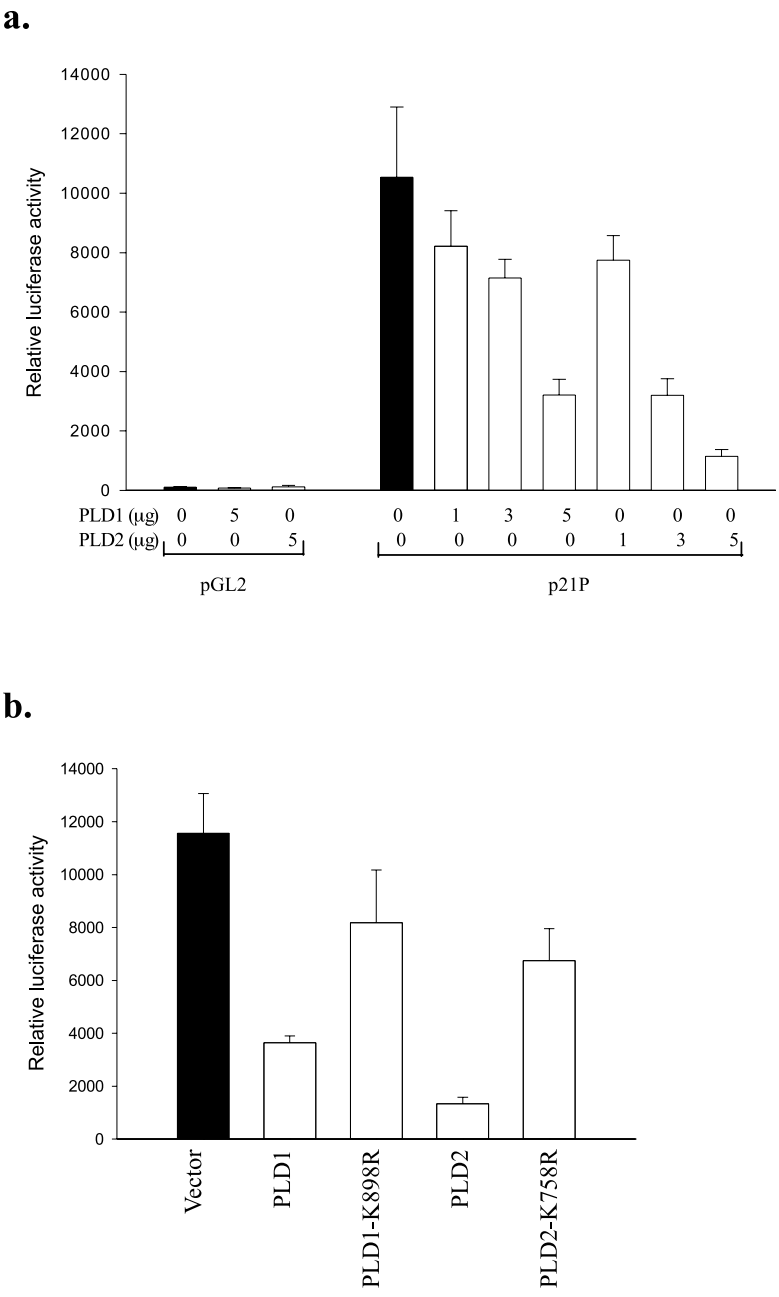


Fig. 2. Repression of p21 promoter by PLD1 and PLD2. a: Increasing amount of either pLD1 or pLD2 expression plasmid was cotransfected with 5 μg of the luciferase construct p21P [22] into HeLa cells and luciferase assay was performed. The empty reporter plasmid pGL2 was included as a control. b: 5 μg of p21P was cotransfected with 5 μg each of plasmid expressing wild-type PLD1, PLD2, or their catalytically inactive mutant forms into HeLa cells. Relative luciferase activities from p21 promoter in the absence or presence of PLD overexpression are indicated by the solid bars and blank bars, respectively. Error bars indicate standard deviations obtained from three different experiments.

erase assay was performed using another series of p21 reporter constructs [22]. As shown in Fig. 4c, deletion of the p21 promoter up to −93 did not reduce the effect of PLD2. However, the minimal promoter of p21P smaΔ1, which consists of only 61 bp proximal to the transcriptional initiation site, was not affected by PLD2. In addition, internal deletion of the sequence between −93 and −61 in p21P smaΔ2 almost completely abolished the effect of PLD2. Therefore, we determined the PLD2-responsive region between nucleotide positions −93 and −61.

To more precisely define the PLD2-responsive region in the p21 promoter, five mutant constructs (93-S mut#1 to 93-S mut#5), each containing 10 consecutive mutated bases between −93 and −44 in the promoter construct 93-S [22], were examined (Fig. 5a). The promoter in 93-S mut#1 was repressed by PLD2 in a similar manner to that of the 93-S. However, the effect was decreased in other mutant constructs and almost completely lost in the case of 93-S mut#2. Therefore, we conclude that the major PLD2-responsive element is located between −84 and −75, based on the result with 93-S mut#2. Interestingly, the PLD2-responsive element defined above is exactly overlapped with the TGF-β-responsive ele-

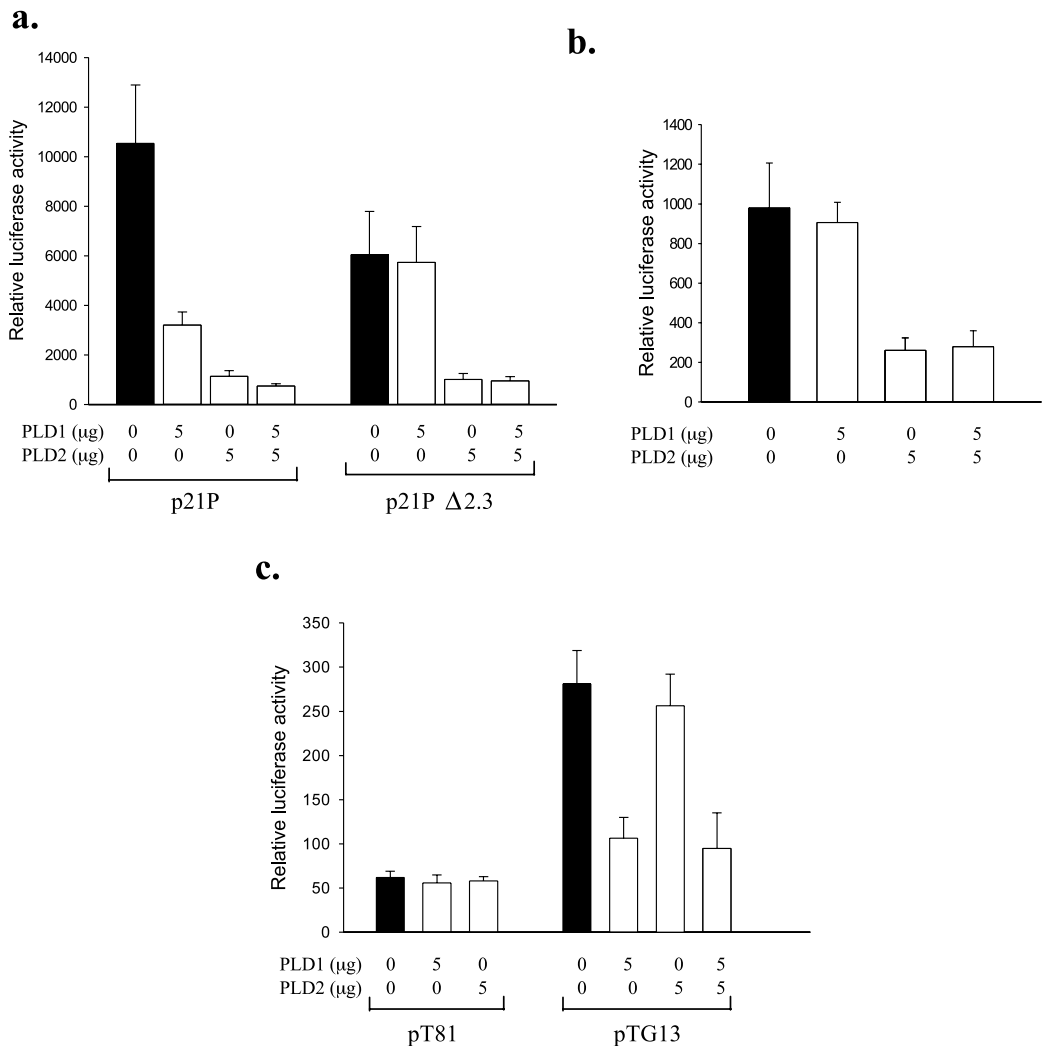


Fig. 3. p53-dependent repression of the p21 promoter by PLD1. a: Either PLD1- and/or PLD2-expression plasmid was cotransfected with luciferase construct which transcription is driven by either a full-length (p21P; left panel) or a truncated form (p21P Δ2.3; right panel) of p21 promoter into HeLa cells and luciferase assay was performed. b: p21P luciferase plasmid was cotransfected with either PLD1- and/or PLD2-expressing plasmid into p53-negative Hep3B cells and luciferase activity was measured. c: pTG13-luc that contains 13 copies of p53 binding site in pT81-luc [24] was cotransfected with either an empty vector or PLD-expressing plasmid into HeLa cells. pT81-luc reporter plasmid that contains a basic promoter element (TATA box) was included as a control.

ment (TβRE), which is known to mediate transcriptional activation of the p21 gene by TGF-β [22] and a Sp1 binding site (Sp1-1). Among them, the latter might be responsible for the effect because all other constructs that showed lower PLD2 responsiveness had at least one mutated Sp1 site. More direct evidence that the Sp1 binding site is responsible for the effect by PLD2 was obtained with 93-S mut#2.3. This construct contains a mutation of bases −76 and −77 from CT to GG, thus maintains the consensus Sp1 binding site but showed a significantly reduced ability to be activated by TGF-β [22]. As the promoter activity of 93-S mut#2.3 was successfully repressed by PLD2 (Fig. 5a), destruction of the Sp1 site in 93-S mut#2 might be responsible for the loss of PLD responsiveness.

To determine if the Sp1 binding site in the p21 promoter is sufficient for the repression by PLD2, the site was used in an attempt to confer PLD2 response to a heterologous promoter. pGL2-2×Sp1 [25] in which two copies of Sp1 binding site were inserted 5′ of the TATA box was responsive to PLD2 (Fig. 5b). As the Sp1 binding site was sufficient to confer HBx responsiveness to a previously non-responsive promoter, we conclude that PLD2 represses the transcription of p21 through the Sp1 binding site, possibly by down-regulating the activity of Sp1.

## 4. Discussion

Several reports suggest that the aberrant expression of PLD is implicated in oncogenesis, although the exact mechanism is not understood. Activation of PLD may result in the prolonged formation of diacylglycerol [30], and consequently causes the long-term activation of PKC, which is necessary for proliferation and tumorigenesis [31]. PLD activity has been found to be markedly elevated in various cancer tissues [13–17], cancer cell lines and transformed cells [6,17–19]. The relationship between PLD and cancer is further supported by the recent findings that some antitumor drugs act as inhibitors of PLD [32]. In addition, overexpression of PLD enzymes transforms murine fibroblast into tumorigenic forms [9]. However, it is not understood how the elevated PLD activity leads

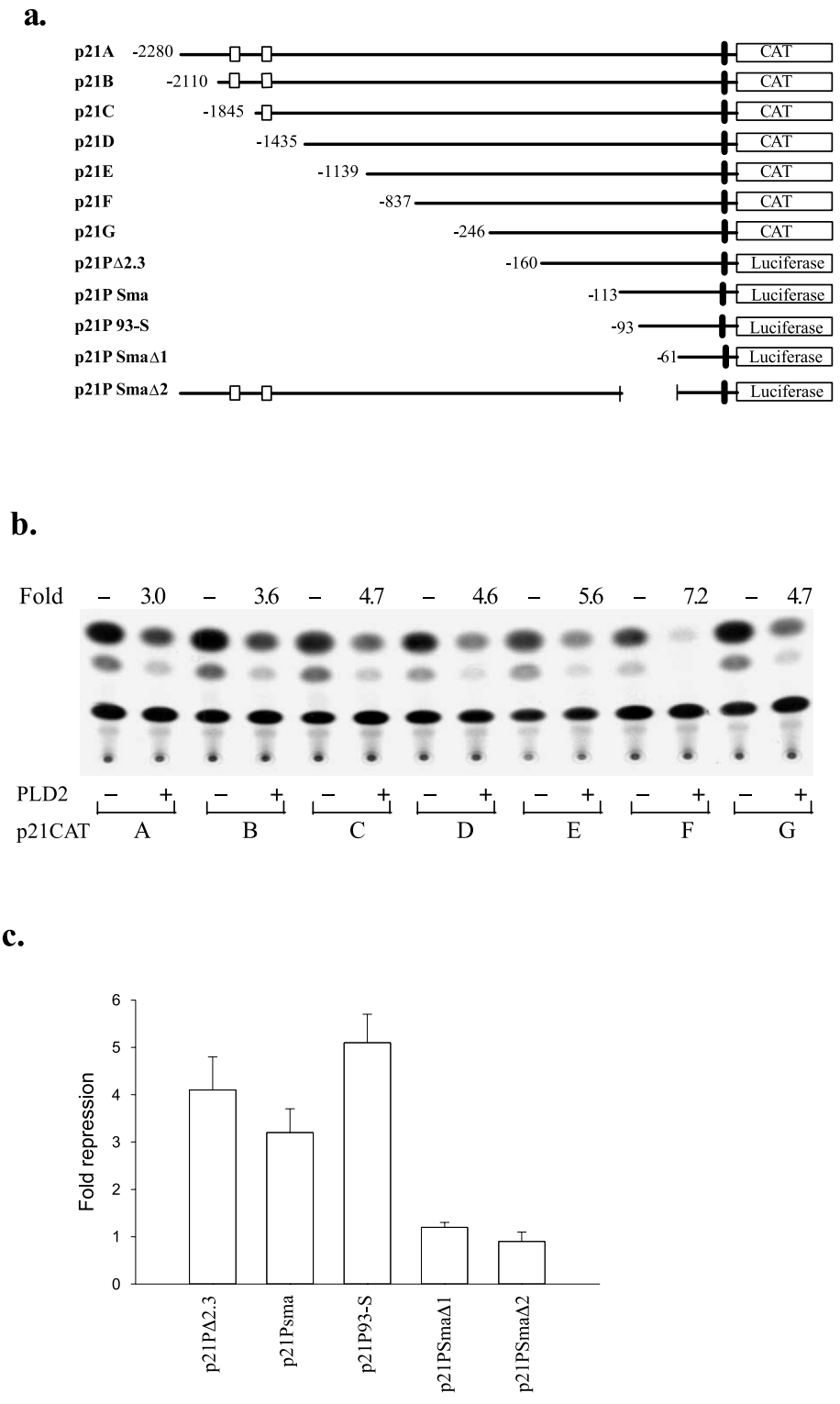


Fig. 4. Determination of PLD2-responsive elements in the p21 promoter. a: Schematic diagram of the various p21CAT [23] or p21 luciferase [22] constructs used in this study. The p53 binding sites located at −2285 and −1394 are indicated by open boxes whereas TATA box in a closed box. b: Full-length and truncated p21CAT constructs were cotransfected with either an empty vector or PLD2-expressing plasmid into HeLa cells and CAT activity was measured. A quantitative estimate of chloramphenicol acetylation was obtained by excision of the substrate and products from thin-layer chromatography plates, and subsequent measurement by liquid scintillation counting in an LKB 216 scintillation counter. The relative chloramphenicol acetylation ratio was indicated as fold repression over control. c: Truncated forms of p21 promoter luciferase constructs were cotransfected with either an empty vector or PLD2-expressing plasmid into HeLa cells and luciferase assay was performed. The data are presented as fold repression over control.

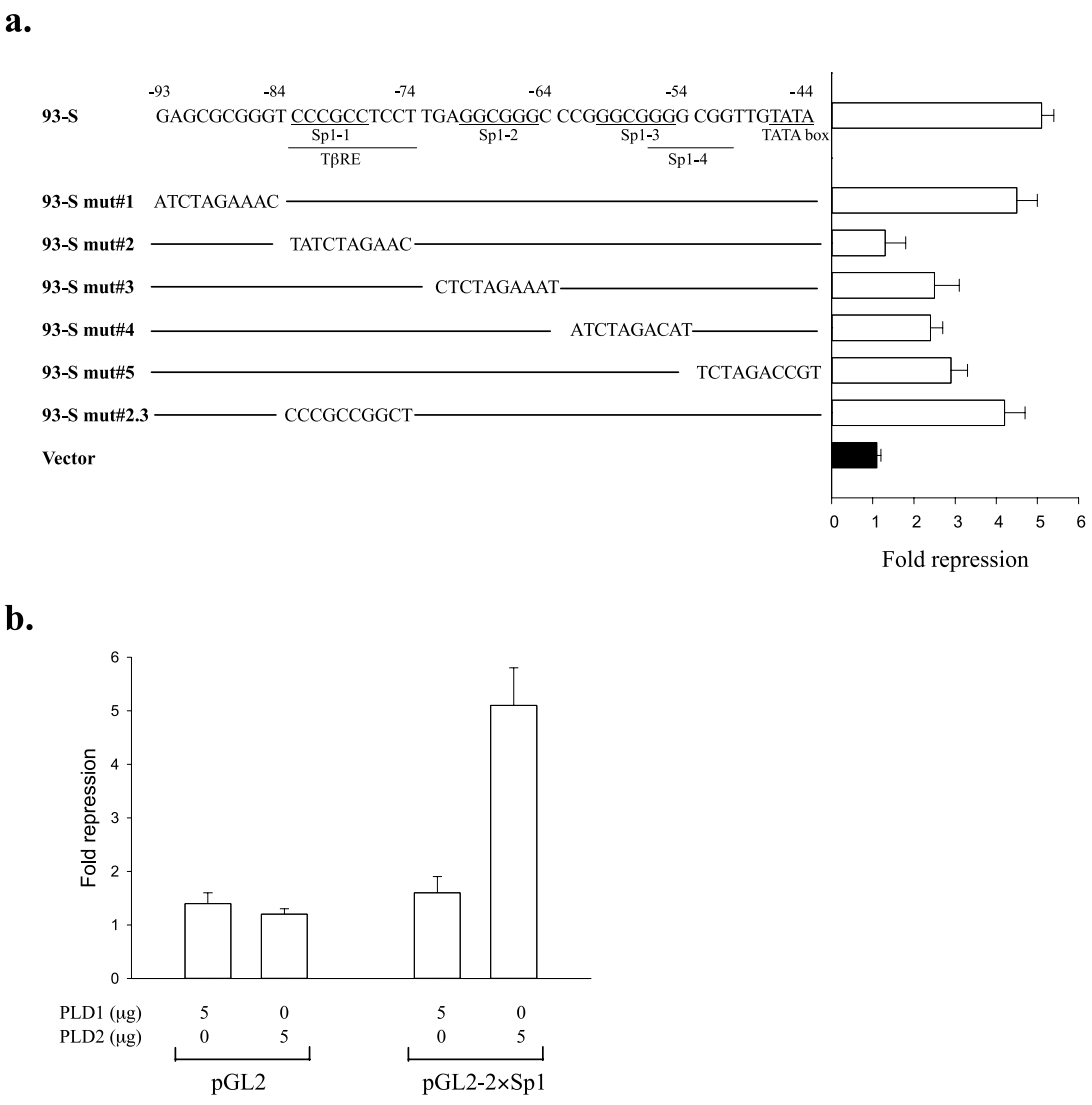


Fig. 5. Repression of the p21 promoter by PLD2 through Sp1 binding sites. a: 93-S mutant constructs [22], identical to the wild-type p21P 93-S sequence with the exception of the sequences shown for each mutant construct were cotransfected with PLD2-expressing plasmid into HeLa cells. The resulting luciferase activity was indicated as repression fold against the basal activity of the control. The positions of transcription factor binding sites are underlined. b: pGL2-2×Sp1 [25] was cotransfected with either PLD1- or PLD2-expressing plasmid into HeLa cells. Repression fold was calculated by dividing the luciferase activity by the basal activity obtained with the control vector.

to cellular transformation. One possibility is that the increased PLD activity causes cell cycle deregulation, which leads to growth stimulation. Actually, overexpression of PLD isozymes stimulates growth of murine fibroblast cells [9]. Recently, we have found that c-Src acts as a kinase of PLD and PLD acts as an activator of c-Src. This transmodulation between c-Src and PLD may contribute to the promotion of cellular proliferation via amplification of mitogenic signaling pathways [33].

Cell growth is regulated at several points in the cell cycle, called checkpoints, at which the cycle can be arrested if previous events have not been completed. Among several checkpoints, both extracellular and intracellular signals, which can either promote or inhibit cell proliferation, tend to act by regulating progression through a $G_1$ checkpoint. Cell cycle regulators critical for error-free execution of this event include $G_1$-Cdk composed of D-type cyclins (cyclins D1, D2 and D3) and Cdk partners (Cdk4 and Cdk6) [34]. In addition, the activities of G1 cyclin–cdk complexes are negatively regulated by the binding of Cdk inhibitor proteins such as p21 and p27. According to a previous report [9], overexpression of PLD1 or PLD2 in mouse fibroblast cells induces the expression of cyclin D3 protein, which may leads to the cell growth stimulation by increasing $G_1$ to S phase transition. In this study, we demonstrated that PLD1 and PLD2 down-regulate the expression level of p21 through different mechanisms. PLD1 might repress it by decreasing the level of p53, which is an important activator for the p21 transcription. The stability of p53 might be decreased by PLD1, although transcriptional repression of the p53 by PLD1 cannot be excluded. On the other hand, PLD2 represses p21 expression through the Sp1 binding site located proximal to the TATA box. Therefore, PLD2 might repress transcription of p21 by down-regulating the activity of Sp1. Although PLD1 and PLD2 show differential repression of p21 gene, both PLD1 and PLD2 repress p21 expression to stimulate cell cycle progression, which may

ultimately contribute to carcinogenesis. Therefore, this study may provide a clue to explain oncogenic processes induced by the elevated expression of PLD isozymes.

The phospholipase activity of PLD1 and PLD2 might be important for the repression of p21 expression. However, the catalytically inactive mutants of PLD1 and PLD2 still showed a weak but significant level of repression activity. The residual activity of PLD mutants might be due to their leaky enzyme activities. Another possibility is that PLD might regulate expression of p21 via phospholipase activity-independent pathways. PLD may interact with p53 or other transcription factors involved in the regulation of p21 expression, either directly or indirectly. Further studies are scheduled to identify the domain of PLD responsible for the effect. Elucidation of the mechanism by which PLD1 destabilizes p53 might provide several critical answers to understand the roles of PLD overexpressed in several human cancers. In addition, the down-regulation of Sp1 activity by PLD2 will be investigated in detail.

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## References

[1] Exton, J.H. (1994) Biochim. Biophys. Acta 1212, 26–42.
[2] Hammond, S.M., Altshuller, Y.M., Sung, T.C., Rudge, S.A., Ross, K., Engebrecht, J., Morris, A.J. and Frohman, M.A. (1995) J. Biol. Chem. 270, 29640–29643.
[3] Colley, W.C., Sung, R., Jenco, R.L., Hammond, S.M., Altshuller, Y., Bar-Sagi, D., Morris, A.J. and Frohman, M.A. (1997) Curr. Biol. 7, 191–201.
[4] Siddiqi, A.R., Srajer, G.E. and Leslie, C.C. (2000) Biochim. Biophys. Acta 1497, 103–114.
[5] Lopez, I., Arnold, R.S. and Lambeth, J.D. (1998) J. Biol. Chem. 273, 12846–12852.
[6] Frankel, P.A., Ramos, M., Flom, J., Bychenok, S., Kerkhoff, E., Rapp, U.R., Feig, L.A. and Foster, D.A. (1999) Biochem. Biophys. Res. Commun. 255, 502–507.
[7] Lu, Z., Hornia, A., Joseph, T., Sukezane, T., Frankel, P., Zhong, M., Bychenok, S., Xu, L., Feig, L.A. and Foster, D.A. (2000) Mol. Cell. Biol. 20, 462–467.
[8] Joseph, T., Wooden, R., Bryant, A., Zhong, M., Lu, Z. and Foster, D.A. (2001) Biochem. Biophys. Res. Commun. 289, 1019–1024.
[9] Min, D.S., Kwon, T.K., Park, W.S., Chang, J.S., Park, S.K., Ahn, B.H., Ryoo, Z.Y., Lee, Y.H., Lee, Y.S., Rhie, D.J., Yoon, S.H., Hahn, S.J., Kim, M.S. and Jo, Y.H. (2001) Carcinogenesis 22, 1641–1647.
[10] Hornia, A., Lu, Z., Sukezane, T., Zhong, M., Joseph, T., Frankel, P. and Foster, D.A. (1999) Mol. Cell. Biol. 19, 7672–7680.
[11] Exton, J.H. (2002) FEBS Lett. 531, 58–61.
[12] Exton, J.H. (1997) Phys. Rev. 77, 303–320.
[13] Uchida, N., Okamura, S. and Nagamachi, Y. (1997) J. Cancer Res. Clin. Oncol. 123, 280–285.
[14] Zhao, Y., Ehara, H., Akao, Y., Shamoto, M., Nakagawa, Y., Banno, Y., Deguchi, T., Ohishi, N., Yagi, K. and Nozawa, Y. (2000) Biochem. Biophys. Res. Commun. 278, 140–143.
[15] Uchida, N., Okamura, S. and Kuwano, H. (1999) Anticancer Res. 19, 671–676.
[16] Yoshida, M., Okamura, S., Kodaki, T., Mori, M. and Yamashita, S. (1998) Oncol. Res. 10, 399–406.
[17] Song, J., Pfeffer, L.M. and Foster, D.A. (1991) Mol. Cell. Biol. 11, 4903–4908.
[18] Joseph, T., Bryant, A., Frankel, P., Wooden, R., Kerkhoff, E., Rapp, U.R. and Foster, D.A. (2002) Oncogene 21, 3651–3658.
[19] Jiang, Y.-W., Song, J., Zang, Q. and Foster, D.A. (1994) Biochem. Biophys. Res. Commun. 255, 1195–1203.
[20] Han, J.M., Kim, Y., Lee, J.S., Lee, C.S., Lee, B.D., Ohba, M., Kuroki, T., Suh, P.G. and Ryu, S.H. (2002) Mol. Biol. Cell. 13, 3976–3988.
[21] Kim, J.H., Lee, B.D., Kim, Y., Lee, S.D., Suh, P.G. and Ryu, S.H. (1999) J. Immunol. 163, 5462–5470.
[22] Datto, M.B., Yu, Y. and Wang, X.F. (1995) J. Biol. Chem. 48, 28623–28628.
[23] El-Deiry, W.S., Tokino, T., Waldman, T., Oliner, J.D., Velculescu, V.E., Burrell, M., Hill, D.E., Healy, E., Rees, J.L., Hamilton, S.R., Kinzler, K.W. and Vogelstein, B. (1995) Cancer Res. 55, 2910–2919.
[24] Lee, C.W., Sørensen, T.S., Noriko, S. and La Thangue, N.B. (1998) Oncogene 16, 2695–2710.
[25] Ahn, J.Y., Chung, E.Y., Kwun, H.J. and Jang, K.L. (2001) Gene 275, 163–168.
[26] Gorman, C.M. (1986) in: DNA Cloning (Glover, D., Ed.), pp. 143–190, IRL Press, Oxford.
[27] Otsuka, M., Kato, N., Lan, K.-H., Yoshida, H., Kato, J., Goto, T., Shiratori, Y. and Omata, M. (2000) J. Biol. Chem. 275, 34122–34130.
[28] Min, D.S., Ahn, B.-H., Rhie, D.-J., Yoon, S.-H., Hahn, S.J., Kim, M.-S. and Jo, Y.H. (2001) Neuropharmacology 41, 384–391.
[29] Xiong, Y., Hannon, G.J., Zhang, H., Casso, D., Kobayashi, R. and Beach, D. (1993) Nature 366, 701–704.
[30] Liscovitch, M. (1997) Trends Biochem. Sci. 17, 393–399.
[31] Boarder, M.R. (1994) Trends Pharmacol. Sci. 15, 57–62.
[32] Gratas, C. and Powis, G. (1993) Anticancer Res. 13, 1239–1244.
[33] Ahn, B., Kim, S.Y., Kim, E.H., Choi, K.S., Kwon, T.K., Lee, Y.H., Chang, J.S., Kim, M., Jo, Y. and Min, D.S. (2003) Mol. Cell. Biol., in press.
[34] Resnitzky, D., Gossen, M., Bujard, H. and Reed, S.I. (1994) Mol. Cell. Biol. 14, 1669–1679.