Protein phosphorylation on tyrosine restores expression and glycosylation of cyclooxygenase-2 by 2-deoxy-D-glucose-caused endoplasmic reticulum stress in rabbit articular chondrocyte

Seon-Mi Yu & Song-Ja Kim*
Department of Biological Sciences, College of Natural Sciences, Kongju National University, Gongju 314-701, Korea

2-deoxy-D-glucose (2DG)-caused endoplasmic reticulum (ER) stress inhibits protein phosphorylation at tyrosine residues. However, the accurate regulatory mechanisms, which determine the inflammatory response of chondrocytes to ER stress via protein tyrosine phosphorylation, have not been systematically evaluated. Thus, in this study, we examined whether protein phosphorylation at tyrosine residues can modulate the expression and glycosylation of COX-2, which is reduced by 2DG-induced ER stress. We observed that protein tyrosine phosphatase (PTP) inhibitors, sodium orthovanadate (SOV), and phenylarsine oxide (PAO) significantly decreased expression of ER stress inducible proteins, glucose-regulated protein 94 (GRP94), and CCAAT/enhancer-binding-protein-related gene (GADD153), which was induced by 2DG. In addition, we demonstrated that SOV and PAO noticeably restored the expression and glycosylation of COX-2 after treatment with 2DG. These results suggest that protein phosphorylation of tyrosine residues plays an important role in the regulation of expression and glycosylation during 2DG-induced ER stress in rabbit articular chondrocytes. [BMB reports 2012; 45(5): 317-322]

INTRODUCTION

Cyclooxygenases (COXs) are the rate-limiting enzyme in the biosynthesis of PGs and have been linked to inflammatory diseases, including rheumatoid arthritis (RA) and osteoarthritis (OA) (1). In arthritis, COX-2 is highly expressed in the chondrocytes and in the synovial tissue (2). The activity and expression of COX are modulated at the levels of transcription, post-transcription, and translation during inflammation. However, there have been few studies that examined the regulation of COX-2 at post-translational levels.

It has been previously demonstrated that protein phosphorylation results in an increase in COX-2 at the transcription and translation levels, and these results are related to protein kinase C (PKC) and protein tyrosine kinase (PTK) (3). In addition, COX-2 activity has been shown to be modulated at the post-translational level by protein tyrosine phosphorylation (4). The function of proteins and cellular processes are regulated by protein phosphorylation at tyrosine residues. The amount of protein phosphorylation at tyrosine residues is dependent on the balance between the activities of tyrosine kinase and tyrosine phosphatase (5). The overall levels of protein phosphorylation at tyrosine residues are low in most cells. Treatment with a protein tyrosine phosphatase inhibitor in cells results in protein phosphorylation at tyrosine residues in unspecified proteins that are generally maintained in dephosphorylated forms.

The ER is a sub-cellular organelle that is involved in the synthesis of secretory and membrane proteins and lipids responsible for several specialized functions, such as protein folding and translocation (6). Several physiological and pathological conditions, such as glucose starvation, disturbance of intracellular stores of Ca\(^{2+}\), oxidative stress, viral infection, and inhibition of protein glycosylation, can lead to ER stress (7). To effectively cope with ER stress, cells initiate two distinct responses in the ER lumen to protect against the accumulation of unfolded proteins. The first involves the unfolded protein response (UPR), which increases genes coding chaperones, such as GRP78 and GRP94. This increases proper protein folding and inhibits the aggregation of unfolded proteins in the ER (8). The second is a profound and transient attenuation of protein synthesis. This response reduces the stress associated with protein folding in the ER (9). Both the unfolded protein response and the repression of protein synthesis may lead to cytoprotection or death, depending on the nature of the stress and the cellular environment (9). ER stress negatively or positively regulates COX-2 expression through a variety of signal pathways in normal or abnormal cells (10).

2DG is an analogue of glucose, where the 2-hydroxyl group is replaced with hydrogen. It is generally believed to be a cru-
Tyrosine phosphorylation affects glycosylation of COX-2
Seon-Mi Yu and Song-Ja Kim

RESULTS

COX-2 expression was detected in control cultured chondrocytes, but was not detected in 2DG-treated cells. However, doublet COX-2 (66-70-KDa; arrowheads) bands were weakly detected in 2DG-treated cells. Our previous study demonstrated that doublet bands corresponded to the unglycosylated form of COX-2 (12). Addition of 2DG to chondrocytes strongly decreased protein phosphorylation at tyrosine residues (Fig. 1A). Therefore, we sought to explore whether down-regulation of both COX-2 expression and glycosylation rate by 2DG-induced ER stress was related to protein phosphorylation at tyrosine residues. To examine this relation, cells were treated with 2DG for 24 h in the absence or presence of SOV, PAO, GN, or OA (Fig. 1A).

We first examined the role of protein phosphorylation at tyrosine residues on the reduction of expression and glycosylation of COX-2 by 2DG. Cells were treated with 2DG for 24 h in the absence or presence of SOV, PAO, GN, or OA (Fig. 1A). Inhibition of PTPs with SOV or PAO resulted in increased expression and glycosylation of COX-2, while expression of GADD153 and GRP94 were markedly decreased in 2DG treated cells (Fig. 1A). In our previous studies, we showed that PP2, a specific inhibitor of Src tyrosine kinase, could recover the decrease in COX-2 expression caused by 2DG treatment (12). Therefore, in this study, we investigated the effect of GN, an inhibitor of tyrosine kinase, and PP2 on the 2DG-induced reduction in expression and glycosylation of COX-2. In these experiments, GN, an inhibitor of tyrosine kinase, did not restore the 2DG-induced reduction in COX-2 expression (Fig. 1B), indicating that PP2 and GN had different effects on COX-2 expression after 2DG treatment.

Consistent with the Western blot data, the RT-PCR results also showed that inhibitors of PTPs recovered the transcription levels of COX-2 (Fig. 1C). To examine whether the reduced expression and glycosylation of COX-2 by 2DG were regulated by protein phosphorylation on serine or threonine, 2DG-treated cells were incubated with GN, an inhibitor of protein tyrosine kinase, or OA, an inhibitor of serine/threonine phosphatase (Fig. 1A). OA and GN treatment did not affect GADD153 and GRP94 expression (Fig. 1A). Thus, based on the results of Western blot analysis and RT-PCR (Fig. 1A and C), the reduced expression and glycosylation of COX-2 by 2DG were not recovered by GN and OA, either at the translation or transcription levels.

The effect of protein tyrosine phosphorylation on COX-2 activity was investigated using a PGE2 assay (Fig. 2A). 2DG-induced ER stress inhibited the production of PGE2 and inhibitors of PTPs, SOV, and PAO increased the production of PGE2 under these conditions (Fig. 2A). In contrast, GN did not have any effects on PGE2 production when compared to that of 2DG treated chondrocytes. These results indicate that maintenance of protein tyrosine phosphorylation with PTPs inhibitors recuperated the loss of COX-2 expression caused by 2DG, as determined by PGE2 production (Fig. 2A). Taken to-

Fig. 1. Protein phosphorylation at tyrosine residues recovers the reduction in COX-2 expression caused by 2DG treatment. (A, B) Chondrocytes were treated or not treated with 5 mM 2DG for 24 h in the absence or presence of various pharmacological agents (2 μM SOV, 0.5 μM PAO, 40 μM GN, 5 ng/ml OA, 10 μM PP2). Protein levels of COX-2, GADD153, GRP94, ptyrosine, pSrc and actin were detected by Western blot analysis. The arrow indicates expression of the reduced molecular mass of COX-2 (∼66 kDa) following 2DG treatment. (C) Transcript levels of COX-2 and GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) were determined using RT-PCR. GAPDH was used as a loading control. (A-C) The data represent the typical results from independent experiments.

Fig. 2. Protein phosphorylation at tyrosine residues modulates PGE2 production and GRP94 expression and restores the reduced COX-2 expression caused by 2DG treatment. (A) Chondrocytes were treated or not treated with 5 mM 2DG for 24 h in the absence or presence of various pharmacological agents (2 μM SOV, 0.5 μM PAO or 40 μM GN). Levels of cellular and secreted PGE2 were determined using a kit assay. The data represent typical results and average values with standard deviations (SD) from independent experiments are shown. *P < 0.05 compared with untreated cells. (B) Primary chondrocytes were treated or not treated with 5 mM 2DG in the absence or presence of 2 μM SOV for 24 h. Distribution of COX-2, ptyrosine, and GRP94 was detected by immunofluorescence staining. Nuclei were stained with DAPI (4',6-diamidino-2-phenylindole).
Together, these results indicate that protein tyrosine phosphorylation not only regulates expression and glycosylation of COX-2 but also modulates the activity of COX-2 (Fig. 2A). Immunofluorescence microscopy was used to assess the expression patterns of COX-2 and GRP94 (Fig. 2B). Similar with the results of the Western blot analysis, double staining of COX-2 and GRP94 in chondrocytes treated with SOV in the presence or absence of 2DG, cells with reduced levels of GRP94 were positive for COX-2 staining, indicating that protein tyrosine phosphorylation by SOV plays a crucial role in the expression of COX-2 (Fig. 2B).

In order to clearly determine the effect of protein phosphorylation at tyrosine residues with respect to the decrease in COX-2 expression after 2DG treatment, chondrocytes were treated with 2DG for 24 h in the presence of different concentrations of SOV, PAO, or GN (Fig. 3). Western blot analysis revealed that expression and glycosylation of COX-2 were substantially recovered by SOV or PAO in a dose dependent manner (Fig. 3A and B). On the other hand, GN treatment did not alter COX-2 expression but rather affected GADD153 and GRP94 expression when compared with 2DG treated cells (Fig. 3C).

To further elucidate the effects of protein phosphorylation at tyrosine residues on the expression and glycosylation of COX-2 after treatment with 2DG, we assessed the effects of SOV and PAO on another gene that is suppressed under ER stress conditions (Fig. 4). Tunicamycin (TN), which inhibits the initial step in N-glycosylation, has been shown to increase the unfolded protein response and alter the protein folding ability of ER by several mechanisms, including transcriptional up-regulation of chaperones, such as GRP94 and GADD153 (8).

As was observed for 2DG treatment, TN also increased the expression of ER stress inducible proteins, GRP94 and GADD153, and reduced protein phosphorylation at tyrosine residues (Fig. 4). To examine the association between protein phosphorylation at tyrosine residues and the reduced expression and glycosylation of COX-2 by TN, cells were treated with TN for 24 h in the absence or presence of SOV or PAO (Fig. 4).

Treatment with SOV or PAO in combination with TN restored the expression and glycosylation of COX-2. In addition, SOV and PAO completely abolished TN induced expression levels of GRP94 and GADD153 (Fig. 4A).

To decipher the link between protein phosphorylation at tyrosine residues and ER stress-mediated regulation of COX-2, the effects of GN and OA on TN modulated COX-2 expression were examined (Fig. 4). Western blot analysis showed that GN and OA did not affect the reduced expression and glycosylation of COX-2 by TN (Fig. 4A). RT-PCR analysis was performed to investigate the effect of protein tyrosine phosphorylation on the transcriptional levels of COX-2 mRNA (Fig. 4B). Consistent with the Western blot analysis, RT-PCR showed that the COX-2 transcript levels were not change by GN and OA treatment (Fig. 4B).

To further verify the effect of protein tyrosine phosphorylation on the reduced expression and glycosylation of COX-2 by TN, chondrocytes were treated with various concentrations of SOV, PAO, or GN in the presence of TN. Chondrocytes were incubated with SOV, PAO, or GN for 24 h, and the expression patterns of COX-2 were measured by Western blot analysis (Fig. 4C-E). Treatment with SOV or PAO in the presence of TN produced a concentration dependent increase in COX-2 expression when compared with cells treated with TN.

**Fig. 4.** Protein phosphorylation at tyrosine residues decreases COX-2 expression caused by TN treatment. (A, B) Rabbit articular chondrocytes were treated or not treated with 1 μg/ml TN for 24 h in the absence or presence of different pharmacological agents (2 μM SOV, 0.5 μM PAO, 40 μM GN, 5 ng/ml OA). (A) Protein levels of COX-2, GADD153, GRP94, ptyrosine, and actin were determined by Western blot analysis. (B) The mRNA levels of COX-2 and GAPDH were detected by RT-PCR. (C, D, E) Chondrocytes were treated or not treated with 1 μg/ml TN for 24 h in the absence or presence of the indicated concentrations of SOV, PAO or GN. Expressions of COX-2, GADD153, GRP94, ptyrosine, and actin were determined by Western blot analysis. (AE) The data represent typical results. The arrow indicates expression of the reduced molecular mass of COX-2 (∼66 kDa) following 2DG treatment.
(Fig. 4C and D), while GN did not have any effects on expression or glycosylation of COX-2 relative to TN treated chondrocytes (Fig. 4E). These results clearly show that maintenance of protein tyrosine phosphorylation is crucial in regulating the reduced expression and glycosylation of COX-2 by TN (Fig. 4).

The above results indicate that the reduced expression and glycosylation of COX-2 by 2DG were regulated through protein phosphorylation at tyrosine residues in rabbit articular chondrocytes.

**DISCUSSION**

In this study, we demonstrate that protein phosphorylation at tyrosine residues regulates the reduced expression and glycosylation of COX-2 by 2DG. We first investigated the effects of inhibitors of PTPs on COX-2 expression and ER stress by 2DG.

Glycosylation of COX-2 is required for normal physiological processes (13). COX-2 has four sites for glycosylation with a subunit molecular mass of 72 kDa (14). Inhibition of glycosylation leads to a reduced molecular mass and activity of COX-2 (15). COX-2 contains potential glycosylation sites, three of which are always glycosylated and one that may or may not be glycosylated or not glycosylated (16). The glycosylation is able to determine the biologically role of COX (13). The glycosylation of COX-2 is inhibited by molecules such 2DG and TN, which are known to induce ER stress (12) by preventing glycosylation and glycolysis.

COX-2 contains four N-glycosylation sites, at Asn$^{68}$, Asn$^{104}$, Asn$^{144}$, and Asn$^{410}$, with consensus sequences (Asn-X-Ser/Thr) (17). Four mutants were constructed in ovine COX-2 by replacing the Asn at each glycosylation site with a Gln. These mutants were transiently transfected in COS-1 cells and the mobility of the mutant proteins were determined by Western blot analysis (16). Three of the mutant proteins, N68Q, N144Q, and N410Q, exhibited a 2 kDa decrease in the apparent subunit molecular mass when compared with non-transfected cells. Also, glycosylation of COX-2 at Asn$^{144}$ and either Asn$^{68}$ or Asn$^{144}$ is necessary for COX-2 activity during its synthesis.

SOV and PAO are known inhibitors of tyrosine phosphatases, which phosphorylate tyrosine residues on proteins. Protein phosphorylation at tyrosine residues is believed to regulate numerous cellular responses that are associated with signal transduction in a variety of cells (18). A previous report showed that SOV inhibits ER stress responses, such as induction of GRP78 and GADD153 (19). However, the underlying mechanisms have not been elucidated.

We initially found that treatment of 2DG led to a significant reduction in protein bands for tyrosine phosphorylation in chondrocytes (Fig. 1). It is likely that 2DG regulates the activity of PTPs through an unknown mechanism. Also, 2DG treatment induced a decrease in COX-2 expression and a reduction in the molecular mass of COX-2 (72-74 kDa) (Fig. 1).

In order to confirm the role of protein tyrosine phosphorylation in the expression of COX-2, cells were treated with a variety of compounds (2 μM SOV, 0.5 μM PAO, 40 μM GN, 5 ng/ml OA) that inhibit PTK, protein tyrosine phosphatase (PTP), and protein serine/threonine phosphatase (PPs) by different mechanisms, and the expression patterns of ER stress-related proteins, GRP94 and GADD153, and COX-2, were determined via Western blot analysis. The data from this analysis showed that inhibitors of PTPs, SOV, and PAO recovered expression and glycosylation of COX-2 in chondrocytes treated with 2DG (Fig. 1 and 3). On the other hand, ER stress inducible proteins, GRP94 and GADD153, which were induced by 2DG treatment, were markedly inhibited by inhibitors of PTPs (Fig. 1 and 3). OA did not show any effects on the expression or glycosylation of COX-2 in 2DG-treated cells (Fig. 1). These results indicated that the reduced expression and glycosylation of COX-2 by 2DG were modulated through protein tyrosine phosphorylation rather than protein phosphorylation at serine or threonine residues. Consistent with the expression and glycosylation of COX-2, the activity of COX-2 in articular chondrocytes was recovered by inhibitors, PTPs, SOV, and PAO (Fig. 2A).

Although some studies have indicated that the effect of SOV independently increases the activity of COX-2 through protein phosphorylation by rapidly stimulating prostaglandin production from arachidonic acid (AA) (6), we did not detect significant changes of COX-2 expression in SOV treated cells (data not shown). To investigate whether COX-2 itself is a substrate for protein tyrosine phosphorylation, immunoprecipitation using phosphotyrosine antibodies was performed. We did not find a COX-2 immunoreactive protein among the anti-phosphotyrosine-precipitated chondrocytes proteins (data not shown). Our data showed that PTP inhibitors recovered the decrease in COX-2 caused by 2DG treatment, while GN had no effect on the 2DG-induced reduction in COX-2 expression. However, PP2 treatment recovered the expression and glycosylation of COX-2. We investigated the effects of PP2 and GN on the levels of phosphotyrosine protein and COX-2. PP2 and GN showed different effects on the levels of phosphotyrosine protein and COX-2.

These results suggested that Src specific inhibitor PP2 might restore COX-2 expression via inactivation of Src kinase, which also inhibits protein tyrosine phosphatase 1B, a major tyrosine phosphatase (20).

Taken together, these data indicate that protein tyrosine phosphorylation acts as an important regulator for restoration of COX-2. However, the precise underlying mechanism of the effect of GN on COX-2 expression is not well understood, and future studies are needed.

**MATERIALS AND METHODS**

**Reagents**

Reagents were purchased from Sigma (St. Louis, MO, USA), Calbiochem (La. Jolla, CA, USA) and Biomol (Plymouth, PA, USA).
Culture of primary chondrocytes and experimental conditions
Joint cartilage of 2-week-old New Zealand White rabbits were dissociated for 6 h in 0.2% collagenase type II (381 units/ml of solid, Sigma) in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen, CA, USA). After collecting individual cells by brief centrifugation at 230 × g for 10 min and 20°C, the cells were suspended in DMEM supplemented with 10% (v/v) bovine calf serum (Invitrogen), 50 µg/ml streptomycin (Sigma), and 50 units/ml penicillin (Sigma). The cells were then plated on culture dishes at a density of 5 × 10^4 cells/cm². After 3 days in culture, the medium was replaced with a glucose-free medium and cells were treated with the indicated pharmacological reagents.

Western blot analysis
Whole cell lysates were prepared by extracting proteins using a buffer containing 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% Nonidet P-40, and 0.1% SDS supplemented with protease inhibitors and phosphatase inhibitors. Proteins were size-fractionated by SDS-PAGE and transferred to a nitrocellulose membrane. The nitrocellulose sheet was blocked with 5% non-fat dry milk in Tris-buffered saline. The following antibodies were used: anti-COX-2 (Cayman Chemical, Ann Arbor, MI, USA), anti-GRP94 (Santa Cruz), anti-GADD153 (Santa Cruz), anti-actin (Santa Cruz), anti-phospho-tyrosine (Cell Signaling). The cells were washed and incubated with secondary antibodies for 1 h and washed with PBS. DAPI was added to stain the nuclei, and the cells were observed under a fluorescence microscope.

Immunofluorescence staining
Cells were fixed in PBS containing 3.5% paraformaldehyde for 15 min at room temperature and permeabilized with 0.1% Triton X-100. The fixed cells were washed three times with PBS and incubated for 2 h with antibodies against anti-COX-2 (Cayman), anti-phospho-tyrosine (Cell Signaling) or anti-GRP94 (Santa Cruz). The cells were washed and incubated with secondary antibodies for 1 h and washed with PBS. DAPI was added to stain the nuclei, and the cells were observed under a fluorescence microscope.

Data analysis and statistics
The results are expressed as mean values with standard deviation. Values were calculated from a specified number of determinations. A one way-ANOVA test was used to compare individual treatments with their respective control values. Significance was defined at the P < 0.05 level.

Acknowledgements
This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea Government (MEST/2011-0005892 & 2009-0084569). Authorship: Yu, Seon-Mi: Designed experiments, conducted research and wrote manuscript. Kim, Song-Ja: Designed experiments, conducted research and analyzed data, wrote manuscript.

REFERENCES
1. Dubois, R. N., Abramson, S. B., Crofford, L., Gupta, R. A., Simon, L. S., Van De Putte, L. B. and Lipsky, P. E. (1998) Cyclooxygenase in biology and disease. FASEB J., 12, 1063-1073.
2. Amin, A. R., Attur, M., Patel, R. N., Thakker, G. D., Marshall, P. J., Rediske, J., Stuchin, S. A., Patel, I. R. and Abramson, S. B. (1997) Superinduction of cyclooxygenase-2 activity in human osteoarthritis-affected cartilage. Influence of nitric oxide. J. Clin. Invest., 99, 1231-1237.
3. Ambesi-Imponiambto, F. S. and Coon, H. G. (1979) Thyroid cells in culture. Int. Rev. Cytol. Suppl. 163-172.
4. Parfenova, H., Balabanova, L. and Leffler, C. W. (1998) Posttranslational regulation of cyclooxygenase by tyrosine phosphorylation in cerebral endothelial cells. Am. J. Physiol. 274, C72-81.
5. Mori, A., Yasuda, Y., Murayama, T. and Nomura, Y. (2001) Enhancement of arachidonic acid release and prostaglandin F(2alpha) formation by Na3VO4 in PC12 cells and GH3 cells. Eur. J. Pharmacol. 417, 19-25.
6. Hammond, C. and Helenius, A. (1995) Quality control in the secretory pathway. Curr. Opin. Cell. Biol. 7, 523-529.
7. Schroder, M. and Kaufman, R. J. (2005) ER stress and the unfolded protein response. Mutat. Res. 569, 29-63.
8. Lee, A. S. (2001) The glucose-regulated proteins: stress induction and clinical applications. Trends Biochem. Sci. 26, 504-510.
Tyrosine phosphorylation affects glycosylation of COX-2
Seon-Mi Yu and Song-Ja Kim

9. Rao, R. V., Ellerby, H. M. and Bredesen, D. E. (2004) Coupling endoplasmic reticulum stress to the cell death program. Cell Death Differ. 11, 372-380.
10. Hung, J. H., Su, I. J., Lei, H. Y., Wang, H. C., Lin, W. C., Chang, W. T., Huang, W., Chang, W. C., Chang, Y. S., Chen, C. C. and Lai, M. D. (2004) Endoplasmic reticulum stress stimulates the expression of cyclooxygenase-2 through activation of NF-kappaB and pp38 mitogen-activated protein kinase. J. Biol. Chem. 279, 46384-46392.
11. Jain, V. K., Kalia, V. K., Sharma, R., Maharajan, V. and Menon, M. (1985) Effects of 2-deoxy-D-glucose on glycolysis, proliferation kinetics and radiation response of human cancer cells. Int. J. Radiat. Oncol. Biol. Phys. 11, 943-950.
12. Yu, S. M. and Kim, S. J. (2010) Endoplasmic reticulum stress (ER-stress) by 2-deoxy-D-glucose (2DG) reduces cyclooxygenase-2 (COX-2) expression and N-glycosylation and induces a loss of COX-2 activity via a Src kinase-dependent pathway in rabbit articular chondrocytes. Exp. Mol. Med. 42, 777-786.
13. Sevigny, M. B., Li, C. F., Alas, M. and Hughes-Fulford, M. (2006) Glycosylation regulates turnover of cyclooxygenase-2. FEBS Lett. 580, 6533-6536.
14. Nemeth, J. F., Hochgesang, G. P., Jr., Marnett, L. J. and Caprioli, R. M. (2001) Characterization of the glycosylation sites in cyclooxygenase-2 using mass spectrometry. Biochemistry 40, 3109-3116.
15. Smith, W. L. and Marnett, L. J. (1991) Prostaglandin endoperoxide synthase: structure and catalysis. Biochim. Biophys. Acta. 1083, 1-17.
16. Otto, J. C., DeWitt, D. L. and Smith, W. L. (1993) N-glycosylation of prostaglandin endoperoxide synthases-1 and -2 and their orientations in the endoplasmic reticulum. J. Biol. Chem. 268, 18234-18242.
17. Merlie, J. P., Fagan, D., Mudd, J. and Needleman, P. (1988) Isolation and characterization of the complementary DNA for sheep seminal vesicle prostaglandin endoperoxide synthase (cyclooxygenase). J. Biol. Chem. 263, 3550-3553.
18. Seglen, P. O. and Gordon, P. B. (1981) Vanadate inhibits protein degradation in isolated rat hepatocytes. J. Biol. Chem. 256, 7699-7701.
19. Hosoi, T., Saito, A., Kume, A., Okuma, Y., Nomura, Y. and Ozawa, K. (2008) Vanadate inhibits endoplasmic reticulum stress responses. Eur. J. Pharmacol. 594, 44-48.
20. Bjørge, J. D., Pang, A. and Fujita, D. J. (2000) Identification of protein-tyrosine phosphatase 1B as the major tyrosine phosphatase activity capable of dephosphorylating and activating c-Src in several human breast cancer cell lines. J. Biol. Chem. 275, 41439-41446.