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Luteolin prevents PDGF-BB-induced proliferation of vascular smooth muscle cells by inhibition of PDGF β-receptor phosphorylation

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Abstract

Luteolin occurs as glycosylated forms in celery, green pepper, perilla leaf and camomile tea, and has been shown to possess antimutagenic, antitumorigenic, antioxidant and antiinflammatory properties. In this study, we have investigated the antiproliferable effect and its mechanism of luteolin on platelet-derived growth factor (PDGF)-BB-induced proliferation of rat aortic vascular smooth muscle cells (VSMCs). Luteolin significantly inhibited PDGF-BB-induced proliferation and DNA synthesis of rat aortic VSMCs in a concentration-dependent manner. In addition, flow cytometry analysis of DNA content revealed blocking of the PDGF-BB-inducible cell cycle progression by luteolin. Pre-incubation of rat aortic VSMCs with luteolin significantly inhibited the PDGF-BB-induced extracellular signal-regulated kinase 1/2 (ERK1/2), Akt and phospholipase C (PLC)-γ1 activation as well as *c-fos* gene expression. Consisted with these findings, luteolin inhibited PDGF-Rβ phosphorylation induced by PDGF-BB in a concentration-dependent manner. These results suggest that the inhibitory effect of luteolin on the PDGF-BB-induced proliferation of rat aortic VSMCs may be mediated by blocking phosphorylation of PDGF-Rβ.

Keywords: Luteolin; Vascular smooth muscle cell; Proliferation; Cell cycle; PDGF-Rβ

1. Introduction

Proliferation and migration of vascular smooth muscle cells (VSMCs) in the neointima are major events in the formation of atherosclerosis, including the process of restenosis after successful angioplasty [1,2]. Although the precise mechanisms of progression of atherosclerosis are uncertain, VSMCs appear to play a key role in the development of atherosclerosis. Since platelet-derived growth factor (PDGF) is one of the principal regulators of mitogenesis in VSMCs and the expression of PDGF is increased in atherosclerotic lesions, PDGF-induced mitogenesis and proliferation are shown to be prerequisites for intimal thickening after angioplasty [3].

PDGF binding to the PDGF-Rβ leads to its phosphorylation on multiple tyrosine residues. The activated PDGF-Rβ is associated with a number of SH2 domain-containing proteins including the p85 regulatory subunit of PI3-kinase and phospholipase C (PLC)-γ1. Of these downstream molecules of the PDGF-Rβ, involvement of Shc, Grb2, PLC-γ1 and PI3-kinase in PDGF-Rβ-induced mitogenesis has been relatively well characterized. PLC-γ1 and PI3-kinase pathways have also been shown to be important for PDGF-induced cell cycle progression in VSMCs [4]. PDGF-BB activates the extracellular regulated kinases 1 and 2 (ERK1/2) pathway with triggering phosphorylation and activation of Raf-1, (MEK, MAPK and ERK kinase) and ultimately ERK1 and ERK2 themselves. Activation of the ERK1 and ERK2 mitogen-activated protein kinases (MAPK) is required for mitogenic signaling through a number of tyrosine kinase growth factor receptors. ERK1/2 transduce mitogenic signals to the nucleus by phosphorylating and activating specific transcription factors, such as Elk-1, which induce expression of *c-fos* and

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; ERK, extracellular signal-regulated kinase; FBS, fetal bovine serum; FACS, fluorescence activated cell sorter; MAP, mitogen-activated protein; PDGF, platelet-derived growth factor; RASMC, rat aortic smooth muscle cell

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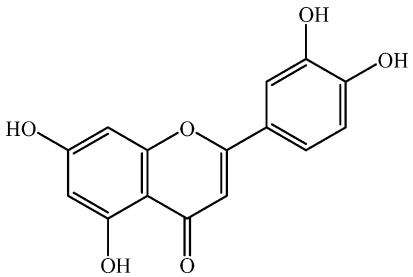

Fig. 1. Chemical structure of luteolin.

other early growth response genes that control the transition from quiescence to proliferation [5]. It has been reported that up-regulations of PDGF ligand and receptor gene expression are associated with the development and progression of proliferative cardiovascular diseases, including hypertension [6] and atherosclerosis [7,8]. Therefore, the regulatory mechanism of PDGF signaling in inhibition of VSMCs proliferation is one of the key pharmacological strategies for prevention of atherosclerosis.

Flavonoids are naturally occurring polyphenolic compounds presented in a variety of fruits, vegetables and seeds. Flavonoids have many biological and pharmacological activities including antioxidative, antiinflammatory and antitumor effects [9]. Luteolin (Fig. 1), a polyphenolic compound available in foods of plant origin, belongs to the flavone subclass of flavonoids, usually occurring as glycosylated forms in celery, green pepper, perilla leaf and camomile tea [10,11]. It has been reported to display antimutagenic, antiplatelet aggregation and anticancer effects [12,13]. However, the antiproliferative effect of luteolin on VSMCs is not yet clearly understood.

In this study, we have investigated the effect of luteolin on PDGF-BB-induced VSMCs proliferation. The intracellular signaling pathways responsible for the action of luteolin in PDGF-BB-induced VSMCs proliferation were also investigated.

2. Materials and methods

2.1. Materials and reagents

Luteolin (Sigma Chemical Co., MO, USA) was dissolved in DMSO and further diluted in DMEM without FBS. Cell culture materials were purchased from Gibco-BRL (MD, USA). Tyrophostin AG1295 was obtained from ALEXIS Biochemicals (CA, USA). U0126, LY294002 and U73122 were obtained from Tocris (BS, UK). Phospho-ERK1/2 antibody, phospho-Akt and phospho-PLC-γ1 antibodies were purchased from New England Biolabs (MA, USA). PDGF-BB and phospho-PDGF-Rβ polyclonal antibody were obtained from Upstate Biotechnology (NY, USA). Other chemicals were of analytical grade.

2.2. Cell culture

Rat aortic VSMCs (RASMCs) were isolated by enzymatic dispersion as previously described [14] according to the modified method of Chamley et al. [15]. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 100 IU/ml penicillin, 100 μg/ml streptomycin, 8 mM HEPES and 2 mM L-glutamine at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 incubator. The purity of VSMCs culture was confirmed by immunocytochemical localization of α-smooth muscle actin. The passage of VSMCs used in this experiment was 4–8.

2.3. Measurement of cell proliferation and DNA synthesis

The cell proliferation and DNA synthesis of RASMCs were measured as previously described [14]. In brief, for cell counting, RASMCs were seeded in 12-well culture plates at 1×10^5 cells/ml and cultured in DMEM with 10% FBS at 37 °C for 24 h. Under these conditions, a cell confluence of ~70% was reached. The medium was then replaced by serum-free medium containing luteolin. The cells were stimulated by 50 ng/ml PDGF-BB, and then trypsinized by trypsin-EDTA and counted by using hemocytometer under microscopy. For [^{3}H]-thymidine incorporation experiments, RASMCs were seeded in 24-well culture plates under the same conditions, and 2 μCi/ml of [^{3}H]-thymidine was added to the medium. The reactions were terminated by aspirating the medium and subjecting the cultures to sequential washes on ice with PBS containing 10% trichloroacetic acid and ethanol/ether (1:1, v/v). Acid-insoluble [^{3}H]-thymidine was extracted into 250 μl of 0.5 M NaOH/well, and this solution was mixed with 3 ml scintillation cocktail (Ultimagold, Packard Bioscience, CT, USA), and quantified using a liquid scintillation counter (LS3801, Beckman, Düsseldorf, Germany).

2.4. Cell cycle progression analysis

RASMCs in 60 mm^2 cell culture dish were incubated in DMEM medium without serum in the presence and absence of 50 μM luteolin for 24 h. RASMCs were then stimulated with 50 ng/ml PDGF-BB for 24 h. And then the cells were trypsinized and were centrifuged at 1500 × *g* for 7 min. The centrifuged pellets were suspended in 1 ml of 1 × PBS and washed twice. The centrifuged pellets were suspended by 70% ethanol and fixed overnight at 4 °C. The fixed cells were briefly vortexed and centrifuged at 15,000 × *g* for 5 min. The ethanol was discarded and the pellets were stained with 0.4 ml propidium iodide (PI) solution (50 μg/ml PI in sample buffer containing 100 μg/ml of RNase A). Before analysis by flow cytometry, each sample was incubated at room temperature for 1 h. The complex in each cell nucleus was measured by

FACSCalibur (Becton & Dickinson Co., USA). The number of dead cells was analyzed by lower fluorescence intensity and expressed as the percentage of total event and setting a marker (M1) on the sub-G_1 peak.

2.5. c-fos mRNA expression

The RNA extraction and analysis of VSMCs were performed as previously described [14]. In brief, total RNA was isolated from each region using a single-step method based on guanidine isothiocyanate–phenolchloroform extraction and TRIzol reagent (Gibco-BRL, MA, USA). RNA concentration was determined by measurement of absorbance at 260 nm. 1 μg of total RNA was reverse-transcribed to synthesize single strand cDNA by first-strand synthesis system kit (Gibco-BRL). Subsequently, 2 μl of the RT reaction was subjected to PCR in order to co-amplify a fragment of *c-fos* and GAPDH gene, which was used as an internal control [16]. The sequences of the oligonucleotide primers used for cDNA amplification were 5′-CCC CTG TCA ACA CAC AGG AC-3′ (sense) and 5′-GCA ATC TCG GTC TGC AAC GC-3′ (antisense) for *c-fos*, and 5′-CCT GCA CCA CCA ACT GCT TA-3′ (sense) and 5′-GAT GCC AGT GAG CTT CCC GT-3′ (antisense) for GAPDH. After an initial denaturation step at 94 °C for 5 min, the PCR (Eppendorf, Hamburg, Germany) reaction was performed for 24 cycles. The cycle profiles for *c-fos* and GAPDH genes amplification were 94 °C, 30 s; 58 °C, 30 s and 72 °C, 1 min. A final extension was performed at 72 °C for 5 min. The *c-fos* gene expression was normalized to GAPDH values. The intensities of bands were quantified using a Scion-Image for Window Program (Scion Corporation, Maryland, USA).

2.6. Immunoblotting assay

The immunoblotting of VSMCs was performed as previously described [14]. Briefly, RASMCs in 12-well plates were incubated in DMEM medium. Rat aortic VSMCs were pre-treated with luteolin for 24 h before the addition of 50 ng/ml PDGF-BB for 5 min for ERK 1/2 and PLC-γ1 phosphorylation assay, and for 15 min for Akt phosphorylation assay. The VSMCs were then lysed with SDS lysis buffer containing 62.5 mM Tris–HCl (pH 6.8), 2% SDS, 10% glycerol, 50 mM dithiothreitol (DTT) and protease inhibitor Cocktail Tablet (Roche, Mannheim, Germany). The lysates were centrifuged at 13,000 × *g* for 10 min, and the supernatants were collected. Protein determination of the supernatant was performed using a BCA Protein Assay Reagent (Pierce, Rockford, IL, USA) according to the manufacturer's manual using bovine serum albumin (BSA) as a standard. Proteins were separated in 7.5–10% SDS polyacrylamide gel (PAGE) using a Mini-Protein II System (Bio-Rad, CA, USA). Proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK) at 250 mA with a transfer buffer containing 25 mM Tris–HCl, 192 mM glysine and 20% methanol (pH 8.3). PVDF membrane was blocked with 5% BSA in TBS-T at room temperature for 1 h. The membrane was washed using TBS-T and incubated with primary phospho-ERK1/2, phospho-Akt, phospho-PLC-γ1 and phospho-PDGF-Rβ primary antibodies at 1:1000 dilution in BSA/TBS-T buffer at 4 °C for overnight and horseradish peroxidase-conjugated IgG secondary antibody (New England Biolabs, MA, USA) at 1:5000 dilution in BSA/TBS-T buffer at 4 °C for 3 h, respectively. After washing, the membrane was detected by chemiluminescent reaction (ECL plus kit, Amersham Pharmacia Biotech, Buckinghamshire, UK) followed by exposure of the membranes to Hyperfilm ECL (Amersham Pharmacia Biotech). The phospho-PDGF-Rβ, phospho-ERK1/2, phospho-Akt or phospho-PLC-γ1 was normalized by total α-actin values, respectively. The intensities of bands were quantified using a Scion-Image for Window Program.

2.7. Statistical analysis

The experimental results were expressed as mean ± S.E.M. A one-way analysis of variance (ANOVA) was used for multiple comparisons followed by Dunnett. Differences with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Effect of luteolin on PDGF-BB-induced proliferation of RASMCs

Luteolin inhibited PDGF-BB-induced proliferation of RASMCs in a concentration-dependent manner. The number of cells was significantly increased after 50 ng/ml PDGF-BB-treatment ($65.66 \pm 5.66 \times 10^4$ cells/well) compared to the non-stimulated group ($43.16 \pm 1.64 \times 10^4$ cells/well), and the increased cells were significantly reduced to 54.83 ± 1.69, 47.91 ± 1.45 and $38.41 \pm 2.20 \times 10^4$ cells/well at concentrations of 5, 20 and 50 μM, respectively (Fig. 2A). Treatment of luteolin (50 μM) for 24 h did not show any cytotoxicity to RASMCs (data not shown), indicating that antiproliferative effect of luteolin on RASMCs was not resulted from cytotoxicity.

3.2. Effect of luteolin on PDGF-BB-induced DNA synthesis of RASMCs

The effect of luteolin on DNA synthesis was assayed by using [^{3}H]-thymidine incorporation. As shown in Fig. 2B, 50 ng/ml PDGF-BB potently increased [^{3}H]-thymidine incorporation from 2360 to 27,025 cpm/well. Luteolin significantly inhibited the PDGF-BB-induced DNA synth-

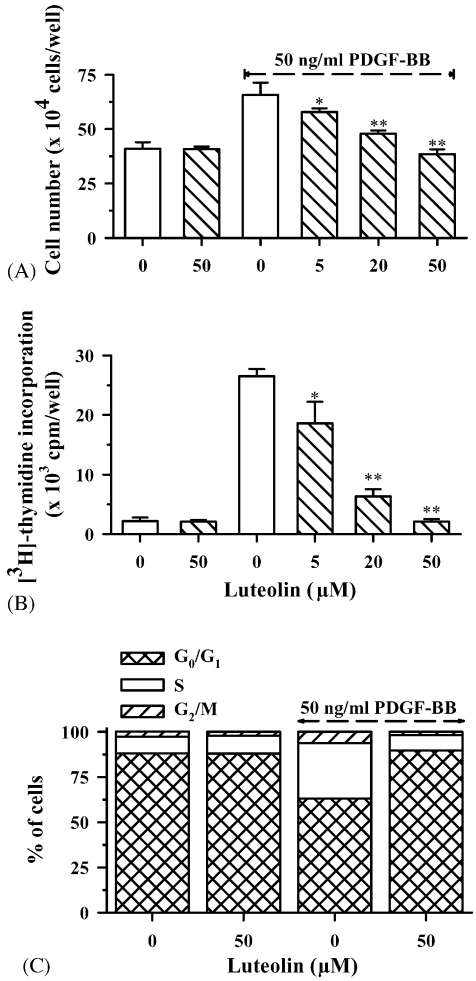

Fig. 2. Effect of luteolin on DNA synthesis, proliferation and cell cycle progression induced by PDGF-BB in RASMCs. (A) RASMCs were pre-cultured in serum-free medium in the presence or absence of indicated concentration of luteolin for 24 h, and then stimulated with 50 ng/ml PDGF-BB. The cells were trypsinized, and were counted using hemocytometer after 24 h. (B) RASMCs were pre-cultured in serum-free medium in the presence or absence of indicated concentration of luteolin for 24 h, and then stimulated with 50 ng/ml PDGF-BB for 20 h. [^{3}H]-thymidine (2 μCi/ml) was added to the medium and incubated for 4 h. The radioactivity was determined by liquid scintillation counter. (C) Cells were pre-cultured in the presence or absence of luteolin in serum-depleted medium for 24 h, and then RASMCs were stimulated with 50 ng/ml PDGF-BB. After 24 h, individual nuclear DNA content is as reflected by fluorescence intensity of incorporated propidium iodide. Each item is derived from a representative experiment, where data from at least 11,000 events were obtained. Data are expressed as mean ± S.E.M. ($n = 4$); $^*P < 0.05$, $^{**}P < 0.01$ compared with PDGF-BB alone.

esis in a concentration-dependent manner. The inhibition percentages were 28.9 ± 8.3, 71.0 ± 3.2 and 91.7 ± 2.3% at the concentrations of 5, 20 and 50 μM, respectively.

3.3. Effect of luteolin on cell cycle in synchronized RASMCs

Effects of luteolin on cell cycle progression were also analyzed by flow cytometry analysis (Fig. 2C). The serum-deprivation of VSMCs in primary culture for 24 h resulted in an approximately 88.2 ± 0.6% synchronization of the cell cycle in the G_0 phase. The percentage of cells in S phase was increased from 9.1 ± 1.5 to 30.6 ± 0.1% for 24 h after PDGF-BB was added. In contrast, luteolin-treated cells showed a significant blocking of cell cycle progression. Luteolin reduced the percentage of cells in S phase to 8.5 ± 1.0% at a concentration of 50 μM. This finding indicates that luteolin may act at the early events of the cell cycle to be effective against DNA synthesis.

3.4. Effect of luteolin on c-fos expression of RASMCs

PDGF-BB was known to stimulate a transient expression of *c-fos* mRNA, which is an early response gene involved in cell proliferation [16]. Therefore, we examined whether luteolin can suppress the *c-fos* mRNA transcription induced by PDGF-BB. RASMCs were pre-treated with luteolin for 24 h and then treated by 50 ng/ml PDGF-BB for 30 min. Consistent with the inhibitory effect of luteolin on RASMCs proliferation, the *c-fos* mRNA expression was also decreased by luteolin in a concentration-dependent manner. The inhibitions of the *c-fos* mRNA expression were 20.3 ± 5.4, 60.4 ± 3.1 and 91.4 ± 3.2% at concentrations of 5, 20 and 50 μM, respectively (Fig. 3).

3.5. Effects of luteolin on ERK1/2, Akt and PLC-γ1 phosphorylations of RASMCs

To examine the underlying mechanism of the antiproliferative effect exerted by luteolin, we tested whether luteolin affects the activation of the MAP kinases (ERK1/2) in view of their importance in the regulation of VSMCs proliferation. Luteolin significantly inhibited ERK1/2 phosphorylation induced by PDGF-BB in a concentration-dependent manner, with inhibition percentages of 27.2 ± 4.0, 43.1 ± 2.7 and 88.5 ± 5.8% at the concentrations of 5, 20 and 50 μM, respectively (Fig. 4A). Pre-

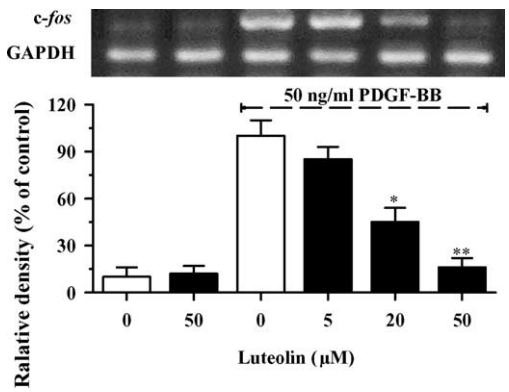

Fig. 3. Effect of luteolin on *c-fos* mRNA expression in RASMCs. Confluent cells were pre-cultured in the presence or absence of luteolin (5–50 μM) in serum-free medium for 24 h, and then stimulated by 50 ng/ml PDGF-BB for 30 min. The RT-PCR was performed as described in Section 2. GAPDH gene was used for normalization. After densitometric quantification, data are expressed as mean ± S.E.M. ($n = 4$); $^*P < 0.05$, $^{**}P < 0.01$ compared with PDGF-BB alone.

treatment with luteolin also inhibited Akt phosphorylation induced by PDGF-BB in a concentration-dependent manner, and the inhibitions were 28.2 ± 5.4, 44.4 ± 3.3 and 87.4 ± 4.0% at the concentrations of 5, 20 and 50 μM, respectively (Fig. 4B). Similarly, PDGF-BB-induced PLC-γ1 phosphorylation was decreased to 41.7 ± 13.4, 56.8 ± 11.1 and 94.6 ± 5.2% at concentrations of 5, 20 and 50 μM, respectively (Fig. 4C). U0126 (ERK1/2 inhibitor; 10 μM), LY294002 (Akt inhibitor; 50 μM) and U73122 (PLC inhibitor; 10 μM) were used as the positive controls, and also significantly inhibited ERK1/2, Akt and PLC-γ1 phosphorylation, respectively.

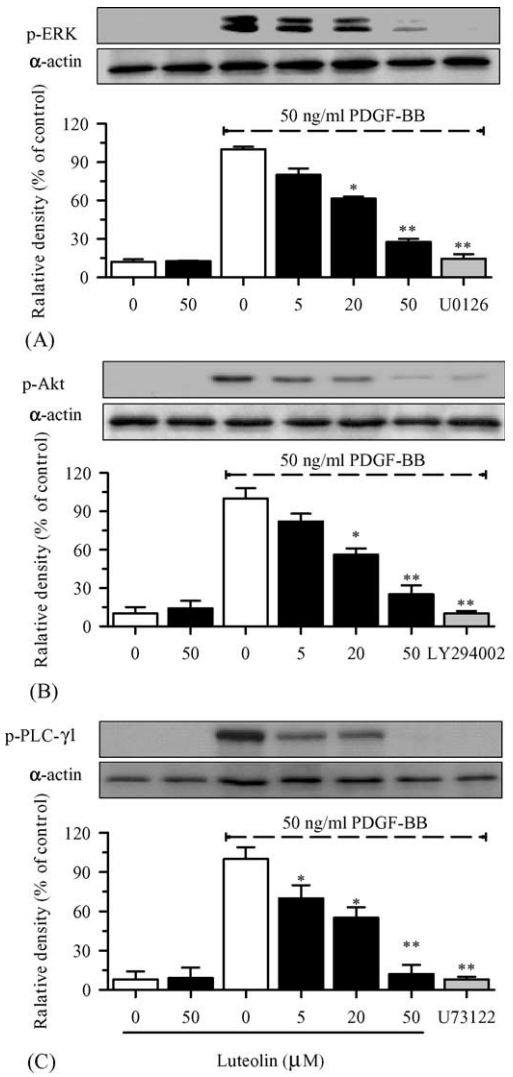

Fig. 4. Effect of luteolin on the phosphorylation of ERK1/2 (A), Akt (B) and PLC-γ1 (C) in PDGF-BB-stimulated RASMCs. Confluent cells were pre-cultured in the presence or absence of indicated concentration of luteolin, U0126 (ERK1/2 inhibitor, 10 μM), LY294002 (Akt inhibitor, 50 μM) or U73122 (PLC inhibitor, 10 μM) in serum-free medium for 24 h. The cells were briefly stimulated with 50 ng/ml PDGF-BB for different time periods (5 min for ERK1/2 and PLC-γ1 phosphorylation, and 15 min for Akt phosphorylation). Cells were lysed, and quantitative proteins were analyzed using 7.5–10% SDS–PAGE and immunoblotting. After densitometric quantification, data are expressed as mean ± S.E.M. ($n = 4$); $^{*}P < 0.05$, $^{**}P < 0.01$ compared with PDGF-BB alone.

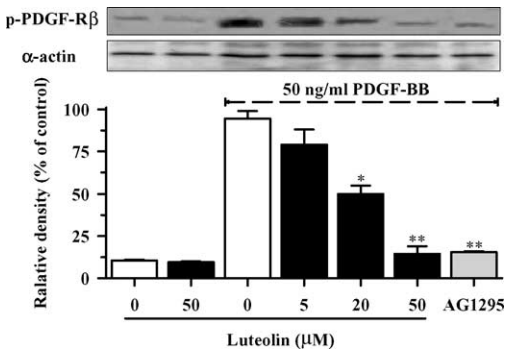

Fig. 5. Effect of luteolin on PDGF-Rβ phosphorylation in RASMCs. Confluent cells were pre-cultured in the presence or absence of indicated concentrations of luteolin or AG1295 (20 μM) in serum-free medium for 24 h, and then stimulated briefly by 50 ng/ml PDGF-BB for 1 min at 37 °C. Cells were lysed, and quantitative proteins were analyzed using 7.5% SDS–PAGE and immunoblotting. Relative activities were quantified by scanning densitometry and showed the levels of each activity as relative value of the total PDGF-Rβ. Western blot was repeated three times. Data are expressed as mean ± S.E.M. ($n = 3$); $^{*}P < 0.05$, $^{**}P < 0.01$ compared with PDGF-BB alone.

3.6. Effect of luteolin on PDGF-Rβ phosphorylation of RASMCs

Luteolin was shown to inhibit the downstream components of PDGF-BB such as ERK1/2, Akt and PLC-γ1 phosphorylation with a similar pattern. Thus, PDGF-Rβ phosphorylation, an upstream of PDGF-BB signaling may be a direct target for luteolin and lead to the inhibition of RASMCs proliferation. Pre-treatment with luteolin significantly inhibited PDGF-Rβ tyrosine phosphorylation induced by PDGF-BB in a concentration-dependent manner, with the inhibition percentages of 25.2 ± 8.8, 50.1 ± 6.5 and 93.4 ± 4.9% at the concentrations of 5, 20 and 50 μM, respectively (Fig. 5). AG1295 at 20 μM, a positive control and selective inhibitor of PDGF-Rβ tyrosine phosphorylation also significantly inhibited PDGF-Rβ tyrosine phosphorylation.

4. Discussion

Vascular smooth muscle cells are the main component of arterial wall, and abnormal proliferation of VSMCs plays a central role in the pathogenesis of atherosclerosis and restenosis after angioplasty, and possibly in the development of hypertension [2]. Therefore, we have investigated the antiproliferative effect and its mechanism of luteolin on VSMCs as an antiproliferating agent for a potential preventive or therapeutic agent of the cardiovascular disease including atherosclerosis.

In the present study, we found that luteolin inhibited rat aortic VSMCs proliferation and DNA synthesis in response to PDGF-BB (Fig. 2A and B). The inhibitory effect of luteolin on incorporation of [^{3}H]-thymidine into the cells gradually declined as the delay between the treatments of

RASMCs with luteolin and PDGF-BB. In addition, the antiproliferative effect of luteolin on RASMCs was not due to cellular cytotoxicity or apoptosis, which were demonstrated by MTT assay and flow cytometry assay (data not shown).

Recent studies [15] have emphasized the role of G-S events in the regulation of cell proliferation through complex stimulant and inhibitory signals driven by cyclin-dependent kinases and their inhibitors, respectively. At the G_0/G_1 transition, the expression of several transcription factors like *c-myc*, *c-myb* and *c-fos* appears to be fundamental [17]. To further investigate the pattern of the antiproliferative effect of luteolin, flow cytometry analysis was performed. As revealed by flow cytometry assay, the antiproliferative effect of luteolin was associated with an accumulation of cells in G_0/G_1 phase of the cycle (Fig. 2C). Since the observed accumulation in G_0/G_1 reflected a specific effect of luteolin on cell cycle progression rather than a decrease of cell number due to apoptosis. We therefore hypothesized that the antiproliferative properties of luteolin are causally related to the modulation of signaling cascade involved in cell cycle progression and proliferation.

MAP kinase is an important player in the early intracellular mitogenic signal transduction for cell growth. Among the MAP kinase family, ERK1/2 has been implicated in proliferation of various cell types [18]. Therefore, we examined whether the anti-proliferation of luteolin could act through down regulation of ERK1/2 cascade. Luteolin inhibited the PDGF-BB-induced ERK1/2 activation, and its downstream *c-fos* gene expression (Figs. 3 and 4A). These data show that the blocking of ERK1/2 and *c-fos* signal pathway may be important in the antiproliferating activity of luteolin. In addition, we also determined the phosphorylated PLC-γ1 and Akt, both of which were also activated by PDGF-BB. As shown in Fig. 4B and C, luteolin showed a marked decrease in the PDGF-BB-induced phosphorylation of PLC-γ1 and Akt in the same pattern as luteolin inhibited ERK1/2 phosphorylation. These data indicated that PDGF-Rβ might be a potential target for luteolin. In fact, luteolin inhibited PDGF-Rβ phosphorylation in a concentration-dependent manner (Fig. 5). These results are also agreed with other studies showing that carvedilol compound inhibited VSMCs proliferation, which was due to the inhibition of PDGF-Rβ tyrosine phosphorylation and its downstream intracellular signal transductions [19]. The precise mechanism in response to the inhibition of PDGF-Rβ phosphorylation by luteolin remains unknown. It is possible that like tyrphostin AG1296, luteolin may induce conformational changes at the ATP-binding site of the PDGF-Rβ, thereby inhibiting its tyrosine phosphorylation [20]. It is also possible that like epigallocatechin-3 gallate (EGCG), a well-known flavonoid, luteolin may incorporate into cell surface membranes, leads to a non-displaceable binding of PDGF-BB to non-receptor sites resulting in a reduced PDGF binding to the respective receptors [21]. Further study to reveal the molecular mechanism is in preparation. In addition, the oxidant species scavenging activity of luteolin may also contribute to the antiproliferative effect of luteolin on PDGF-BB-induced VSMCs proliferation since hydrogen peroxide (H_2O_2) plays a role in signaling transduction induced by PDGF.

Taken together, these results show that luteolin inhibits the proliferation and DNA synthesis of RASMCs induced by PDGF-BB, which is mediated by inhibition of PDGF-Rβ phosphorylation and its downstream signal transductions. This beneficial property of luteolin may be important in prevention of atherosclerosis in which proliferation of VSMCs is an important determinant of atherosclerotic plaque development and stability.

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