Naringin inhibits growth and induces apoptosis by a mechanism dependent on reduced activation of NF-κB/COX-2-caspase-1 pathway in HeLa cervical cancer cells

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Abstract. Naringin (NRG), a bioflavonoid found in citrus fruit extracts, has been pharmacologically evaluated as a potential anticancer agent. This study confirmed a novel mechanism of the anticancer effects of NRG in the human cervical cancer HeLa cell line (HeLa cells). Exposure of HeLa cells to NRG resulted in growth inhibition, as evidenced by a decrease in cell viability. In addition, NRG treatment induced apoptosis, as indicated by the increased apoptotic percentage and the cleaved caspase-3 expression. Importantly, exposure of the cells to NRG attenuated the expression levels of phosphorylated (p) nuclear factor κB (NF-κB) p65 subunit, cyclooxygenase-2 (COX-2) and cysteinyl aspartate proteinase-1 (caspase-1). Treatment with PDTC (an inhibitor of NF-κB) or NS-398 (an inhibitor of COX-2) or SC-3069 (an inhibitor of caspase-1) markedly induced growth inhibition and apoptosis. Treatment with PDTC or NS-398 also reduced caspase-1 expression. Interestingly, PDTC treatment blocked the expression of COX-2 and NS-398 reduced the p-NF-κB p65 expression. Taken together, this study provides novel evidence that NRG induces growth inhibition and apoptosis by inhibiting the NF-κB/COX-2-caspase-1 pathway and that a positive interaction between NF-κB and COX-2 pathway contributes to the growth and antiapoptotic effect in HeLa cells.

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Introduction

Cervical cancer contributes to be an important world health problem for women. It is the third most commonly diagnosed cancer and the fourth cause of cancer death in females worldwide. The role of human papillomavirus (HPV) infection has been extensively investigated and considered as the pathogenesis of both cervical cancer and its precursor damage (1). Of note, increasing evidence indicates that additional non-viral molecular pathways are involved in the initiation and progression of the desease. Thus, to explore the effects of the signaling pathways on cell growth and apoptosis is useful to identify the underlying mechanisms of carcinogenesis and to find the potential therapeutic targets.

Nuclear factor-κB (NF-κB) is a transcription factor that controls numerous genes regulating various cell functions (2). NF-κB family is composed five subunits, including p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), RelB and c-Rel, each of which may form homo- or heterodimers. Among them, nuclear translocation of p65 subunit is a key step in the activation of NF-κB (3). NF-κB signaling pathway plays important roles in general inflammatory response and cell death (4-6). We have recently shown that the inhibition of NF-κB reduces the levels of inflammatory factors, such as IL-6 and IL-8, as well as COX-2 in chemical hypoxia-treated HaCat cells (4,5). Furthermore, it has reported that NF-κB has a critical role in malignancies related to chronic inflammation, due to the activation of genes that promote cell proliferation, angiogenesis and survival (7,8). The activation of NF-κB pathway triggered by HPV 16E5, E6 and E7 oncoproteins is associated with cervical carcinogenesis and progression (9). NF-κB is constitutively activated, which promotes human cervical cancer progression and poor prognosis (10,11). Notably, NF-κB is also involved in overexpression of COX-2 by HPV 16E5 (9).

COX is the rate-limiting enzyme in the arachidonic acid cascade that produces prostaglandins (PG) (12). There are

two isoforms of COX. COX-1 is constitutively expressed in almost all tissues and involved in maintaining homeostasis. In contrast, COX-2 is induced immediately in response to inflammatory stimuli including mitogens, growth factors and cytokine (13). Increasing evidence has indicated that COX-2 levels are elevated in several malignancies, such as liver, colon, prostate, gall bladder, lung, skin and gynecologic cancer (14-21). Accumulating studies have demonstrated that overexpressed COX-2 in cervical cancer is related to lymph node metastasis (22) and resistance to radiation therapy and chemotherapy (21,23). In addition, oxyphenbutazone (a non-selective COX inhibitor) treatment markedly improves 5-year survival rates in cervical cancer patients receiving radiation therapy (24). A recent study also reported that celecoxib, a selective COX-2 inhibitor, radiosensitizes the human cervical cancer HeLa cells (25). Although NF-κB has been shown to be a positive regulator of COX-2 expression in response to various cytokines and growth factors in some cell types (26,27), such as in human colorectal cancer cells (27), whether COX-2 was implicated in the regulation of NF-κB expression in cervical cancer cells in unclear.

Another signal molecule involved in the activation of NF-κB is caspase-1. Caspase-1, also known as interleukin 1β (IL-1β) converting enzyme, is an initiator caspase that is originally the active proinflammatory cytokine IL-1β (28). Caspase-1 has been shown to be an activator of NF-κB in B cells (29), whereas caspase-1 knockout macrophages have decreased NF-κB activity (30). Although generally categorized as cytokine-processing caspase, caspase-1 also plays important roles in apoptosis in some cells, such as neurons (31), human ovarian cancer cells (32), prostate cancer cells (33) and pancreatic cancer cells (34). However, it is unknown what is the relationship among caspase-1, NF-κB and COX-2, and what are the roles of caspase-1 in cell growth and apoptosis in cervical cancer cells.

Flavonoids are a large class of natural polyphenolic compounds, existing in a wide variety of fruits and vegetables regularly consumed by humans. Naringin (NRG, the glycoside of the flavonone, naringenin), one of the most abundant flavonoids in grape fruit and other citrus fruits, has been reported to have multiple pharmacological effects, for example, antioxidant (35,36), anti-inflammatory (36,37), anti-apoptotic (35,38,39) and antiviral (40) effects. Importantly, recent studies have demonstrated that increased dietary consumption of NRG is related to attenuation of the risk of certain cancers, such as breast cancer and lung cancers (41,42). In addition, the findings from *in vivo* and *in vitro* studies indicated that NRG suppresses colon cancer cells (43), urinary blader cancer cells (44), human breast cancer cells (45) and human cervical cancer (SiHa) cells (46). Although many studies have analyzed the effects of NRG on the tumor growth inhibition and apoptosis induction, in several cell lines (41,46), the signaling pathways involved in these effects on cancer cells remain to be investigated.

The present study soughs to explore: i) the roles of NF-κB-COX-2/caspase-1 pathway in regulating cell growth and apoptosis; ii) the roles of inhibition of this pathway in NRG-induced cell growth inhibition and apoptosis in cervical cancer (HeLa) cells. This study demonstrated that NRG induces not only cell growth inhibition, but also apoptosis by inhibiting the NF-κB-COX-2/caspase-1 pathway in cervical cells.

Materials and methods

Materials. Naringin, Hoechst 33258 and NS-398 were purchased from Sigma-Aldrich (St. Louis, MO, USA). The cell counter kit-8 (CCK-8) was supplied by Dojindo Laboratories (Kumamoto, Japan). Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were obtained from Gibco-BRL (Grand Island, NY, USA). Anti-COX-2 antibody, anti-p-NF-κB p65 antibody, anti-total (t)-NF-κB p65 antibody, anti-caspase-1 antibody and anti-caspase-3 were purchased from Cell Signaling Technology (Boston, MA, USA), HRP-conjugated secondary antibody and BCA protein assay kit were obtained from KangChen Bio-tech, Inc (Shanghai, China). Enhanced chemiluminescence (ECL) solution was purchased from KeyGen Biotech (Nanjing, China).

Culture and treatments. The HeLa cells, a human cervical cancer HeLa cell line, were supplied by Sun Yat-sen University Experimental Animal Center (Guangzhou, China). The cells were cultured in DMEM medium supplemented with 10% FBS at 37˚C under an atmosphere of 5% CO_2 and 95% air.

To explore the anticancer effects of NRG on growth and apoptosis, HeLa cells were treated with 1,000 μmol/l NRG for the indicated times. To determine the roles of NF-κB, COX-2 and caspase-1 in the growth and apoptosis, HeLa cells were treated with 1,000 μmol/l PDTC (an inhibitor of NF-κB) or 10 μmol/l NS-398 (an inhibitor of COX-2) or 0.1 μg/ml SC-3069 for 24 h.

Cell viability assay. HeLa cells were cultured in 96-well plates at a concentration of $1x10^4$/ml, the CCK-8 assay was employed to assess the cell viability of HeLa cells. After the indicated treatments, 10 μl CCK-8 solution at a 1/10 dilution was added to each well and then the plate was incubated for 1.5 h in the incubator. Absorbance at 450 nm was tested with a microplate reader (Molecular Devices, Sunnyvale, CA, USA). The means of the optical density (OD) of five wells in the indicated groups were used to calculate the percentage of cell viability according to the formula below: cell viability (%) = (OD treatment group/OD control group) x100%. The experiment was repeated in triplicate.

Hoechst 33258 nuclear staining for assessment of apoptosis. Apoptotic cell death was measured by the Hoechst 33258 staining followed by photofluorography. Firstly, HeLa cells were plated in 35-mm dishes at a density of $1x10^6$ cells/well. After the indicated treatments, HeLa cells were fixed with 4% paraformaldehyde in 0.1 mol/l phosphate-buffered saline (PBS, pH 7.4) for 15 min. And then the slides were washed three times with PBS. After staining by 5 mg/ml Hoechst 33258 for 10 min, HeLa cells were washed three times with PBS. The cells were visualized under a fluorescence microscope (Bx50-FLA; Olympus, Tokyo, Japan). Viable HeLa cells displayed a uniform blue fluorescence throughout the nucleus and normal nuclear size, however, apoptotic HeLa cells showed condensed, distorted or fractured nuclei. The experiment was carried out in triplicates.

Western blot assay for expression of protein. After the indicated treatments, HeLa cells were harvested and lysed with cell lysis solution at 4˚C for 30 min. The total proteins were quantified using the BCA protein assay kit. Loading buffer was added to cytosolic extracts, and boiled for about 5 min, then the same amount of supernatant from each sample were fractionated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and the total proteins were transferred into polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% fat-free milk for 1 h in fresh blocking buffer [0.1% Tween-20 in Tris-buffered saline (TBS-T)] at room temperature, and incubated with either anti-COX-2 antibody (1:1,000 dilution), anti-p-NF-κB p65 antibody (1:1,000 dilution), anti-total (t)-NF-κB p65 antibody (1:1,000 dilution), anti-caspase-1 antibody (1:1,000 dilution) or anti-caspase-3 antibody (1:1,000 dilution) in freshly prepared TBS-T with 3% free-fat milk overnight with gentle agitation at 4˚C. The membranes were washed 3 times with TBST and then incubated with a secondary antibody for 1.5 h at room temperature in TBST with 3% fat-free milk [horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody, 1:2,500 dilution; Kangchen Biotech, Shanghai, China]. Then membranes were washed three times with TBST for 5 min. The immunoreactive signals were visualized by using enhanced chemiluminescence (ECL) detection. In order to quantify the protein expression, the X-ray film was scanned and analyzed with ImageJ 1.47 i software. The experiment was repeated 3 times.

Statistical analysis. All data are presented as the mean ± SEM. Differences between groups were analyzed by one-way analysis of variance (ANOVA) by using SPSS 13.0 (SPSS, Chicago, IL, USA) software, and followed by LSD post hoc comparison test. A significance of $p<0.05$ was considered significant.

Results

Naringin attenuates the expression of phosphorylated NF-κB p65 in HeLa cells. In order to observe the effect of NRG on activation of NF-κB pathway, HeLa cells were treated with 1,000 μmol/l NRG for 3, 6, 9, 12 and 24 h, respectively. As shown in Fig. 1, the expression level of phosphorylated (p) NF-κB p65 was markedly reduced after exposure of the cells to NRG for the indicated times. The maximal inhibition of p-NF-κB p65 expression appeared after exposure to NRG for 12 to 24 h. However, exposure of HeLa cells to 1,000 μmol/l NRG did not alter the total (t) expression of NF-κB p65 at the indicated times.

Naringin ameliorates the expression of COX-2 in HeLa cells. Similarily, the expression of COX-2 was significantly depressed after exposure of HeLa cells to 1,000 μmol/l NRG for the indicated times (3, 6, 9, 12 and 24 h), respectively. The maximal inhibition of COX-2 expression by NRG was recorded at 12 to 24 h (Fig. 2).

Naringin inhibits growth in HeLa cells. To explore the effect of NRG on growth of HeLa cells, we observed the effect of NRG at different concentrations (200, 400, 600, 800, 1,000 and 1,500 μmol/l). As shown in Fig. 3A, HeLa cells were exposed to the indicated concentrations of NRG for 24 h. At the range from 400 to 1,500 μmol/l, NRG dose-dependently attenuated the cell viability of HeLa cells. NRG at 1,000 μmol/l reduced the cell viability to 51.1-3.1% ($p<0.01$), compared with the control group. Based on these data, NRG at 1,000 μmol/l was used to perform a time response experiment on growth of HeLa cells. As presented in Fig. 3B, the cell viability was time-dependently inhibited after exposure of HeLa cells to 1,000 μmol/l RNG for the indicated times (12, 24, 36 and 48 h).

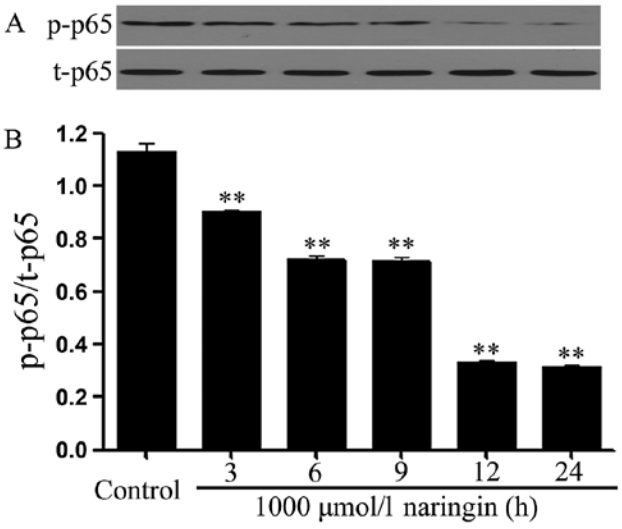

Figure 1. Naringin alleviates the activation of NF-κB in HeLa cells. The cells were exposed to 1,000 μmol/l naringin (NRG) for the indicated times. The expression of p-NF-κB p65 was (A) measured by western blot assay and (B) quantified by densitometric analysis with Image J 1.47i software. Data are shown as the means ± SE (n=3). **p<0.01, compared with the control group. p, phosphorylated; t, total.

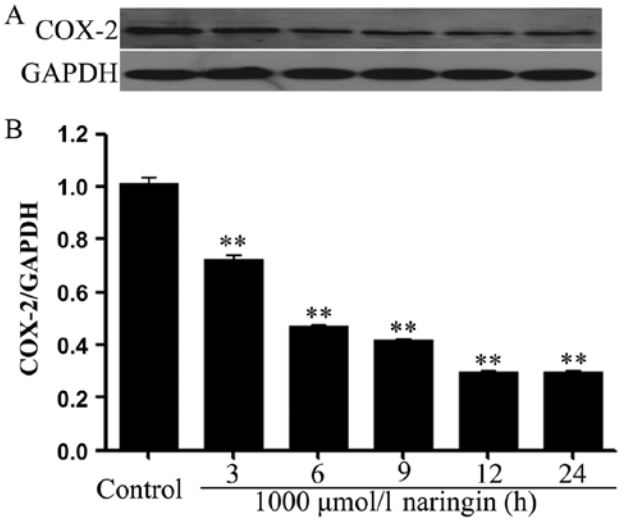

Figure 2. Naringin inhibits the expression of COX-2 in HeLa cells. The cells were treated with 1,000 μmol/l naringin (NRG) for the indicated times. The expression level of COX-2 was (A) tested by western blot assay and (B) quantified by densitometric analysis with Image J 1.47i software. Data are shown as the means ± SE (n=3). **p<0.01, compared with the control group.

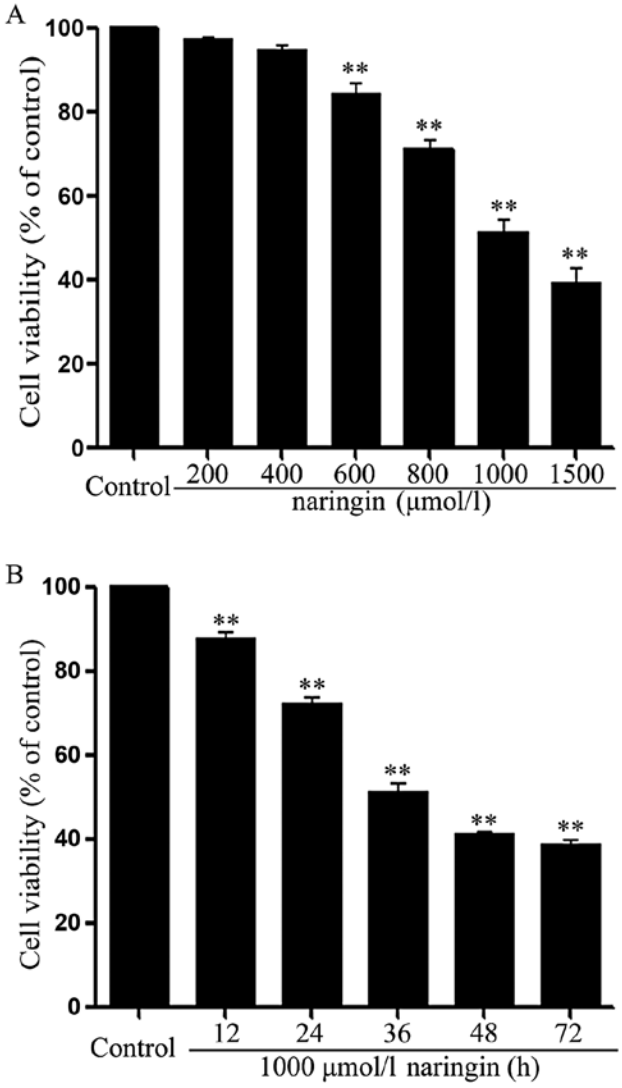

Figure 3. Naringin suppresses growth of HeLa cells. HeLa cells were treated with (A) NRG at the indicated concentrations for 24 h or (B) with 1,000 μmol/l NRG for the indicated times. Cell viability was measured by CCK-8 assay. Data are presented as the means ± SE (n=6). **p<0.01, compared with the control group.

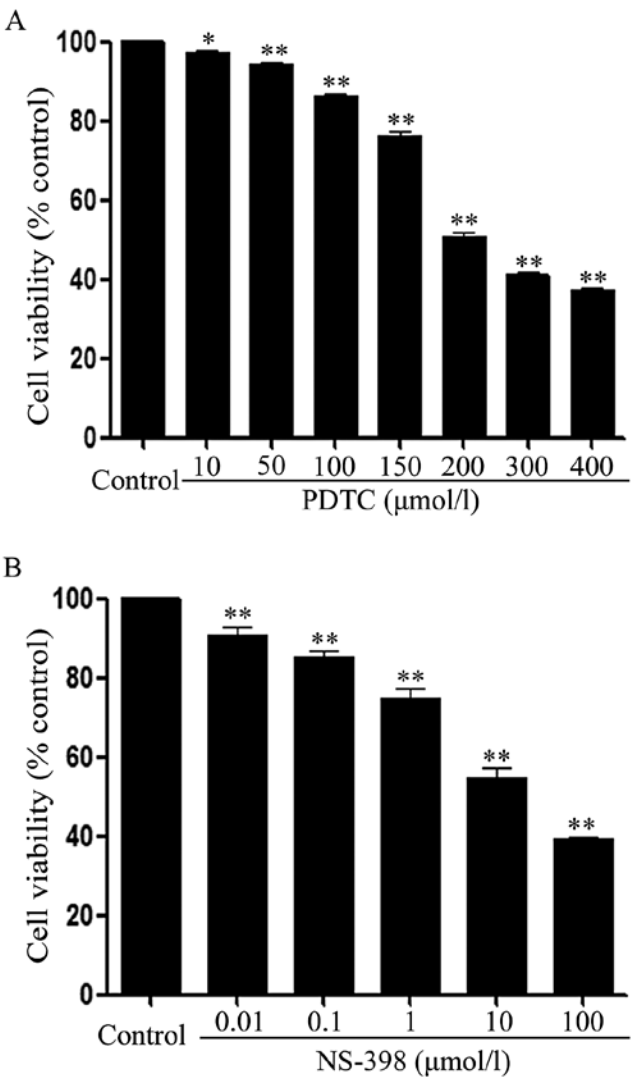

Figure 4. Both NF-κB and COX-2 pathways are involved in growth of HeLa cells. HeLa cells were exposed to the indicated concentrations of (A) PDTC or (B) NS-398 for 36 h, respectively. Cell viability was detected by CCK-8 assay. Data are presented as the means ± SE (n=6). *p<0.05, **p<0.01, compared with the control group.

NF-κB inhibitor and COX-2 inhibitor block growth of HeLa cells. To investigate the roles of both NF-κB and COX-2 pathways in the inhibitory effect of NRG on HeLa cell growth, the effects of PDTC (an inhibitor of NF-κB) and NS-398 (an inhibitor of COX-2) on the growth were observed. As shown in Fig. 4A, after HeLa cells were exposed to different concentrations (10, 50, 100, 150, 200, 300 and 400 μmol/l) of PDTC for 36 h, the cell viability was decreased in a dose-dependent manner. Similarly, exposure of the cells to the indicated concentrations (0.01, 0.1, 1, 10 and 100 μmol/l) of NS-398 for 36 h dose-dependently attenuated the cell viability (Fig. 4B).

Naringin, PDTC and NS-398 induce apoptosis in HeLa cells. As shown in Fig. 5B, exposure of HeLa cells to 1,000 μmol/l NRG for 24 h induced typical characteristics of apoptosis, as evidenced by the condensation of chromatin, the shrinkage of nuclei and the formation of apoptotic bodies. Similar to the apoptotic effect of NRG, exposure of the cells to 200 μmol/l PDTC or 10 μmol/l NS-398 for 24 h also enhanced the number of apoptotic HeLa cells (Fig. 5C and E).

On the other hand, the effect of NRG on the expression of cleaved caspase-3 (an apoptotic effector) was observed. As illustrated in Fig. 5F and G, exposure of HeLa cells to 1,000 μmol/l NRG for the indicated times (3, 6, 9, 12 and 24 h) markedly upregulated the expression level of cleaved caspase-3, peaking at 9 h. Similarly, exposure of the cells to 200 μmol/l PDTC or 10 μmol/l NS-398 for 24 h also increased the expression level of cleaved caspase-3, respectively (Fig. 5H and I).

Naringin, PDTC and NS-398 downregulate the expression of caspase-1 in HeLa cells. Caspase-1 is one member of inflammasome family (47,48), which has been reported to contribute to tumor growth. Thus, we explored the influences of NRG, PDTC and NS-398 on caspase-1 expression in HeLa cells. As shown in Fig. 6, expression of caspase-1 was observed in the cells. Of note, exposure of the cells to 1,000 μmol/l NRG

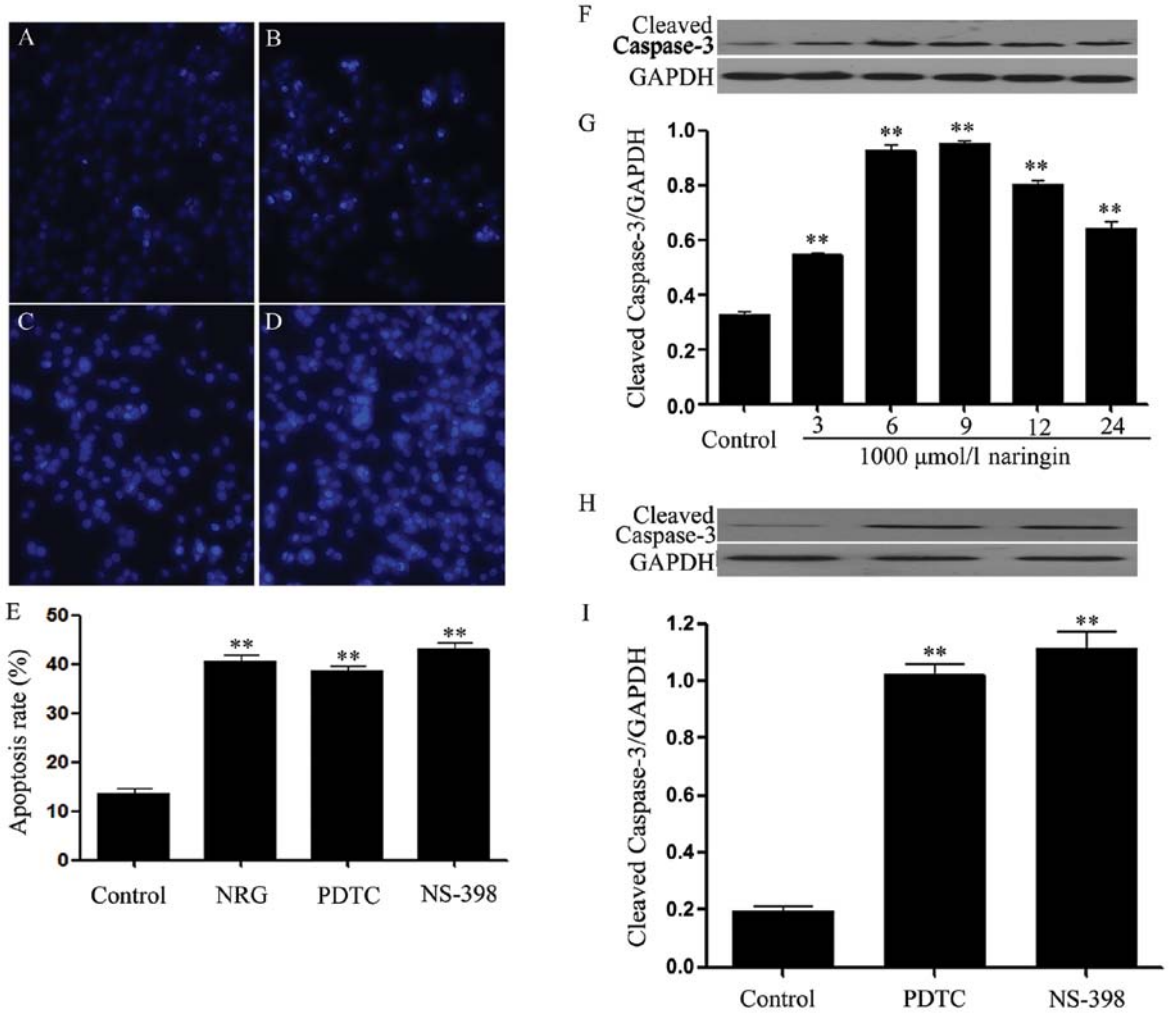

Figure 5. Naringin, PDTC and NS-398 elicit apoptosis in HeLa cells. The cells were treated with 1,000 μmol/l NRG or 200 μmol/l PDTC or 10 μmol/l NS-398 for the indicated times. (A-D) Hoechst 33258 nuclear staining followed by photofluorography was carried out to test cell apoptosis. (A) Control group. (B) The cells exposed to 1,000 μmol/l NRG for 24 h. (C) The cells exposed to 200 μmol/l PDTC for 24 h. (D) The cells exposed to 10 μmol/l NS-398 for 24 h. (E) The apoptotic rate was analyzed using a cell counter and Image J 1.47i software. The expression levels of cleaved caspase-3 were (F and H) measured by western blot assay and (G and I) quantified by densitometric analysis with Image J 1.47i software. Data are shown as the means ± SE (n=3). **p<0.01, compared with the control group.

for 24 h obviously reduced the expression level of caspase-1. In addition, treatment of the cells with 200 μmol/l PDTC or 10 μmol/l NS-398 for 24 h also attenuated caspase-1 expression.

Caspase-1 inhibitor reduces growth and induces apoptosis in HeLa cells. To investigate the roles of caspase-1 in growth of the cells, we examined the effects of SC-3069 (an inhibitor of caspase-1) on the cell viability and apoptosis. As illustrated in Fig. 7A, exposure of the cells to SC-3069 at different concentrations (0.01, 0.02, 0.05, 0.1, 0.5 and 1 μg/ml) for 24 h dose-dependently decreased cell viability.

Furthermore, as shown in Fig. 7B, treatment of HeLa cells with 0.1 μg/ml sc-3096 for 12 h significantly increased expression level of caspase-3, compared with the control group.

There is a positive interaction between NF-κB and COX-2 pathway in HeLa cells. The data from western blot assay showed that exposure of the cells to 200 μmol/l PDTC for 24 dramatically block the expression of COX-2 (Fig. 8A and B, p<0.01), indicating contribution of NF-κB to the activation

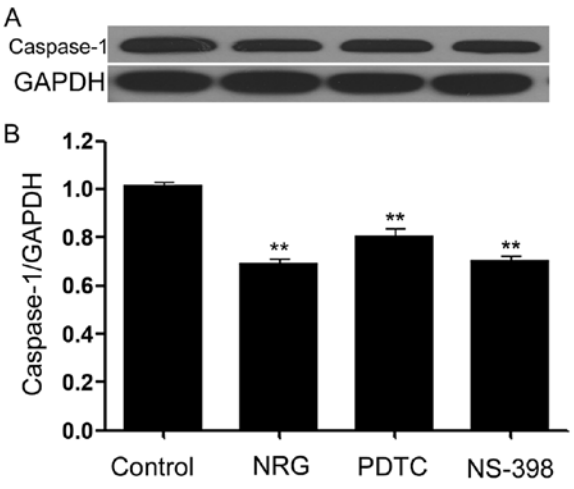

Figure 6. Naringin, PDTC and NS-398 inhibit the expression of caspase-1 in HeLa cells. The cells were treated with 1,000 μmol/l NRG or 200 μmol/l PDTC or 10 μmol/l NS-398 for 24 h. (A) The expression level of caspase-1 was detected by western blot assay. (B) Densitometric analysis of the data shown in (A). Data are presented as the means ± SE (n=3). **p<0.01, compared with the control group.

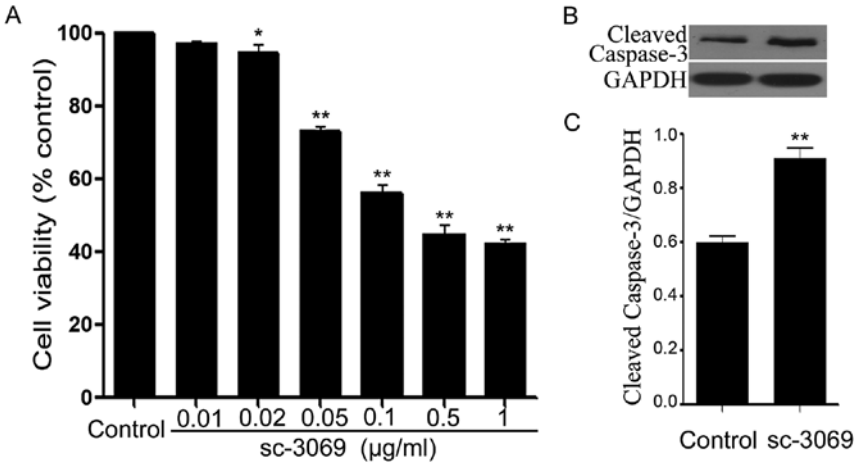

Figure 7. Caspase-1 contributes to growth and induces anti-apoptosis in HeLa cells. The cells were exposed to SC-3069 (an inhibitor of caspase-1) at the indicated concentrations for 24 h. (A) Cell viability was measured by CCK-8 assay. (B) The expression level of cleaved caspase-3 was detected by western blot assay. (C) Densitometric analysis of the data shown in (B). Data are presented as the means ± SE (n=3). $^{*}p<0.05$, $^{**}p<0.01$, compared with the control group.

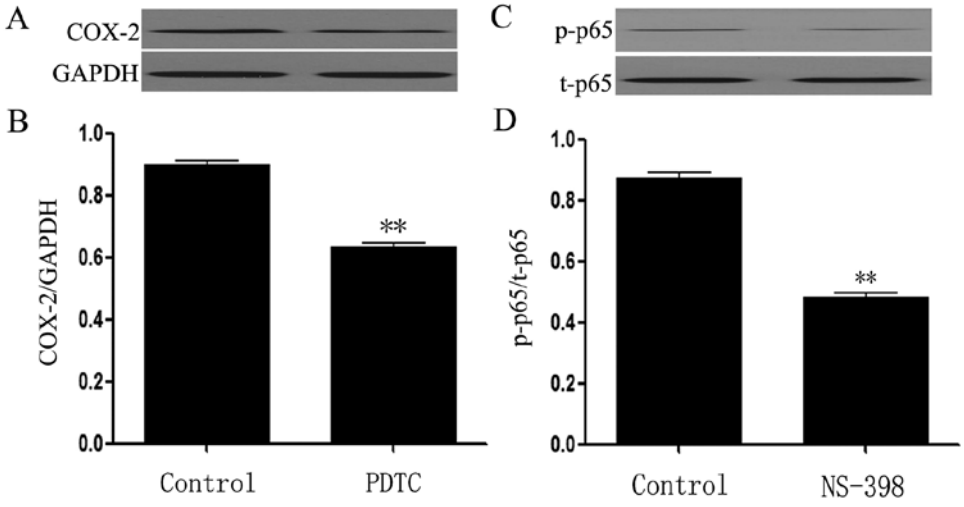

Figure 8. A positive interaction between NF-κB and COX-2 pathway exists in HeLa cells. The cells were treated with 200 μmol/l PDTC or 10 μmol/l NS-398 for 24 h. The expression level of (A) COX-2 or (C) p-NF-κB p65 was detected by western blot assay. (B and D) Densitometric analysis of the data from (A and C), respectively. Data are presented as the means ± SE (n=3). $^{**}p<0.01$, compared with the control group.

of COX-2 pathway. Interestingly, treatment of the cells with 10 μmol/l NS-398 for 24 h considerably antagonized the expression of p-NF-κB p65 (Fig. 8C and D, $p<0.01$), revealing involvement of COX-2 in the activity of NF-κB pathway.

Discussion

The activation of NF-κB (9,10,12) or COX-2 pathway (21-25,49) has been correlated with cervical carcinogenesis and progression. Furthermore, NF-κB is implicated in overexpression of COX-2 by HPV 16E5 (9). However, the relationship between NF-κB and COX-2 in cell proliferation and apoptosis of cervical cancer cells is incompletely understood. In the present study, we provided novel evidence that there is a positive interaction between NF-κB and COX-2 pathway, which may be an important mechanism responsible for cell growth and antiapoptosis in cervical cancer cells. This mechanism is supported by the following results: i) treatment with PDTC, an inhibitor of NF-κB, attenuated the expression level of COX-2; ii) NS-398, an inhibitor of COX-2, reduced the expression of NF-κB p65 subunit; iii) exposure of HeLa cells to PDTC or NS-398 induced growth inhibition and apoptosis, as demonstrated by the decreased cell viability and increased amount of apoptotic cells and cleaved caspase-3 expression.

Apoptosis is the physiologically relevant model of programmed cell death that counterbalances cell proliferation. Activation of the caspase cascade is involved in the execution of apoptosis in a variety of cellular systems. Caspase-1, a member of the so-called 'inflammatory caspases' group, is an initiator caspase. Recently, the role of caspase-1 in apoptosis of cancer cells has been receiving attention, but previous studies concering the role of caspase-1 are controversial. Jarry *et al* (50) have demonstrated obvious downregulation of caspase-1 expression in human colon cancer. Overexpression of caspase-1

enhances the rate of apoptosis *in vitro* and *in vivo* in renal cancer cell lines (51). In constrast, Schlosser *et al* (34) reported that caspase-1 has antiapoptotic function in pancreatic carcinoma. Thus, to explore the functional roles of caspase-1 in cell growth and apoptosis will enhance the understanding of the molecular basis of cervical carcinogenesis and progression. The findings of this study showed that both NF-κB inhibitor (PDTC) and COX-2 inhibitor (NS-398) significantly reduced the expression level of caspase-1, indicating the contribution of NF-κB-COX-2 pathway to activation of caspase-1 in HeLa cells. In addition, SC-3069, an inhibitor of caspase-1, suppressed cell viability and upregulated the expression of cleaved caspase-3 (an executioner of apoptosis), suggesting involvement of caspase-1 in cell growth and antiapoptotic effect in cervical cancer cells. Our results are comparable with those of a previous study that caspase-1 has antiapoptotic effect in pancreatic cancer cells (34). However, our data contrast with previous observations that caspase-1 contributes to apoptotic induction in human ovarian cancer cells (32), prostate cancer cells (33), human colon cancer cells (50) and renal cancer cell lines (51). The apparent discrepancy may reflect specific differences in the molecular mechanisms responsible for the development of various human malignancies. Alternatively, a potential antiapoptotic function for this caspase may be a result of alternative splicing of caspase-1, since caspase-1 has four known isoforms, at least two of which may have antagonistic effect against apoptosis (52).

Another novel finding of the present study is that NRG, a citrus flavonone, exerts its anticancer effects by inhibiting the NF-κB-COX-2/caspase-1 pathway in cervical cancer cells. Citrus fruits contain various flavonoids, and among these natural compounds, NRG has been pharmacologically considered as a potential anticancer agent, due to its anticancer effect on various cancer cells, such as colon cancer cells (43,53), urinary blader cancer cells (44), human breast cancer cells (45) and human cervical cancer (SiHa) cells (46). However, the molecular mechanisms responsible for the anticancer effect of NRG have yet to be complely understood. To the best of our knowledge, no previous studies have focused on NRG-induced cell growth inhibition and apoptosis through the inhibition of NF-κB-COX-2/caspase-1 pathway in human cervical cancer (HeLa) cells.

To investigate this topic, firstly, we observed the roles of NRG in cell growth inhibition and apoptosis in HeLa cells. In agreement with the results of previous studies (43-46), our data showed that NRG had marked anticancer effects, as evidenced by a decrease in cell viability and increases in number of apoptotic cells as well as the expression level of cleaved caspase-3 in HeLa cells. In human cervical cancer (SiHa) cells, administration of NRG also has anti-proliferative effect and increases the expression of caspase-3 and -9 (46), which support our results. Based on the above, it is suggested that NRG may be a potential inducer of cleaved caspase-3 (an executioner of apoptosis) in human cervical cancer (including SiHa and HeLa) cells.

As described above, the present study demonstrated the involvement of NF-κB-COX-2/caspase-1 pathway in cell growth and anti-apoptosis, thus, we further explored the effects of NRG on the activation of NF-κB-COX-2/caspase-1 pathway. The findings of the current study showed that treatment of HeLa cells with NRG markedly attenuated the expression levels of NF-κB p65 subunit, COX-2 and caspase-1. These results revealed that NRG induces growth inhibition and apoptosis, at least in part, through the inhibition of NF-κB-COX-2/caspase-1 pathway in HeLa cells.

In summary, the present study provides novel evidence that the activation of NF-κB-COX-2/caspase-1 pathway contributes to cervical carcinogenesis, including cell proliferation and antiapoptosis. The understanding of the roles of such a signaling pathway is important, as it may lead to the development of novel treatment strategies designed to inhibit this signal cascade for cervical cancer cells. Additionally, the present study provides important new insight into the molecular mechanisms underlying the anticancer effect of NRG in cervical cancer cells. First, NRG reduces cell viability and induces apoptosis. Second, the NRG-induced growth inhibition and apoptosis appears to be linked to the inhibition of activation of NF-κB-COX-2/caspase-1 pathway. The findings of the present study may, in part, explain the therapeutic effects of NRG in treatment of human cervical cancer cells.

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