

Notoginsenoside R1 (NGR1) induce DNA damage to inhibit cervical cancer cells proliferation by inhibiting PHF6 activity

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Abstract

Notoginsenoside R1 (NGR1) is isolated from the panax notoginseng which is a kind of Traditional Chinese Medicine and edible plant with good healthful effect that using range is very wide for medical treatment and health care. It had been demonstrated to inhibit various tumors proliferation, but whether it inhibited cervical cancer cells proliferation and its mechanism was not unclear. In this study, we showed that NGR1 could inhibit cervical cancer cells to proliferate with a time and dose dependent manner, induce cervical cancer cells apoptosis and arrest cervical cancer cells in G1/S-phase. We also found that NGR1 could make H2AX phosphorylation and inhibit PHF6 expression with a time and dose dependent manner. Furthermore, when over expression PHF6 gene, the γ H2AX haven't any change, but silenced PHF6 gene with siRNA, the γ H2AX increased significantly. That mean PHF6 has negative correlation with γ H2AX. Subsequently, we added NGR1 to intervene, something interesting happened that PHF6 protein fell even more, but the γ H2AX more up regulation in the siPHF6 and NGR1 group. In the PEGFP-C1-PHF6 plasmid vector and NGR1 group, inversely, the PHF6 protein declined, and the γ H2AX still up regulation. All those results indicate d that NGR1 caused DNA injury by inhibiting PHF6 activity pathway and arrested cervical cancer cells in G1/S-phase.

Introduction

Cervical cancer is the most common malignant tumor of female reproductive system. It has a significant adverse impact on a women's quality of life and health, as well as increases their risk of morbidity and mortality year by year[1, 2]. The pathogenesis of cervical cancer is still not clear, which is related to multiple factors. Some studies indicate that cervical cancer associate with many factors, for examples, HPV virus infection, gene mutation, telomerase activation, hormone abnormalities, abnormal immune function of the body and so on[3–8]. So, it is strongly necessary to study the pathogenesis of cervical cancer for clinical therapeutics.

DNA damage is one of the most common factors of cancer, which is caused by the biological environment or endogenous metabolic products of cells. As we known, γ H2AX, an important member of the cellular stress response to DNA damage, is a marker of DNA double strand breaks(DSBs), because it will be form a focal point by γ H2AX to recruitment other proteins assemblage in order to repair when DNA double strand is broken[9–11]. To protect genome stability,an intricate DNA repair systems have evolved in the process. They make up tangle some DNA-damage response (DDR) signaling networks to repair damaged DNA depending on the degree of DNA damage[12, 13]. When DNA double strand is repaired, the cells continue to grow, otherwise the cells initiates apoptosis or lead to cancer[14]. So, abnormal of DNA repair systems is an important factor in the pathogenesis of cancer[9, 15]. DNA damage response and mitosis are relate to plant homeodomain finger protein 6 (PHF6). PHF6 is identified from borjeson-forssman-lehmann syndrome that is a rare X-linked mental retardation syndrome[16], but its cellular function is remain unknown.

PHF6 is a X-linked tumor suppressor gene, as a high conservative transcription factor, which is belong to the zinc finger-like homologous domain proteins[17]. It is located in Xq26.3 and contains 11 exons[18]. In protein structure, PHF6 protein contains two nuclear localization sequences (NLS1: aa13-16;NLS2: aa 129–133), a nucleolus localization sequence (NoLS: 157–169), and two nearly identical zinc finger domains(ZaP1: aa14-134;ZaP2: aa 209–332)[19, 20]. It may play an important role in nucleolar transcriptional regulation and/or chromatin remodeling[21, 22]. When PHF6 protein binding to dsDNA, it may be beneficial for nucleosome remodeling. It also facilitate the nucleosome remodeling and deacetylase complex(NuRD) –CHD3/4, HDAC1 and RBBP4/7 to recruit relevant target genes assembling, which is involved in embryonic development, neurogenesis, hematopoiesis and tumorigenesis[19, 20, 23–25]. Recent studies have shown that deletion of the PHF6 gene can lead to instability increased of the rDNA genome and block the cells in G2/M to repair DNA[26].

In currently, chemotherapy also is an important method for therapy cervical cancer, especially suit for the patients with terminal cancers or metastatic cancers. But, chemotherapy is easy to cause different degrees of drugs tolerance, furthermore, most chemotherapy drugs are highly toxic and prone to many adverse reactions[27, 28]. So, it lead to a series of serious impacts on the quality of life of cancer patients. Therefore, it has great significances to find anti-cancer drugs with high efficiency and low toxicity to enhance the patient immunity, strengthen the therapeutic effect of drug chemotherapy, prolong and improve the quality life of patients.

Panax notoginseng is a kind of Traditional Chinese Medicine and edible plant with good healthful effect that is widely used in clinic and food industry. It has many favorable effects on patients such as strengthening and filling deficiency, relieving swelling and pain, promoting blood circulation and removing blood stasis. Futhermore, it also has important effects on prevention and treatment cardiovascular and cerebrovascular system diseases, promoting angiogenesis, treatment central nervous system diseases, depressing blood-fat and blood pressure, antioxidant, improving immunity and other functions[29–34]. Recently, some studies have exciting found that panax notoginseng has important anti-cancer effects[35]. It may become the first choice for chemotherapy drugs to treat cancer, because, which is not only boosts the body's immunity, but also has anti-cancer activity. Notoginsenoside R1 (NGR1) is the main active ingredient which is isolated from the Panax notoginseng. It can significantly inhibit the activity of colorectal cancer cells to proliferate, and arrest the cells in S phases[36], and effectively reduce the invasion and metastasis of lung cancer[37]. But, Whether NGR1 has an effect on cervical cancer is unknown, and its mechanism is also indistinct. So, in this paper, firstly, we demonstrated that NGR1 has an inhibitory effect on cervical cancer. Secondly, we investigated the molecular mechanisms that NGR1 induce DNA damage to inhibit cervical cancer cells proliferation with down-regulation the expression of PHF6 protein.

Results

NGR1 inhibits cervical cancer cells proliferation

At first, we demonstrated whether the NGR1 could inhibit the cervical cancer cells growth and proliferation. The NGR1 structure was showed in Fig. 1A. HeLa cells were treated with difference concentration of NGR1(0mM, 1mM, 2mM, 4mM, 8mM), by the CCK8 assay, we found that NGR1 could inhibit the cervical cancer cells growth and proliferation compared with non-treated cells, and the inhibition ratio is also depend on concentration gradient and time duration (Fig. 1B). Soft agar cloning formation experiment is an important symbolic of malignant cancer cells that connected with tumorigenesis, invasiveness and metastasis. Fig. 1C showed that the colony formation ability of HeLa cells were also obviously inhibited by NGR1 with a dose-dependent manner. Compared with the control group, the number of cells colony of the treated groups significantly decreased. Along with increasing the concentration, the cells colony formation was decreased. That mean the NGR1 could effectively inhibit the cervical cancer cells growth and proliferation.

NGR1 induces cervical cancer cells apoptosis

In this step, we observed whether NGR1 induces cervical cancer cell apoptosis. After HeLa cells were treated with NGR1 for 24h, follow on, stained with DAPI. In the treatment group(8mM, NGR1), the results showed that the cell nucleus became irregular and small, even had some typical apoptotic bodies after NGR1 treated. However, in the control group, the cell nucleus morphology were roundish and they nucleus color were homogeneous (Fig. 2A). Thereafter, HeLa cells were treated with a series concentration of NGR1 (0mM, 1mM, 2mM, 4mM, 8mM) for 12h. The cells were stained with AnnexinV-FITC/PI, then using flow cytometry to analyse apoptosis rate of the cells. The funny fact was that the cells apoptosis rate had a significant increase with a dose-dependent manner (Fig. 2B). Caspase3 play a key role in the process of cell apoptosis. So we detected the caspase3 of HeLa cells after NGR1 treatment for 24h. The results showed that caspase3 was activated after NGR1 treatment, and caspase3 up-regulated after NGR1-treated cells compared to the control cells (Fig. 2C).

DNA damaged and PHF6 protein activated caused by NGR1

DNA damage is an early event, it may immediately trigger DNA repair response. When damaged DNA can not be repaired, cells are likely to activate the apoptotic system to apoptosis. In the present study, HeLa cells were treated with different hours (0, 3, 6, 12, 24,48h) by NGR1(4mM), cells harvest for protein assay by western blot, we found that NGR1 could damage DNA and activate PHF6 protein with time-dependent manner (Fig. 3A). In concretely describe, compare to the control group, DNA was damaged by NGR1 in early stage, because γ H2AX increased from 3h to 12h, but it gradually returned to normal levels as time extension(from 24h to 48h). This mean NGR1 may sustained to destroy the DNA double stranded structure with time during until the DNA double stranded were completely destroyed. Meanwhile, we founded that PHF6 protein decreased by NGR1 treatment with the stress time going on. In addition, we also observed the relationship of γ H2AX and PHF6 protein with the concentration of NGR1. HeLa cells were treated with difference concentration of NGR1(0, 1, 2, 4, 8mM) for 12h, we found that γ H2AX increased and PHF6 protein decreased with dose-dependent manner(Fig. 3B).

NGR1 induce DNA damage by inhibiting PHF6 activity

PHF6 protein is a nucleolus protein which contains two zinc finger domains (ZaP1: aa14-134;ZaP2: aa 209-332). So we observed whether PHF6 protein locate in nucleolus though immunofluorescence assay. First, compare with DAPI group, we found PHF6 protein location in cell nucleus (Fig. 4A). Further investigation, compare with DAPI and nucleolin(C23, a nucleolus indicator protein) group , we found PHF6 protein location in nucleolus(Fig. 4B). Nucleolus is not only a site for storage rRNA, synthesis and assembly of ribosome subunits, but it is also involved in a number of important biological processes. Recently study have shown that PHF6 may play an important role in nucleolar transcriptional regulation and/or chromatin remodeling[21]. So, we investigated the relation of DNA damage and PHF6 protein. When the PHF6 gene was independently over expressed by PEGFP-C1-PHF6 plasmid vector, we found the γ H2AX haven't any change, but PHF6 gene silenced solely by siRNA, the γ H2AX increased significantly. That mean PHF6 has negative correlation with γ H2AX. It indicate that PHF6 deletion may have triggered a series of stress responses in the nucleus to repair the rDNA genome. Subsequently, we added NGR1 to intervene. We found, compared to the siPHF6 or control group, PHF6 protein fall even more, but the γ H2AX more up regulation in the siPHF6 and NGR1 group. In the PEGFP-C1-PHF6 plasmid vector and NGR1 group, inversely, the PHF6 protein declined, and the γ H2AX still up regulation(Fig. 4C). The results indicate that NGR1 induce DNA damage by inhibiting PHF6 activity.

NGR1 arrest cervical cancer cells in G1/S phase

The previous results showed that NGR1 inhibited cervical cancer cells proliferation, So we continue to verify whether NGR1 affected the cells cycle progression. HeLa cells were treated with NGR1 at 2, 4, 8mM for 24h, respectively.

To collect and analyze the cells by flow cytometry assay. The specific procedures were similar to the apoptosis rate experiment. Compared with the control group (25.90%), both NGR1 at 2 mM, 4 mM and 8mM groups had more percentage in G1/S-phase, 34.13%, 44.32% and 58.63% respectively(Fig. 5). All those results clearly indicate that NGR1 arrest cervical cancer cells in G1/S phase and had a significant increase with a dose-dependent manner.

Discussion

NGR1 is isolated from panax notoginseng, a valuable Chinese herbal medicine and edible plants with good healthful effect for human. Several studies have found it has anti-tumor activity[37-39]. However, there are few reports that NGR1 has an anti-cervical cancer activity and its mechanism. In this study, we showed that NGR1 could effectively inhibit cervical cancer cells proliferation with time and dose dependent manner. The inhibition rate of cells were increased with extension the time or increasing the concentration of NGR1(Fig. 1). It indicate that NGR1 has some symptomatic therapeutic effect on cervical cancer.

Apoptosis is a programmed cell death, a common phenomenon at the special developing stages in the prokaryotic and eukaryotic cells, which is an active mode of death and is controlled by certain genes[40-42]. It is very important for reproductive development, organogenesis and keeping the organism to

maintain dynamic homeostasis[43, 44] Recently, increasing NGR1 could induce SW480 human colorectal cancer cells apoptosis and arrest them in G1/S phase[36]. We also demonstrated that NGR1 induced cervical cancer cells to apoptotic and arrest them in G1/S phase(Fig. 2 and Fig. 5). The result is supported by nuclear morphology and cells cycle has change in NGR1 treated group. Furthermore, the apoptosis rate of cells were increased with dose-dependent manner of NGR1(Fig. 2B), the augment of G1/S ratio in the proliferation cycle of cells presents a dose-dependent manner(Fig. 5). As we known, Caspase3 activation plays an important role in the development of cell apoptosis[45-47]. In the NGR1 treated cells groups, we observed that caspase3 was increased relay on increasing the concentration of NGR1(Fig. 2C). The results suggested that NGR1 activated caspase3 to apoptotic with dose dependent manner.

DNA damage is an early event, it may immediately trigger DNA repair response[48, 49]. When damaged DNA can not be repaired, cells are likely to activate the apoptotic system to apoptosis. DNA damage response (DDR) is a series of regulatory events which including DNA damage, cell cycle arrest, and repair the damaged DNA to ensure the maintenance of genomic stability and cell viability[50]. In order to clarify its initial mechanism, we found that NGR1 indeed induce cervical cancer apoptosis, when we increased the NGR1 concentration to treat HeLa cells, An interesting phenomenon happened that NGR1 could make H2AX phosphorylation with a time and dose dependent manner(Fig. 3). Above all, our data suggest that DNA damage is an early event after NGR1 treatment and may trigger DNA repair processes, and that the failure of repair of DNA damage may trigger apoptosis program.

PHF6 deletion may have triggered a series of stress responses in the nucleus to repair the rDNA genome, blocking the progression of the cell cycle and inhibiting the proliferation of cancer cells, and delay the damaged genome to repair[26, 51]. In this study, we found that NGR1 inhibited PHF6 expression with a time and dose dependent manner and PHF6 has negative correlation with γ H2AX. Furthermore, when over expression PHF6 gene, the γ H2AX haven't any change, but silenced PHF6 gene with siRNA, the γ H2AX increased significantly(Fig. 3). That mean PHF6 has negative correlation with γ H2AX. Subsequently, we added NGR1 to intervene, more interestingly were that PHF6 protein fall even more, but the γ H2AX more up regulation in the siPHF6 and NGR1 group. In the PEGFP-C1-PHF6 plasmid vector and NGR1 group, inversely, the PHF6 protein declined, and the γ H2AX still up regulation(Fig. 4).. All those results indicate that NGR1 caused DNA injury by inhibiting PHF6 activity.

In summary, our data showed NGR1 can effectively inhibit cervical cancer proliferation with a time and dose dependent manner, due to it induced cervical cancer cells apoptosis and arrests cells at G1/S-phase. In addition, our study also suggested that NGR1 induce DNA damage by activation the nucleolus protein of PHF6. It will provide a new target and therapeutic strategy for cancers from the molecular mechanism researches. Finally, It is worth noting that DNA repair function defects can cause tumors, but in cancer cells it can rapid and complete to repair DNA damage to resist chemotherapy drugs. This is the reason why most anticancer drugs do not work. But in this research we found that NGR1 could induce early DNA damage and lead to apoptotic. Its mean cervical cancer cells could not resist NGR1 to work. Furthermore, NGR1 has a function to enhance the organic immunity[52]. So, we believe that NGR1 may be extensively

applied in clinical to treat various cancers in the future, due to it can improve patients' immunity to reduce drug resistance of cancers.

Materials And Methods

Reagents and antibodies

PEGFP-C1-PHF6 plasmid was gift from Ottawa Hospital Research Institute (professor [Picketts DJ](#)). NGR1 was obtain from Chen du Pure Chem-Standard Co.,Ltd (Chen du, China). Its chemical structure is shown at Fig. 1A, and the purity is no less than 98%. NGR1 was dissolved in dimethyl sulphoxide (DMSO) which as 100 mM stock solution stored at -20°C, the stock solution was diluted using cell culture medium for the indicated concentration. CCK-8, DAPI and BCA Protein Assay were purchased from Dalian Meilun Biotechnology Co., Ltd (Dalian, China). Sodium dodecyl sulfate (SDS), phenylmethane-sulfonyl fluoride (PMSF), BCA Protein Assay kit, AnnexinV-FITC/ PI reagents were purchased from [Beyotime Biotechnology Co., Ltd](#) (Shanghai, China). Primary antibodies against γ H2AX, Casepase3, PHF6, GAPDH were purchased from Abcam Company (MA, USA). DMSO, Triton™ X-100 were purchased from [Sigma-Aldrich Company](#) (SL, USA). Horseradish peroxidase and fluorescein labeled secondary were obtained from Santa Cruz Biotechnology Co., Ltd (CA, USA). siRNA-PHF6 and Lipofectamine™2000 were purchased from Thermo Fisher Scientific Company (MA, USA)

Cell culture

HeLa cells as cervical cancer cells were obtained from American Type Culture Collection (ATCC, ML, USA). HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Hyclone, UT, USA) with 10% fetal bovine serum (FBS) (Gibco, YK, USA) and 1% double antibiotics (containing 100U/mL of penicillin and 100 μ g/mL of streptomycin), and incubated in a humidified atmosphere with 5% CO₂ at 37°C.

Cell viability assays by CCK8 assay

HeLa cells were digested by trypsin and made the cells suspension concentration as 1 \times 10⁵/ml. Inoculated 100 μ l the cells suspension into a 96-well plate to make per well with 1 \times 10⁴ cells. Cultured them incubator at 37°C for 4h. After cells adherence, used different concentrations of NGR1 treatment them with 1, 2, 4, 8, and 16mM for 24h and 48h, at the same time each concentration were set 6 replicates. Added 100 μ l of DMEM medium with 10% CCK8 to each well as a liquid to change. Be carefully, do not has any bubble in the wells to avoid affecting OD value. After incubated its at 37°C for 4h, the OD value was measured using a spectrophotometer at 450nm. Finally, the cells viability rate were calculated according to the formula:

$$\text{Cell viability rate} = [(As - Ab)/(Ac - Ab)] \times 100\%$$

As: absorbance of experimental wells (culture medium containing cells, CCK-8 and NGR1); Ac: absorbance of control wells (culture medium containing cells and CCK-8, but no NGR1); Ab: absorbance

of blank wells (culture medium without cells, CCK-8 and NGR1).

Soft agar colony formation assay

Cell colony formation assay was performed as previously described[53]. Briefly, the base agarose was prepared which contained 1×DMEM medium, 0.6% agarose, 10% FBS and 1×antibiotics mixture. Then, the top agarose was prepared which contained 1×DMEM medium, 0.3% low-melting-point agarose, 10% FBS, 1×antibiotics mixture and made the mixture contained 1×10^3 cells/ml. The top agarose was inoculated on the base agarose for 1ml to make every well contained 1×10^3 cells, meanwhile, NGR1 was added at 2, 4, 8mM. After being incubated for 12 days, the cells colonies were stained with 0.1% crystal violet for 20 min, and counted them under a microscopy.

Apoptosis rate analysis by flow cytometry

HeLa cells were seed in 6-well plate, when its reached 90% confluence, treated with NGR1 at 1, 2, 4, and 8mM for 12h. Apoptotic cells were quantified by the Annexin V-FITC double staining assay following the manufacturer's instructions. In brief,. Trypsin digested the cells and collected its in plastic tube, each plastic tube with 1×10^6 cells, washed twice with PBS. added 195 μ L Annexin V-FITC binding buffer to suspend the cells, then added 5 μ L Annexin V-FITC and 10 μ L PI staining solution. After being mixed gently, the cell suspensions were incubated at room temperature and away from light for 15 minutes, next, placed them on ice. After being filtrated with 300 mesh nylon membrane, the cells were detected with flow cytometry as soon as possible. All experiments were done independently at least three times per experimental point.

Cell cycle analysis using flow cytometry

HeLa cells were treated exactly same as in apoptosis rate analyses described above, In brief, HeLa cells were seed in 6-well plate, when its reached 90% confluence, treated with NGR1 at 0, 2, 4, and 8mM for 24h. After treatment, should be strictly in accordance with the operating instructions, Briefly, the cells (1×10^6) were collected using trypsinization to digest , washed twice with cold PBS. Cell pellets were fixed in 70% ethanol, washed the cells in cold PBS, resuspended the cells in 0.535 mL of dye buffer containing 10 μ l RNase(50×) and 25 μ l PI(25×), incubated the samples in the dark for 30 min at room temperature, and analyzed with a BD Accuri C6 flow cytometer.

Preparation of siRNA and cell transfection

PHF6-siRNA (siPHF6) and nonspecific siRNA(simock) were synthesized from Shanghai Gene Pharma Technology Co. Ltd (Shanghai, China). SiPHF6 sequence is as follows: si-h-PHF6_101: 5'-GGACAGTTACTAATATCTG-3'; si-h-PHF6_102: 5'-GCACGAAGCTGATGTGTTTC-3'; si-h-PHF6_103: 5'-CCACTGTGCATTGCATGAT-3'; siRNA(simock): 5'-UUCUCCGAACGUGUCACGU-3'. We had already demonstrated that the interference effect of si-h-PHF6_101 is the best(the date is not show).

The siRNA or PEGFP-C1-PHF6 plasmid and Lipofectamine™2000 with Opti-MEM culture medium which without FBS were mixed gently, then incubated them on ice for 20min. The complexes was added to each cell well. After 6h, the culture medium was exchanged that instead of complete medium to keep cultivating.

Immunofluorescence staining

Immunofluorescence staining analyses was performed as previously described[53]. Briefly, HeLa cells were fixed with 4% paraformaldehyde for 20min, and then added 0.1% TritonX-100 incubation for 5min. The cells were blocked with 3% bovine serum albumin(BSA) for 1h, and then incubated with anti-PHF6 over night at 4°C. After washing, fluoresein-conjugated secondary antibody were incubated to conjugate the anti-PHF6. DAPI was use to label the cell nuclear. The all samples in the same experiments were observed under Olympus Confocal laser scanning microscope.

Western-blotting analysis

Western-blotting analysis was performed aspreviously described[53]. Briefly, cells were lysed in RIPA buffer containing 25mM Tris (pH7.6), 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 1mM PMSF (Sigma), and 1% protease inhibitors cocktail(Thermo Fisher Scientific). The protein in lysate was measured with BCA Protein Assay kit. Equal amounts of protein extracts were loaded on 10% sodium dodecyl sulfate polyacrylamide gel, electrophoresed, and transferred them to a PVDF membranes. The membranes were blocked with 5% skim milk for 1h at room temperature and then incubated them with the desired primary antibodies overnight at 4°C. After washing, its were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody. Detection was performed by using a chemiluminescent ECL advance western blotting detection kit. The primary antibodies used were anti-PHF6, anti-γH2AX and anti-caspase3 antibody. GAPDH served as control. Western-blotting radioactive signal was quantified using Image J.

statistical analysis

All experiments were repeated at least three times and the data were presented as the mean ± standard deviation (SD). Student's t-test was used to compare the differences in all the measurable variables in this study. P values less than 0.05 considered statistical significance.

Declarations

Conflicts of interest

The authors declare that they have no competing interests in regards to this work.

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Authors' contributions

PHM conceived the research and designed it; TC carried out the molecular experiments and drafted the manuscript; WQW, QM, YWX, LMZ and FDP participated in the experiments; LHG, YHL and XXJ conceived of the study and provided some of the reagents and advice. All authors read and approved the final manuscript. All authors reviewed the manuscript.

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Figures

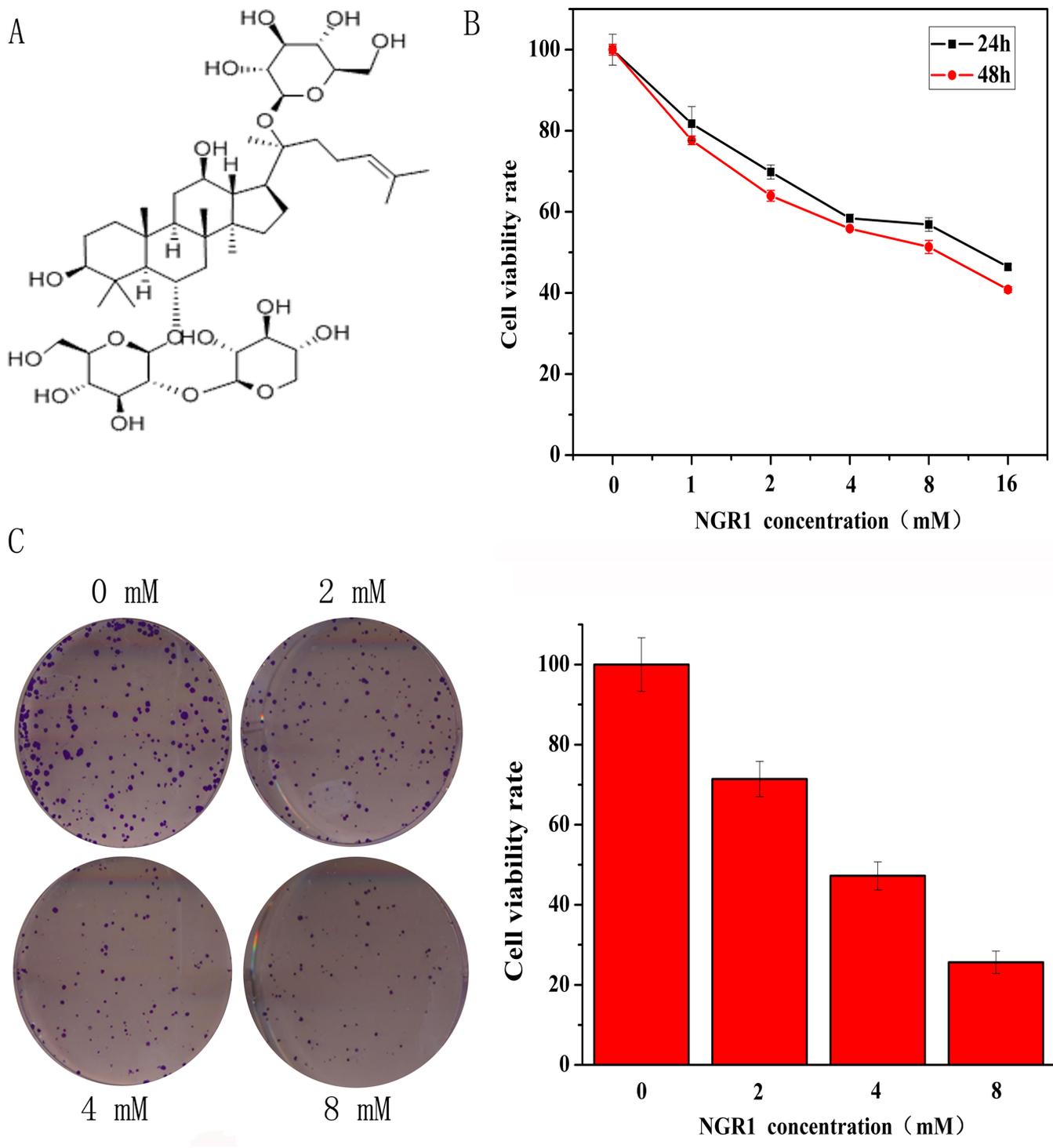


Figure 1

Inhibitory effects of NGR1 on cervical cancer cells. (A) Chemical structure of NGR1. (B) The inhibition rate of different concentrations of NGR1(0,1,2,4,8,16mM) after 24h, 48h treatment Hela cells. (C) The effects of different concentrations NGR1 (0,2,4,8mM) on cells survival rate were detected by Soft agar cloning formation experiment.

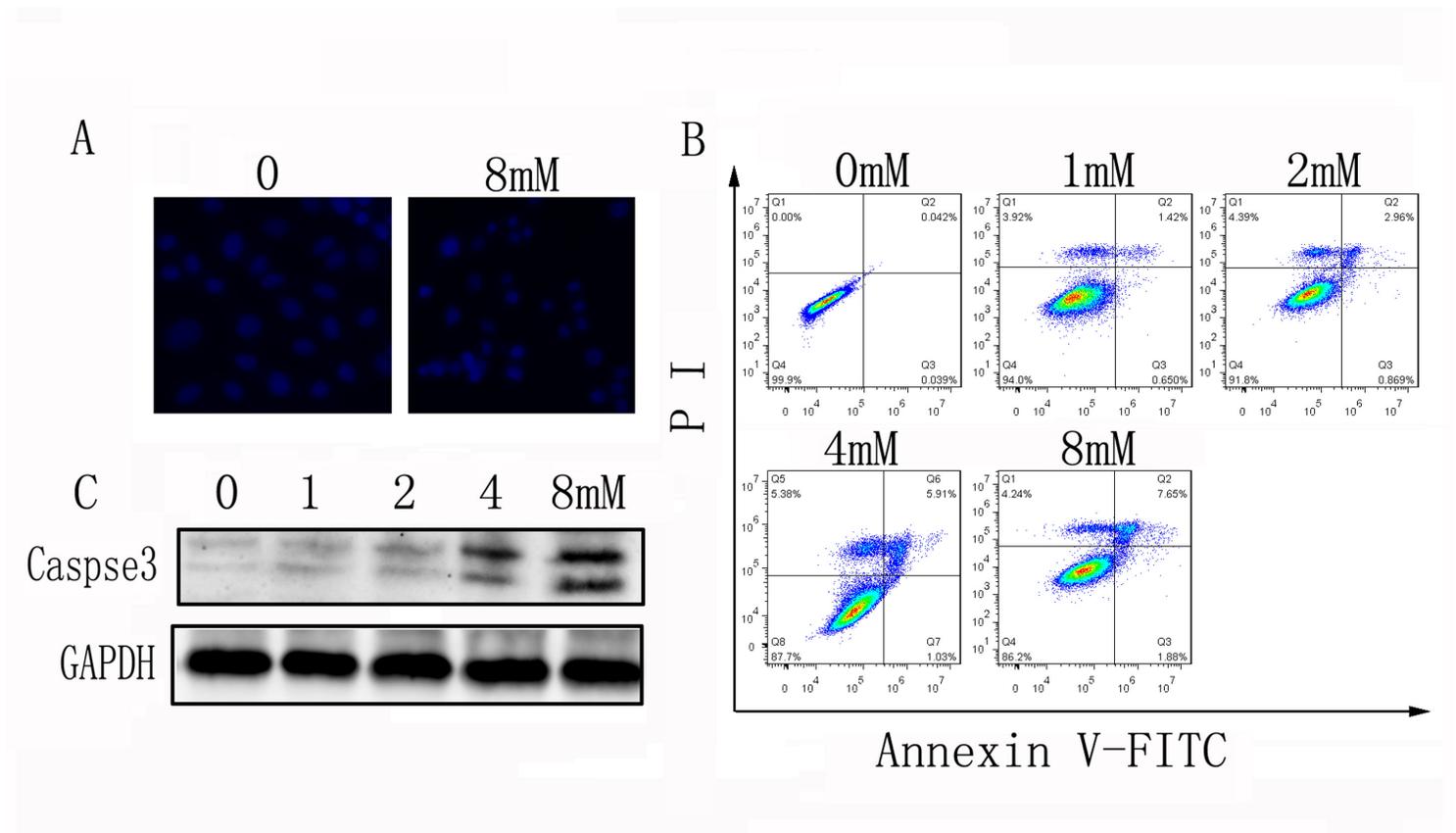


Figure 2

NGR1 induces cervical cells apoptosis. (A) HeLa cells were treated with NGR1 at the indicated concentrations for 24h, The nuclear morphology were observed under a fluorescence microscope (400×). (B) After different concentrations of NGR1 treatment(0,1,2,4,8mM), the cells were stained with Annexin V-PI, the cells apoptosis rates were counted using flow cytometry. (C) After NGR1 treated HeLa cells, Caspase3 was detected by western blotting assay.

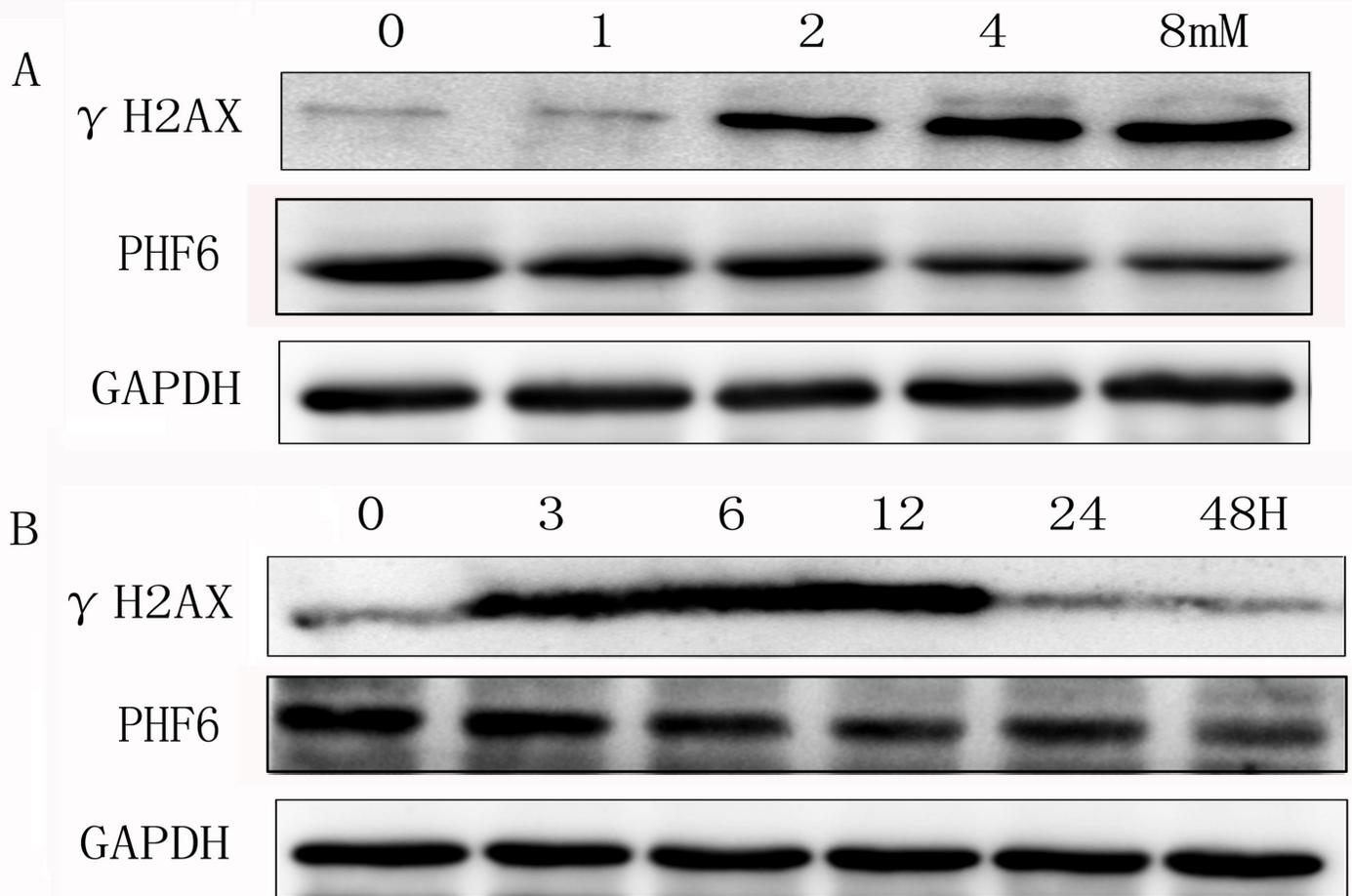


Figure 3

Effect of NGR1 on PHF6 and γ H2AX. (A) After different concentrations of NGR1 treatment(0,1,2,4,8mM), the effect on PHF6 and γ H2AX. (B) Hela cells were treated with different time (0,3,6,12,24,48h) by NGR1, the PHF6 and γ H2AX had change.

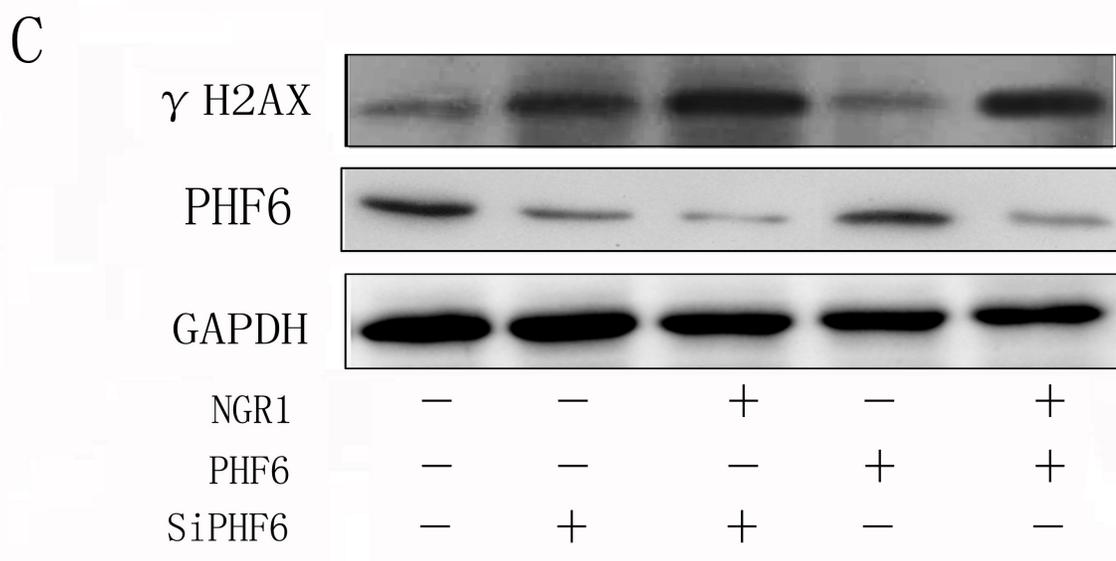
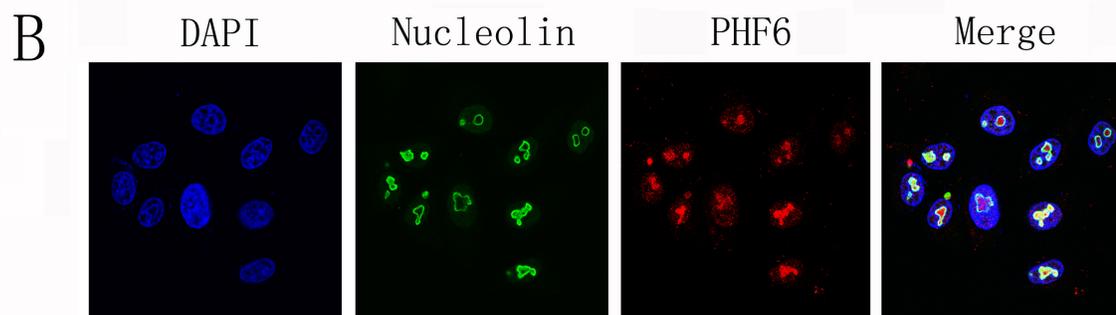
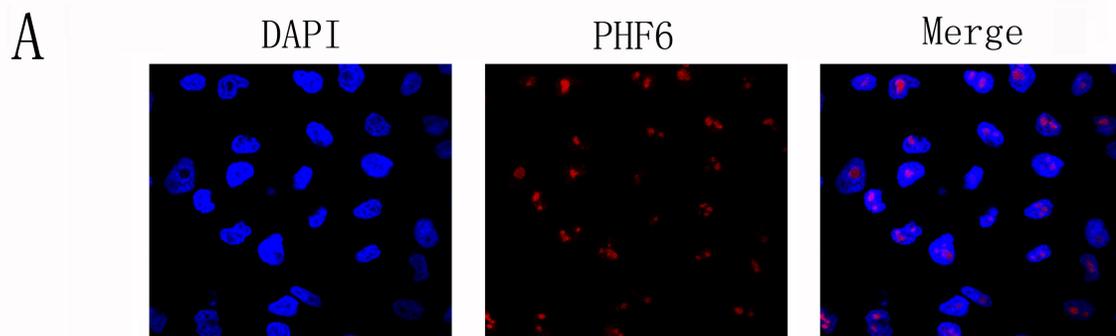


Figure 4

NGR1 induce DNA damage by activating PHF6 protein. (A) PHF6 protein location in cell nucleus. (B) Furthermore PHF6 protein location in nucleolus. (C) The changing of PHF6 and γ H2AX proteins after Hela cells were treated by NGR1.

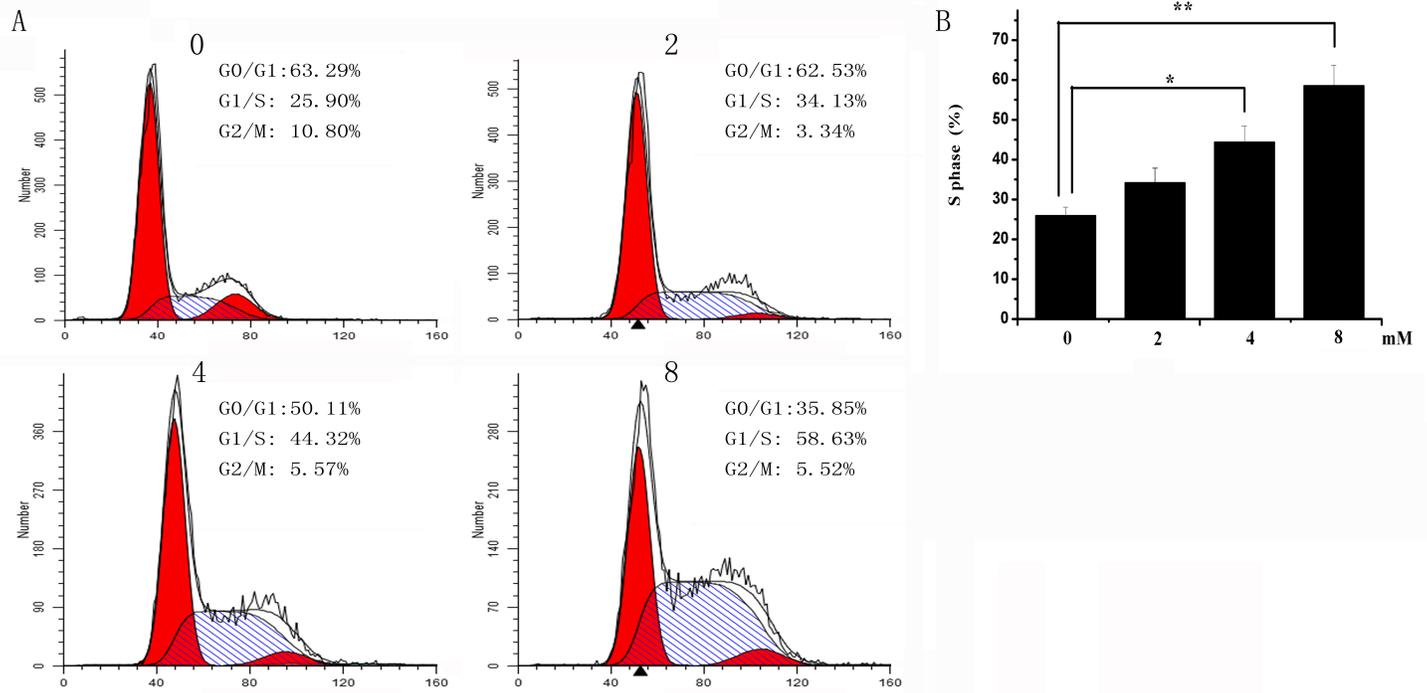


Figure 5

The effect of NGR1 on cell cycle. (A) The effects of different concentrations of NGR1(0, 2, 4, 8mM) on cell cycle. (B) The proportion of different concentrations of NGR1(0, 2, 4, 8mM) arrest Hela cells in G1/S phase. The data are presented as the mean \pm SD (n = 3 in each group).