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RESEARCH ARTICLE

miR-34a regulates cisplatin-induce gastric cancer cell death by modulating PI3K/AKT/survivin pathway

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Abstract The purposes of this study were to determine the expression profiles of microRNA-34a (miR-34a) in human gastric cancer cell line (SGC-7901) and cisplatin-resistant cell lines (SGC-7901/DDP), and to establish the correlation between miR-34a expression profile and the sensitivity of human gastric cancer cell to cisplatin-based pattern, thereby providing new methods and strategies for treating gastric cancer. Gastric cancer cell line (SGC-7901) and cisplatin-resistant cell line (SGC-7901/DDP) were cultivated in vitro, respectively. Quantitative real-time PCR (qRT-PCR) and Western blot were utilized to determine the expression profiles of miR-34a and survivin in both gastric cancer cell lines. With miR-34a mimic and miR-34a inhibitor transfected into SGC-7901 and SGC-7901/DDP for 48 h, post-transfection changes of miR-34a expression was determined; the effects of miR-34a ectopic expression on the viability of cisplatin-induce gastric cancer cell were assayed by the MTT method. The effects of miR-34a ectopic expression on apoptosis of cisplatin-induce gastric cancer cell were determined by Annexin V/propidium iodide (PI) double staining method and flow cytometry. The effects of miR-34a ectopic expression on the AKT and p-AKT expression of cisplatin-induce gastric cancer cells were determined by Western blot and flow cytometry with the PI3K pathway inhibitor Wortmannin. As shown by qRT-PCR and Western blot analyses, the expression of miR-34a in cisplatin-resistant cell lines decreased significantly in comparison to that of SGC-7901 cell line ($p<0.05$), while significant up-regulation of survivin expression was also observed ($p<0.05$). Compared with the control group, the expression of miR-34a increased significantly in SGC-7901 cells transfected with miR-34a mimic for 48 h ($p<0.01$). After miR-34a inhibitor transfection, the expression of miR-34a decreased significantly ($p<0.05$). The viability of cisplatin-induce gastric cancer cells increased significantly ($p<0.05$) with significant decrease of apoptosis after miR-34a expression inhibition, as demonstrated by MTT and flow cytometry with miR-34a over-expression, the viability of cisplatin-induce gastric cancer cells decreased significantly ($p<0.05$), with significant apoptosis increase ($p<0.05$). As shown by Western blot and flow cytometry, in comparison to the control group, Wortmannin could inhibit miR-34a inhibitor and DDP induced up-regulation of p-AKT significantly ($p<0.05$) and stimulated apoptosis. In conclusion, miR-34a expression was down-regulated in cisplatin-resistant cell lines. miR-34a over-expression could improve the sensitivity of gastric cancer cells against cisplatin-based chemotherapies, with PI3K/AKT/survivin signaling pathway possibly involved in the mechanism.

Keywords Gastric cancer · miR-34a · Cisplatin · Apoptosis · PI3K/AKT/survivin

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Introduction

Gastric cancer is a type of malignant tumor with a high incidence. Its pathogenesis is strongly connected to precancerous lesions such as chronic atrophic gastritis and intestinal metaplasia [1, 2]. Its incidence and mortality rates are ranked fourth and second among all tumors, respectively [3]. The

relative prevalence of gastric cancer is high in China, with morbidity and mortality more than twice of world averages [4]. Due to its asymptomatic presentation in the early stage, most patients experienced metastases at diagnosis. At this time, metastasis is still difficult to treat and it directly correlates to the prognosis of gastric cancer [5, 6]. Surgical intervention remains to be the only possible curable regimen for gastric cancer, two third of patients were diagnosed with their advanced stages with high incidence of post-operative relapse and metastasis. Even after a comprehensive treatment of chemotherapy and radiotherapy, the 5-year survival remained approximately 40 % only [7–9]. Therefore, the chemotherapy for advanced gastric cancer is important during the course of gastric cancer treatment, which has been highlighted in recent new drug investigations and appropriate chemotherapy regimen. More effective clinical therapies have been available for advanced gastric cancer. Platinum-based antineoplastic agents (e.g., cisplatin, oxaliplatin), paclitaxels, 5-FU derivatives (e.g., Xeloda) and S-1 have been used increasingly in the clinical treatment of gastric cancer. The response rate was reported to be approximately 40–60 % for the combination chemotherapy in gastric cancer [10–13]. However, many patients with gastric cancers did not respond to chemotherapies, although they have to tolerate the relevant toxic and adverse effects. It is clinically urgent to solve the problem of how to improve chemotherapy efficacy against gastric cancer and to avoid ineffective regimens [14, 15]. Therefore, it is of the utmost importance to clarify gastric cancer pathogenesis and to investigate the genes responsible for the progress, metastasis, relapse and tolerance of gastric cancer, for the purpose of further improving the outcome of gastric cancer treatments.

miRNAs have become a major research focus in the field of cancer research in recent years [16, 17]. miRNA is a family of small, noncoding RNAs in eukaryotes that are 17–25 nucleotides in length and are involved in the posttranscriptional regulation of gene expression [18, 19]. In animal cells, mature miRNAs and proteins form the RNA-induced silencing complex (RISC, also known as miRNA/RISC), which binds to the 3′-UTR loci of its target gene's mRNA, leading to the degradation or translational inhibition of the target mRNA [20]. miRNA. The binding of miRNA/RISC with its target mRNA only requires partial base pairing, which usually consists of six to eight miRNA nucleotides, also called the seed sequence. Therefore, one miRNA might interact with hundreds or thousands of target mRNAs. This characteristic enables these small RNA molecules to reduce the expression of a myriad of genes significantly or weakly, depending on their characteristics of 3′-UTR target loci [21, 22]. These small, regulatory RNA molecules are involved in the biological processes of cancer [23]. Researchers found that many miRNAs are encoded in cancer-related gene regions, suggesting that there may be a causal relationship between changes in miRNA expression and tumorigenesis [24, 25].

The miR-34 family is located on human chromosome 1p36, a region that is often deleted in human tumors [26]. For example, microdeletions of genes that include miR-34b/c are often found in breast and lung cancers, and studies have also determined that miR-34b/c expression is decreased in non-small cell lung cancer cell lines [27]. Studies have shown that miR-34a is a direct transcriptional target of p53 and is often deleted in various types of cancers [28]. The down-regulated expression of miR-34a genes may occur due to mutations that inactivate p53 in tumor cells [29]. In our previous studies, down-regulation of miR-34a expression was observed in human gastric cancer cell lines; miR-34a can negatively regulate survivin protein expression and inhibit gastric cancer cell proliferation and invasion [30]. As reported in recent studies, miR-34a are involved in the sensitivity of breast and lung cancers to chemotherapies [31, 32]. However, the relationship between miR-34a and the sensitivity of gastric cancer to chemotherapies has not been established. In order to provide new methods and strategies for the treatment of gastric cancer, we investigated the relationship between miR-34a and the sensitivity of gastric cancer cells to cisplatin based chemotherapy, as well as the signaling pathways.

Materials and methods

Major reagents

Human gastric cancer cell line SGC-7901 was purchased from American Type Culture Collection (Manassas, VA, USA). Cisplatin-resistant cell lines (SGC-7901/DDP) were purchased from Nanjing KeyGEN Biotech. Co., Ltd. (Nanjing, China). Fetal bovine serum, RPMI 1640 medium, L-glutamine, HEPES, and Lipofectamine 2000 were purchased from Invitrogen (Carlsbad, CA, USA).

TaqMan miRNA isolation Kit, TaqMan microRNA assay kit, and TaqMan microRNA Assay and TaqMan Universal PCR Master Mix were purchased from Applied Biosystems (California, USA). The miR-34a mimic, inhibitor, and non-specific control were synthesized by Genepharma (Shanghai, China).

MTT (3-(4,5)-dimethylthiahiazo(−*z*-y1)-3,5-di-phenytetrazoliumromide), trypsin, and phosphate buffer solution (PBS) were purchased from Sigma-Aldrich, USA. Cell culture plates and dishes were purchased from Corning, USA. Cisplatin (DDP) were purchased from Sigma-Aldrich (San Diego, CA, USA). PI3K pathway inhibitors Wortmannin was purchased from Axxora (San Diego, CA, USA). Annexin V and PI were purchased from Roche. The primary antibodies including rabbit anti-human survivin polyclonal antibody and mouse anti-human β-actin monoclonal antibody were purchased from Abcam, UK. AKT and phospho-AKT(p-AKT, Ser473) primary antibody were purchased from Cell Signaling

Technology (Beverly, MA, USA). The secondary antibodies including IRDye 800 conjugated affinity purified goat anti-mouse IgG and IRDye 800 conjugated affinity purified goat anti-rabbit IgG were purchased from Odyssey. Protein extraction and quantification kits were purchased from Bio-Rad (Hercules, CA, USA).

Culture and treatment of human gastric cancer cell lines

The SGC-7901 and Cisplatin-resistant cell lines (SGC-7901/DDP) were cultured in RPMI 1640 medium containing 10 % fetal bovine serum and incubated at 37 °C, 5 % CO_2, and saturated humidity. Cell growth was observed under an inverted microscope. When cell growth reached 70–80 % confluence, the cells were digested with 0.25 % trypsin and passaged. The culture medium was changed every other day, and the cells were passaged every 3 to 4 days. Cells in the logarithmic growth phase were collected for experiments.

SGC-7901 or SGC-7901/DDP cells that were cultured under normal conditions were inoculated uniformly into 6- or 96-well culture plates at a concentration of 3×10^5 cells/ml. After adherent cell culture, transfections were conducted for miR-34a mimic (5′-UGG CAG UGU CUU AGC UGG UUG U-3′ and 5′-ACA ACC AGC UAA GAC ACU GCC A-3′), non-specific control (5′-UUC UCC GAA CGU GUC ACG UTT-3′ and 5′-ACG UGA CAC GUU CGG AGA ATT-3′) and miR-34a inhibitor according to the Lipofectamine 2000 transfection manual. The normal control group was also established. miR-34a mimic, inhibitor and non-specific control were diluted by MEM medium free of serum components, and the liposomes Lipofectamine 2000 was added to the MEM medium. After being mixed mildly and incubation under ambient temperature for 5 min, the diluted Lipofectamine 2000 was mixed with diluted miR-34a mimic, inhibitor and non-specific control, respectively. After being mixed mildly, the mixture was incubated under ambient temperature for 20 min to form the mixture. The mixture was added to the culture plate with SGC-7901 or SGC-7901/DDP cells. The obtained mixture was incubated in 5 % CO_2 atmosphere at 37 °C. The culture medium was exchanged to RPMI 1640 medium containing 10 % fetal bovine serum after 5 h and the mixture was incubated for another 48 h.

Detection of expression of miR-34a and survivin in human gastric cancer cell lines

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and Western blot were utilized to determine the expression of miR-34a and survivin in human gastric cancer cell lines (SGC-7901 and SGC-7901/DDP). Both cells cultured in vitro were collected and RNA was extracted using the TaqMan miRNA Isolation kit. The expression of mature miR-34a was detected using the TaqMan microRNA Assay and the TaqMan Universal PCR Master Mix with U6 as an internal control gene. All reactions were performed in triplicate wells. The cycle threshold (CT) value of the samples in each reaction well was recorded. The experimental results were analyzed using the relative quantification method of qRT-PCR.

To each of the six-well cell culture plates, 1 ml RIPA lysis buffer [150 mM NaCl, 1 % NP40, 0.5 % sodium deoxycholate, 0.1 % sodium dodecyl sulfate (SDS), 50 mM Tris (pH 7.9), 10 mM NaF, 10 mM PMSF and 1× protease inhibitors] (Complete cocktail tablets, Roche) was added. Cell lysates were transferred to 1.5-ml microfuge tubes and centrifuged at $16{,}000\times g$ for 30 min. The supernatants were collected, and the protein concentration was determined using the bicinchoninic acid method. After the 5 % stacking gel and 15 % separating gel were cast, 50 μg protein was loaded into each lane, separated by electrophoresis, and wet transferred to a polyvinylidene difluoride membrane (Bio-Rad, USA). After blocking in Tris-buffered saline and Tween-20 (TBST) solution (10 mM Tris–HCl (pH 7.5), 150 mM NaCl, and 0.1 % Tween-20) containing 5 % nonfat dry milk at room temperature for 1 h, the membranes were separately incubated with either the rabbit antihuman survivin polyclonal antibody (1:500 dilution) or the mouse anti-human β-actin monoclonal antibody (1:1,000 dilution) and incubated overnight at 4 °C. The appropriate secondary antibodies labeled with IRDye 800 (1:2,000 dilutions in PBS) were then added, followed by an overnight incubation at 4 °C. After washing with TBST, the Odyssey infrared imaging system (Rockland) was used to scan the membranes. The relative content of survivin was represented as the grayscale ratio of survivin/β-actin, and the grayscale was analyzed using the QuantityOne software (Bio-Rad, USA).

MTT assay detection of the effects of miR-34a on the viability of cisplatin-induce gastric cancer cell

SGC-7901 gastric cancer cells cultured under normal conditions were inoculated uniformly into six-well culture plates at a concentration of 3×10^5 cells/ml. After adherent cell culture, transfections were conducted for miR-34a mimic, non-specific control and miR-34a inhibitor according to the Lipofectamine 2000 transfection manual. The normal control group was also established. The RNA of cells in each group was extracted using the TaqMan miRNA Isolation kit and qRT-PCR was used to determine the changes of expression in SGC-7901 cells.

SCG-7901 or SGC-7901/DDP cells cultured under normal conditions were inoculated uniformly into 96-well culture plates at a concentration of 3×10^5 cells/ml. The transfection procedure was described above. miR-34a inhibitor and non-specific control were transfected into SGC-7901 cells, while miR-34a mimic and non-specific control were transfected to SGC-7901/DDP cells. After transfection, cisplatin (DDP)

with a final concentration of 0.5 μg/ml was added to the SGC-7901 cell culture; the final concentration of cisplatin (DDP) added to the SGC-7901/DDP culture was 5 μg/ml. Forty-eight hours after transfection, 100 μl of MTT (0.5 mg/ml) solution was added to each well, and the plates were placed in a 37 °C/5 % CO_2 incubator for 4 h. A total of 100 μl of 20 % SDS (cosolvent 50 % dimethyl formamide) was added to each well, and the plates were incubated at 37 °C for 24 h. A microplate reader (Bio-Tek, USA) was used to measure the OD values at 570 nm. Each experimental group contained ten replicate wells, and the experiment was repeated three times.

Flow cytometry detection of the effects of miR-34a on the cellular apoptosis of cisplatin-induce gastric cancer cells

SCG-7901 or SGC-7901/DDP cells cultured under normal conditions were inoculated uniformly into six-well culture plates at a concentration of 3×10^5 cells/ml. miR-34a inhibitor and non-specific control were transfected into SGC-7901 cells, while miR-34a mimic and non-specific control were transfected to SGC-7901/DDP cells. After transfection, cisplatin (DDP) with the final concentration of 0.5 μg/ml was added to the SGC-7901 cell culture; the final concentration of cisplatin (DDP) added to the SGC-7901/DDP culture was 5 μg/ml. Normal control groups were established for both SGC-7901 or SGC-7901/DDP cells. After the transfection for 48 h, the cells were washed with PBS for 1–2 times, followed by addition of Annexin V-FITC and PI staining reagents. The mixture was incubated in darkness for 15 min. After being filtrated with a screen cloth, the cells were analyzed using flow cytometry (BD, USA). FCM CellQuest software was used to count the cells, and Macquit software was used to analyze the data.

Signaling pathway of effects of miR-34a ectopic expression on the sensitivity of cisplatin-induce gastric cancer cells

SCG-7901 or SGC-7901/DDP cells cultured under normal conditions were inoculated uniformly into six-well culture plates at a concentration of 3×10^5 cells/ml. miR-34a inhibitor and non-specific control were transfected into SGC-7901 cells, while miR-34a mimic and non-specific control were transfected to SGC-7901/DDP cells. After transfection, cisplatin (DDP) with a final concentration of 0.5 μg/ml was added to the SGC-7901 cell culture; the final concentration of cisplatin (DDP) added to the SGC-7901/DDP culture was 5 μg/ml. To SGC-7901 or SGC-7901/DDP cell cultures, the PI3K pathway inhibitor Wortmannin was added, with a final concentration as 5 μM. Normal control groups were established for both SGC-7901 or SGC-7901/DDP cells. After the transfection for 48 h, the cells were washed with PBS for 1–2 times. Then, 1 ml RIPA lysis buffer was added. The supernatants were collected, and the protein concentration was determined using the bicinchoninic acid method. The proteins were separated by electrophoresis and wet transferred to a polyvinylidene difluoride membrane (Bio-Rad, USA). After blocking in TBST solution containing 5 % nonfat dry milk at room temperature for 1 h, AKT, phospho-AKT (p-AKT, Ser473) primary antibody (1:500 dilution) and mouse anti-human β-actin monoclonal antibody (1:1,000 dilution) and incubated overnight at 4 °C. After washing with TBST, the Odyssey infrared imaging system was used to scan the membranes. The relative content of survivin was represented as the grayscale ratio of survivin/β-actin, and the grayscale was analyzed using the QuantityOne software.

The effect of PI3K pathway inhibitor Wortmannin on the apoptosis of cisplatin-induce gastric cancer cells with miR-34a ectopic expression was determined by flow cytometry.

Statistical analysis

Data were analyzed using SPSS 17.0. software (SPSS, Inc., Chicago, IL, USA). Comparisons between two groups are performed using the *t*-test, whereas comparisons among three or more groups using analysis of variance. A p value <0.05 was considered statistically significant.

Results

Expression profiles of miR-34a and survivin in human gastric cancer cell lines

Using qRT-PCR, we determined that the miR-34a expression profile of cisplatin-resistant gastric cancer cell lines (SGC-7901/DDP) were significantly lower than that of SGC-7901 gastric cancer cell lines ($p<0.05$) (Fig. 1a). Western blot analysis showed up-regulation of survivin protein expression in SGC-7901/DDP cells in comparison to that of SGC-7901 cells ($p<0.05$) (Fig. 1b).

MTT assay detection of the effects of miR-34a on the viability of cisplatin-induce gastric cancer cell

miR-34a mimic was transfected into SGC-7901 cells to establish miR-34a over-expression model. miR-34a inhibitor was also transfected to produce miR-34a inhibition model. As shown by qRT-PCR assays, the expression level of miR-34a mimic transfected group was signficant higher than those of normal control group ($p<0.01$) and negative control (NC) group ($p<0.01$). In the miR-34a inhibitor transfected group, miR-34a expression was significantly lower than those of normal control group ($p<0.01$) and NC group ($p<0.01$), as shown in Fig. 2.

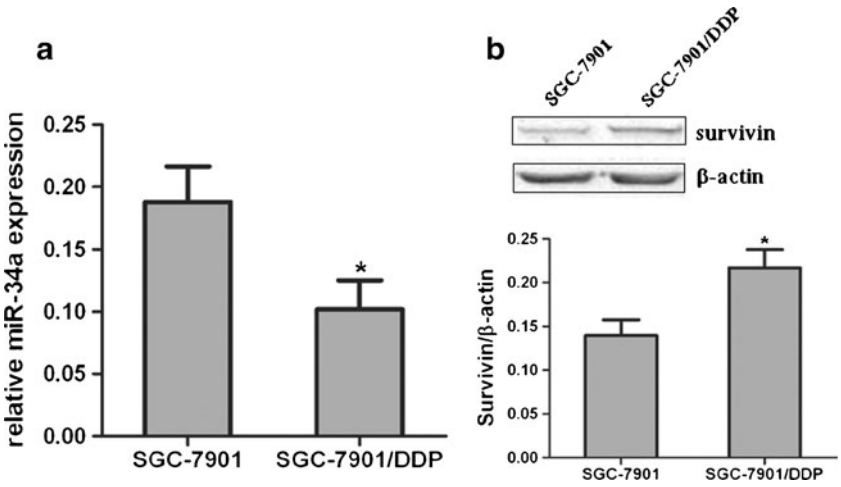

Fig. 1 the expression profiles of miR-34a and survivin in human gastric cancer cell lines. **a** The expression profile of miR-34a in SGC-7901 and SGC-7901/DDP cell lines assayed by qRT-PCR; **b** the expression profile of survivin in SGC-7901 and SGC-7901/DDP cell lines assayed by Western blot. *$p<0.05$ versus SGC-7901 group

The effect of miR-34a ectopic expression on the viability of cisplatin-induce gastric cancer cells was determined by MTT assays. As shown by MTT assays, the viability of SGC-7901 cells transfected with miR-34a inhibitor and incubated with DDP of 0.5 μg/ml for 48 h was significantly higher than that of NC cells ($p<0.05$). The viability of SGC-7901/DDP cells transfected with miR-34a mimic and incubated with DDP of 5 μg/ml for 48 h was significantly lower than that of NC cells ($p<0.05$), as shown in Fig. 3. These results showed that miR-34a over-expression could increase the sensitivity of SGC-7901/DDP cells to cisplatin-based chemotherapies.

Effects of miR-34a on cellular apoptosis by flow cytometry

As shown by flow cytometry assay, the percentage of apoptotic SGC-7901 cells transfected with miR-34a inhibitor and incubated with DDP of 0.5 μg/ml for 48 h was significantly lower than that of the NC group ($p<0.05$), while the percentage of apoptotic SGC-7901/DDP cells transfected with miR-34a mimic and incubated with DDP of 5 μg/ml for 48 h was significantly higher than that of the NC group ($p<0.05$), as shown in Fig. 4. These results showed the inhibition of miR-34a expression contributed to the decreased sensitivity of SGC-7901 cells to cisplatin.

Signaling pathway of effects of miR-34a expression on the sensitivity of cisplatin-induce gastric cancer cells

As shown by MTT and flow cytometry, the inhibition of miR-34a expression contributed to the decreased sensitivity of SGC-7901 cells to cisplatin, while over-expression of miR-34 resulted in increased sensitivity of SGC-7901/DDP cells to cisplatin. Moreover, the pathway of miR-34a mediated mechanism was investigated by using the PI3K pathway inhibitor Wortmannin.

As shown by Western blot, the p-AKT/AKT ratio of SGC-7901 cells transfected with miR-34a inhibitor and incubated with DDP of 0.5 μg/ml for 48 h was significantly lower than that of normal control cells ($p<0.05$), while the p-AKT/AKT ratio of miR-34a inhibitor transfected group was significantly

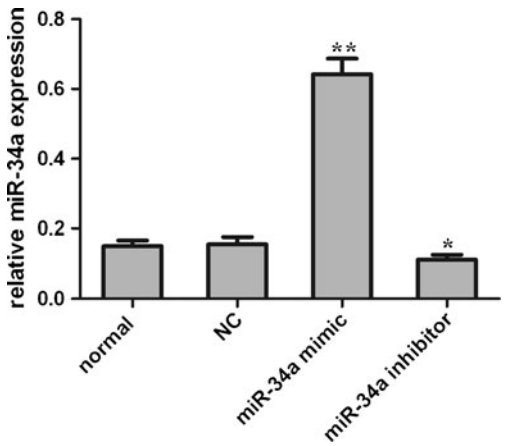

Fig. 2 The effects of miR-34a mimic or miR-34a inhibitor transfection on the expression of miR-34a in gastric cancer cell lines (SGC-7901). **$p<0.01$ and *$p<0.05$ versus normal group or NC group

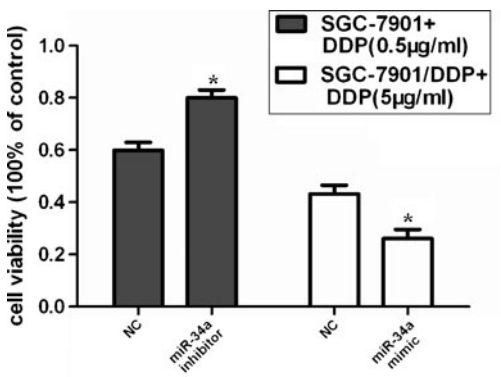

Fig. 3 The effect of miR-34a ectopic expression on the viability of cisplatin-induced gastric cancer cells. *$p<0.05$ versus NC group

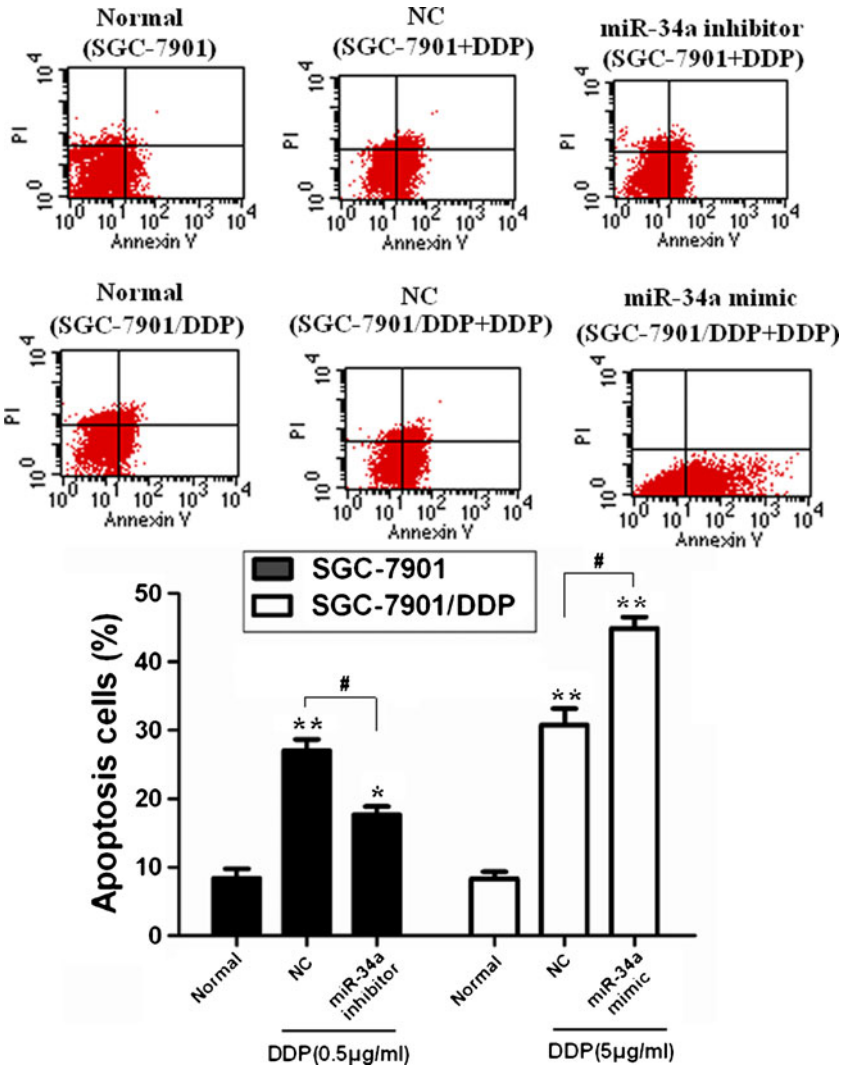

Fig. 4 Effects of miR-34a on cellular apoptosis of cisplatin-induce gastric cancer cells by flow cytometry. $^{**}p<0.01$ versus normal group. $^{\#}p<0.05$ versus NC group

higher than that of Wortmannin treatment group ($p<0.05$), as shown in Fig. 5a. The p-AKT/AKT ratio of SGC-7901/DDP cells transfected with miR-34a mimic and incubated with DDP of 5 μg/ml for 48 h was significantly lower than that of normal control group ($p<0.05$) but was not significantly different from that of Wortmannin treatment group, as shown in Fig. 5b.

As shown by flow cytometry assay, the percentage of apoptotic SGC-7901 cells transfected with miR-34a inhibitor and incubated with DDP of 0.5 μg/ml or 5 μM was significantly higher than that of normal control group ($p<0.01$). The percentage of apoptotic SGC-7901/DDP cells transfected with miR-34a inhibitor and incubated with DDP of 0.5 μg/ml or 5 μM was significantly higher than that of normal control group ($p<0.01$), as shown in Fig. 6.

In summary, Wortmannin could inhibit miR-34a inhibitor and DDP induced p-AKT expression and stimulate apoptosis. miR-34a over-expression could improve the sensitivity of gastric cancer cells against Cisplatin-based chemotherapies, with PI3K/AKT/survivin signaling pathway possibly involved in the mechanism.

Discussion

Research concerning the relationship between miRNAs and gastric cancer has gradually gained attention from researchers in the general surgery field, and this research has become an important research topic in recent years. By investigating the miRNA expression profile in gastric cancers, Yao et al. determined that miR-34a was among the 24 miRNAs displaying a greater than 2-fold expression difference between gastric cancer and normal gastric tissues [33]. The miR-34 family is a class of miRNAs that are highly evolutionarily conserved, and they are widespread in arthropods, nematodes, and mammals [34]. In virtually all vertebrates, there are three miR-34 members, including miR-34a, miR-34b, and miR-34c. In most human tissues, the expression level of miR-34a is much higher than that of miR-34b/c [26]. Kumar et al. [35] and Shen et al. [36] observed that the expression of miR-34a is down-regulated in laryngeal squamous cell carcinoma and HNSCC. The above-mentioned studies confirmed that miR-34a plays a role that is similar to tumor suppressor miRNAs; however, the

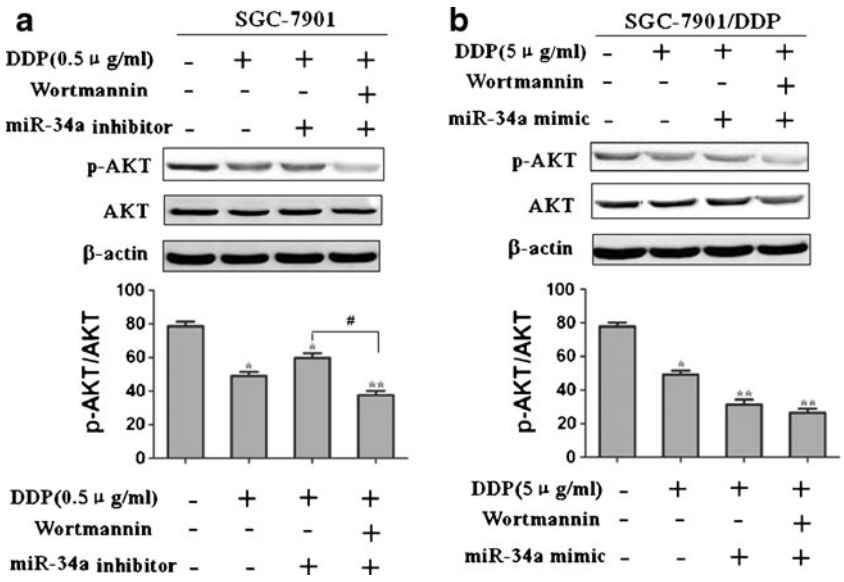

Fig. 5 **a** Expression profiles of p-AKT, AKT, and β-actin in SGC-7901 cells of each group by Western blot analysis. **b** Relative changes of expression of p-AKT, AKT and β-actin in SGC-7901 cells of each group. *$p<0.05$ and **$p<0.01$ versus normal group. #$p<0.05$

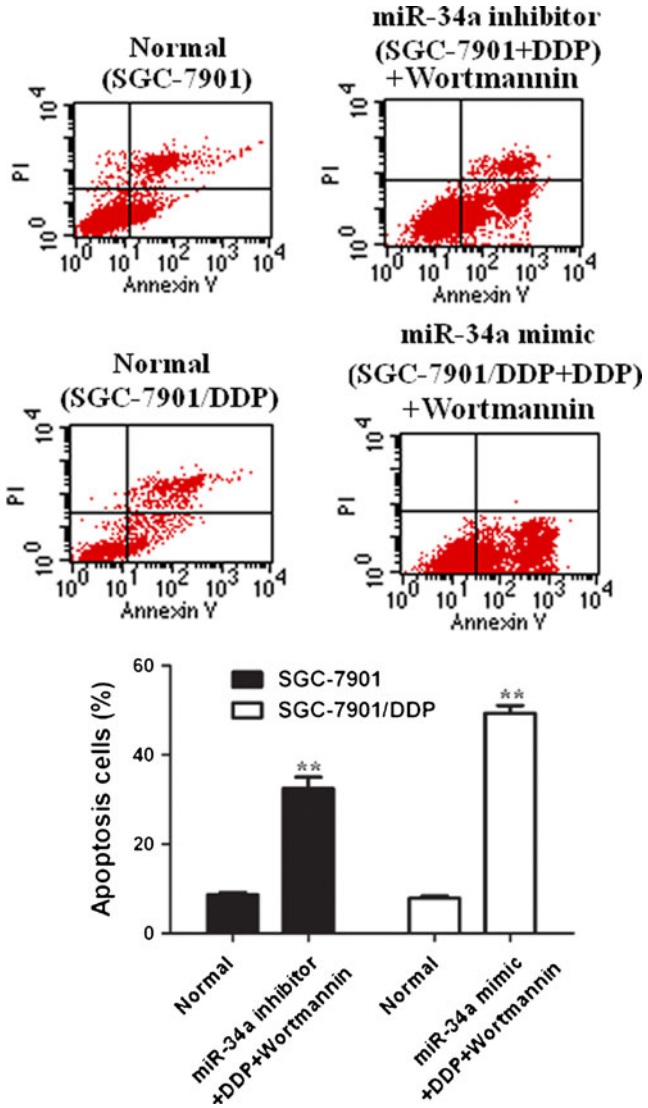

Fig. 6 Apoptosis of gastric cancer cell per group by flow cytometry. **$p<0.01$ versus normal group

relationship between miR-34a and the sensitivity of gastric cancer to chemotherapies has not been established.

It has been demonstrated that resistance to chemotherapy is one of the major causes of failure of gastric cancer chemotherapy. The development of chemotherapy resistance could be influenced by many factors, including isolated intracellular drug efflux, metabolic detoxification, drug target alternation, DNA repair improvement, and apoptosis regulation [37, 38]. The mechanism of drug resistance has been extensively investigated at the levels of genes and proteins. It has been disclosed that several genes correlate positively to the resistance to chemotherapy, such as P-gp, MRP, GST-π, TopoII, et al.-π, and TopoII [39].

However, with intervention in relevant genes and their upstream and downstream targets, there seems to be no significant improvement of clinical outcome for the resistance to chemotherapy [40]. Thus, it is of the utmost importance to investigate more factors contributing to chemotherapy resistance comprehensively in depth.

In our previous studies, down-regulation of miR-34a expression was observed in human gastric cancer cell lines; miR-34a can negatively regulate survivin protein expression and inhibit gastric cancer cell proliferation and invasion [30]. In this study, the correlation between miR-34a expression profile and the sensitivity of Human gastric cancer cell to cisplatin-based regimen was investigated in cisplatin-resistant cell lines (SGC-7901/DDP) and Human gastric cancer cell line (SGC-7901). Moreover, the effects of miR-34a ectopic expression on the sensitivity of cisplatin-induced gastric cancer cells were also investigated by transfecting miR-34a inhibitor into SGC-7901 cells and transfecting miR-34a mimic into SGC-7901/DDP cells.

As shown by MTT and flow cytometry, the viability of SGC-7901 cells transfected with miR-34a inhibitor and incubated with DDP of 0.5 μg/ml for 48 h was significantly higher

than that of NC cells ($p < 0.05$), but the percentage of apoptotic SGC-7901 cells was significantly lower than that of NC group ($p < 0.05$). In contrast, the viability of SGC-7901/DDP cells transfected with miR-34a mimic and incubated with DDP of 5 μg/ml for 48 h was significantly lower than that of NC cells ($p < 0.05$), but the percentage of apoptotic SGC-7901/DDP cells was significantly higher than that of the NC group ($p < 0.05$). As a result, the inhibition of miR-34a expression contributed to the decreased sensitivity of SGC-7901 cells to cisplatin, while over-expression of miR-34 resulted in increased sensitivity of SGC-7901/DDP cells to cisplatin. As disclosed in other previous studies, miR-34a is related to the sensitivity of breast cancer and NSCLC to chemotherapies [31, 32]. Our current study further confirms that miR-34a is related to the sensitivity of gastric cancer to chemotherapies.

PI3K/AKT signaling pathways could be activated by cytokines, growth factors, and physical stimulation. The activated AKT could induce downstream phosphorylation cascade, target protein interaction, and regulation of cell growth and survival, proliferation and apoptosis, carbohydrate metabolism, gene transcription, angiogenesis, cell migration and cell cycle, and other cellular activities and biological effects [41, 42]. Du et al. [43] demonstrated the acquisition of paclitaxel resistance via PI3K dependent signaling pathway. In this study, miR-34a over-expression has been demonstrated to improve the sensitivity of gastric cancer cells to cisplatin and this effect was considered to be mediated via the PI3K/AKT/survivin signaling pathway.

In conclusion, the miR-34a expression was down-regulated in cisplatin-resistant cell lines. The over-expression of miR-34a could improve the sensitivity of gastric cancer cells to cisplatin-based chemotherapies. This effect was considered to be mediated via the PI3K/AKT/survivin signaling pathway.

Conflicts of interest The authors have no commercial, proprietary, or financial interest in the products or companies described in this article.

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