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Original Article

THY-1 (CD90) expression promotes the growth of gastric cancer cells

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Abstract: Objective: To observe the expression of THY-1 (CD90) in gastric tumour cells and its effect on the growth of gastric cancer and to provide new evidence for the development of possible targets for the treatment of gastric cancer. Methods: The effect of THY-1 on the proliferative ability of HGC-27, MGC-803 and AGS gastric cancer cells was examined by CCK-8 and cell cycle assays. The effect of THY-1 on the ability of gastric cancer cells to avoid apoptosis was analysed by Annexin V/PI double staining. The effect of THY-1 on the tumourigenic ability of gastric cancer cells in vivo was explored by subcutaneous tumour formation assay in nude mice. Results: The CCK-8 assay showed that the proliferative activity of HGC-27 and MGC-803 gastric cancer cells was significantly limited after THY-1 interference in vitro ($P < 0.01$); however, exogenous THY-1 significantly promoted the growth of AGS gastric cancer cells ($P = 0.003$). The cell cycle assay showed that exogenous THY-1 reduced the G0/G1 phase arrest of AGS cells and facilitated cell entry into S phase, which accelerated cell division and proliferation ($P = 0.008$). After interference in the expression of the THY-1 gene, HGC-27 cells showed significant G0/G1 arrest, while the percentage of S phase cells decreased, and cell proliferation was inhibited ($P < 0.001$). The apoptosis assay showed that the average apoptosis rate of AGS cells was significantly lower in the overexpression group versus the control group (7.89 ± 1.08% vs. 11.90 ± 0.45%, $P = 0.004$). In contrast, the average apoptosis rate of HGC-27 cells was significantly increased in the interference group versus the control group (37.88 ± 5.47% vs. 22.84 ± 1.50%, $P = 0.01$). The subcutaneous tumour formation assay in nude mice revealed that at week 3, tumour volume and weight reached 1018.33 ± 521.48 mm^3 and 81.47 ± 41.72 mg, respectively, in the control group, while tumour volume and weight were only 213.72 ± 111.94 mm^3 and 17.10 ± 9.00 mg, respectively, in the interference group; the differences between the two groups were statistically significant ($P < 0.01$). Conclusions: THY-1 promoted the proliferation of gastric cancer cells and reduced the apoptosis rate of gastric cancer cells with a lack of nutrient supply. Moreover, THY-1 promoted subcutaneous tumour formation and growth in nude mice, as indicated by the results of the subcutaneous tumour formation assay.

Keywords: Gastric cancer, THY-1, proliferation, apoptosis

Introduction

THY-1 (CD90), a member of the cell adhesion molecule immunoglobulin superfamily, is expressed at different degrees on the surface of neuronal cells, thymic cells, endothelial cells, glomerular mesangial cells and haematopoietic stem cells [1]. Under normal conditions, THY-1 has roles in various physiological processes including the regulation of neuronal axonal growth, activation of T cells, induction of apoptosis/necrosis and the regulation of fibrosis [2-4]. Under pathological conditions, the THY-1 gene may be abnormally expressed, which has a significant impact on the development and progression of tissue inflammation and fibrosis as well as on various human malignancies. THY-1 expression is often absent in the lung tissue of patients with idiopathic pulmonary fibrosis, which significantly differs from the positive expression of THY-1 observed in the lung tissue of healthy individuals [5, 6]. In the field of tumour research, the dysregulation of THY-1 has been reported in various solid tumours such as liver cancer, oesophageal cancer, nasopharyngeal cancer, ovarian cancer

Table 1. Primer sequences of related genes

Gene name	Primer sequence (5'-3')
THY-1-F	ATCGCTCTCCTGCTAACAGTC
THY-1-R	CTCGTACTGGATGGGTGAACT
18s-F	GTAACCCGTTGAACCCCATT
18s-R	CCATCCAATCGGTAGTAGCG

and prostate cancer; THY-1 is also thought to play various roles in tumour development and progression. For example, THY-1 expression is abnormally elevated in malignant tumours such as melanoma [7], liver cancer [8] and oesophageal cancer [9], where THY-1 functions as an "oncogene". In contrast, THY-1 expression is lost to varying degrees in ovarian cancer and nasopharyngeal cancer, where THY-1 is thought to inhibit the proliferation and metastatic ability of tumour cells [10].

However, the effect of THY-1 on gastric cancer cells has rarely been reported in gastric cancer tissues. More than 90% of clinical samples from gastric cancer patients were detected CD90 positive, these cells are more likely to promote tumor growth and proliferation in vivo [11]. Oikonomou et al. found that Thy-1 was detected in the majority of 57 GIST samples (54 out of 57 patients), and the patients with THY-1 negative expression had better prognosis. Zhu GC et al. found that CD90 is elevated in gastric cancer cell in vitro [12]. THY-1 is a "bad" or "good molecular"? The purpose of the present study was to investigate the dual role of THY-1 as a "promoter" and a "suppressor" in gastric cancer.

Materials and methods

Cells and animals

The human gastric cancer cell lines HGC-27, N87, SGC-7901, AGS, MGC-803, BGC-823 and MKN-45 were purchased from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. Five-week-old male BALB/c-nu nude mice weighed between 18 and 20 g and were reared in a specific-pathogen-free animal laboratory.

qRT-PCR assay of target gene expression in cells

Cells were rinsed with cold phosphate-buffered saline (PBS, 0.01 M, pH 7.2), which was followed by the addition of 1 ml of TRIzol reagent per dish. The cell suspension was fully pipetted, allowed to stand for 10 min and was then transferred into a 1.5-ml centrifuge tube. Subsequently, 200 μl of chloroform was added. The mixture was vortexed and allowed to stand at room temperature for 10 min, at which point it was centrifuged at 12,000 rpm at 4°C for 15 min. Flocculent RNA precipitate was visible, and the supernatant was discarded. The RNA was washed with 1 ml of pre-cooled 75% ethanol followed by 1 ml of pre-cooled anhydrous ethanol. The ethanol was discarded, and the RNA was air-dried. The RNA was dissolved in DEPC water, and the concentration of RNA was measured and adjusted to 3 μg/10 μl. The RNA was stored on ice until further use. Reverse-transcription PCR was performed according to the manufacturer's instructions provided with the Thermo Fisher RT-PCR kit (10928042). After reverse transcription, the cDNA was diluted. A quantitative fluorescence PCR reaction was prepared and performed according to the manufacturer's instructions provided with the Thermo Fisher qRT-PCR kit (11736059). The primer sequences of related genes are shown in **Table 1**.

Western blot assay of target proteins in cells

Cells were washed once with cold PBS, which was followed by the addition of 120 μl of cell lysis buffer and subsequent lysis on ice for 30 min. The lysate was then transferred to a 1.5-ml EP tube and centrifuged at 12,000 rpm at 4°C for 15 min. The supernatant was transferred to a new EP tube, and the protein concentrations were measured by BCA assay. Based on the results, the corresponding loading buffer and PBS were added, and the protein was denatured at 95°C for 10 min. After precooling on ice, the proteins were separated by electrophoresis, transferred onto a membrane, and incubated with blocking solution for 1 h at room temperature. Primary antibody (dilution ratio = 1:1000) was added, and the membrane was incubated at 4°C overnight, followed by three quick washes (5 min per wash) in Tris-buffered saline with Tween. Subsequently, the secondary antibody (dilution ratio = 1:1000) was added, and the membrane was incubated at room temperature for 1 h, followed by three quick washes (5 min per wash) in Tris-buffered saline with Tween. The membrane was then developed to visualize the results.

Stable transfection interference and overexpression of the THY-1 gene in cells

Twenty-four hours before transfection, the gastric cancer cell lines to be transfected were digested with trypsin and adjusted to a cell density of 2×10^5 cells/ml with RPMI1640 medium supplemented with 10% foetal bovine serum (FBS). The cell suspension (1 ml each) was inoculated into 6-well plates and placed in a 37°C thermostat incubator with 5% CO_2. Cell confluence reached 30-50% after 24 h. An appropriate amount of virus solution, which was diluted with RPMI1640 medium supplemented with 10% FBS, was then thawed on ice. Polybrene was also added at a final concentration of 5 μg/ml to dilute the virus and to increase the efficiency of the viral infection. The original medium was aspirated, and the mixed virus dilution was added. The cell culture was incubated for another 24 h after which the virus-containing medium was aspirated and replaced with fresh complete medium. The cell culture was incubated for another 48 h, which was followed by digestion and subculture of the cells in Petri dishes. The cells were screened with RPMI1640 complete medium containing 6 μg/ml of puromycin. One week later, the puromycin concentration was reduced to 2 μg/ml to maintain cell resistance. When the status of transfected cells was stable, the THY-1 expression level in each cell line was assayed by qRT-PCR and Western blot. The required cell lines were selected for subculture and subsequent assays.

CCK-8 assay for cell proliferation

Cells in the logarithmic phase were digested to prepare a single cell suspension. The cell density was adjusted to 2×10^4 cells/ml, and the cells were then added to 96-well plates. The cells were incubated at 37°C in a CO_2 incubator for 6-8 h. Subsequently, 10 μl of CCK-8 reagent was added to each well, and the cells were further incubated for an additional hour. The optical density (OD value) at 450 nm was measured using a microplate reader, and the cell growth curve was plotted.

Cell cycle assay

After adhesion, the cells were cultured in serum-free medium, and 12 hours later, the medium was changed to complete medium supplemented with 10% FBS. The cells were cultured for another 48 h and then harvested. The cells were washed twice with PBS pre-cooled to 4°C and then centrifuged at 1000 rpm for 5 min. The supernatant was discarded, and the cell pellet was resuspended in 300 μl of PBS pre-cooled to 4°C until a single cell suspension was formed. The cell suspension was added dropwise into pre-chilled -20°C anhydrous ethanol and placed in a -20°C freezer overnight for fixation. The cell suspension was then centrifuged at 1000 rpm for 5 min, and the supernatant was discarded. Subsequently, 100 μl of binding buffer containing PI (final concentration, 10 μg/ml) and RNase A (final concentration, 100 μg/ml) was added, and the cell suspension was incubated in the dark for 30 min at room temperature. Thereafter, the cell suspension was transferred to a sample tube for flow cytometry, which was followed by the addition of 200 μl of binding buffer. The cell suspension was thoroughly mixed before the flow cytometric analysis. The results were analysed using the ModFit cell cycle fitting software.

Apoptosis assay

Cells in the logarithmic phase were digested with 0.25% trypsin and then seeded into 6-well plates at the density of 1×10^5 cells per well. The cells were cultured in complete medium for 24-36 h to reach a cell confluence of 50%. Then, the cells were cultured in serum-free medium in order to induce apoptosis by starvation. Forty-eight hours later, the cells were harvested and washed twice with PBS pre-cooled to 4°C, followed by centrifugation at 1000 rpm for 5 min. The supernatant was discarded, the cells were resuspended in binding buffer, and the cell density was adjusted to 1×10^6 cells/ml. Subsequently, 5 μl of FITC Annexin V dye and 5 μl of PI were added, and the cell suspension was incubated in the dark at room temperature for 15 min. Thereafter, 200 μl of binding buffer was added, and the cell suspension was thoroughly mixed before the flow cytometric analysis. Data were collected and scatter plots were drawn using CellQuest software in order to analyse the percentage of cells in each quadrant. The lower left (LL) quadrant represents the normal cell population; the upper left (UL) quadrant represents the necrotic/mechanically damaged cell population; the lower right (LR)

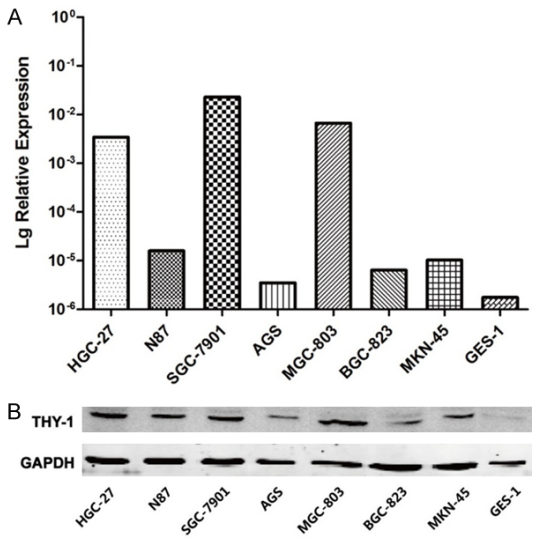

Figure 1. THY-1 gene and protein expression in different gastric cancer cell lines (A. qRT-PCR; B. Western blot).

quadrant represents the early apoptotic cell population; and the upper right (UR) quadrant represents the advanced apoptotic cell population. The sum of the LR and UR was calculated to represent the total percentage of apoptotic cells.

Subcutaneous tumour formation assay in nude mice

Inoculation of tumour cells: The cells in each group were cultured in complete medium supplemented with 10% FBS under standard conditions. Cells in the logarithmic phase were harvested for subsequent use. The cells were digested with trypsin and centrifuged at 1000 rpm. The supernatant was discarded, and the cell pellet was resuspended in serum-free medium. The cell suspension was adjusted to a cell density of 1×10^7 cells/ml and stored on ice before use. Five-week-old male BALB/c-nu nude mice that weighed between 18 and 20 g were selected and divided into groups, with five mice per group. The nude mice were first subjected to routine disinfection. Each group of cells was inoculated into the left inguinal subcutaneous layer of the nude mice by subcutaneous injection. Each mouse received 200 μl of cell suspension (2×10^6 cells).

Observation of tumour formation in nude mice: The general activities and nutritional status of the nude mice were observed on a daily basis. Tumour masses were palpable 5-7 days later. After the formation of the tumour mass, the long diameter (a) and short diameter (b) of each tumour mass were measured by the same observer every 3 days using a Vernier calliper. The volume of each tumour mass was calculated using the following formula: $V\ (mm^3) = ab^2\times \pi/6$. The growth curve was drawn, and three weeks later, the tumour-bearing mice were sacrificed by cervical dislocation, and finally, the weight of each tumour mass was determined.

Statistical analysis

The results were analysed using GraphPad Prism 5. Data are presented as the mean ± SD. The analysis of variance was used for multiple comparisons, and the t-test was used for pairwise comparisons. Differences were considered highly significant at $P < 0.001$, statistically significant at $0.001 < P < 0.05$ and not significant at $P > 0.05$.

Results

THY-1 expression in gastric cancer cells

The expression of the THY-1 gene at the mRNA and protein levels was considerably different among various gastric cancer cell lines. SGY-7901, MGC-803 and HGC-27 cells showed the highest expression, followed by N87, MKN-45 and BGC-823 cells; AGS cells showed the lowest expression. The expression of the THY-1 gene in the normal human gastric mucosal epithelial cell line GES-1 was significantly lower than the THY-1 expression level in gastric cancer cells (**Figure 1A** and **1B**).

Verification of THY-1 overexpression and interference

The THY-1 gene was downregulated in the HGC-27 and MGC-803 cell lines, which normally express high levels of THY-1, and was overexpressed in the AGS cell line, which normally expresses low levels of THY-1. PCR and Western blot assays showed that the efficiency of four interference sequences used to downregulate THY-1 expression at the gene level was 34.8%, 78.6%, 81.4% and 78.2%, respectively, in HGC-27 cells compared with sh-nc cells (**Figure 2A**). The efficiency of four interference sequences

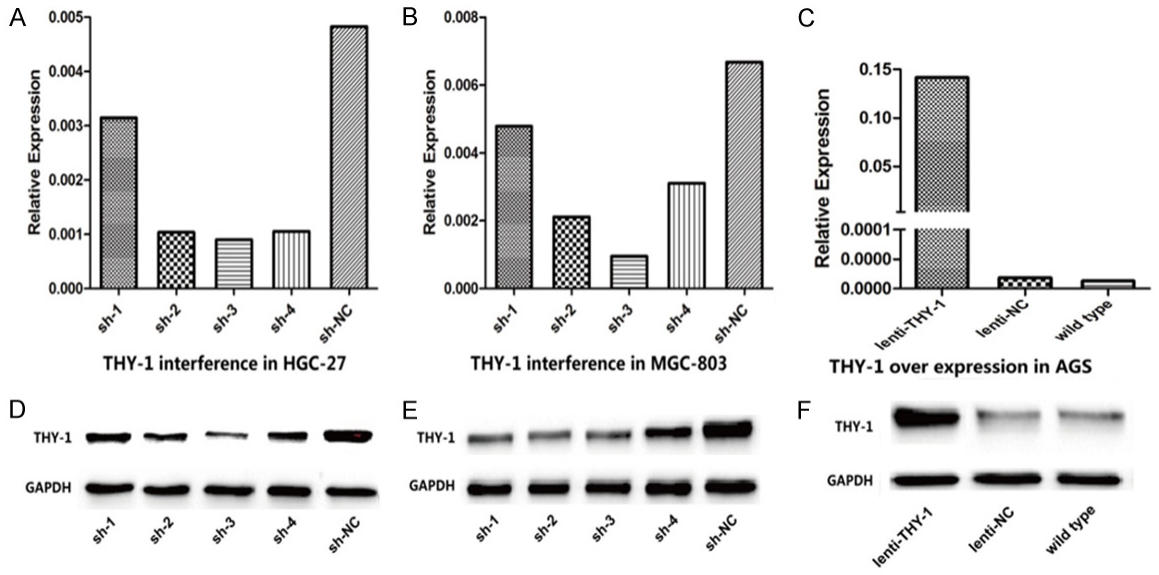

Figure 2. THY-1 expression efficiency in gastric cancer cells after stable transfection with the lentivirus. A, B, D and E: Show interference in the expression of the THY-1 gene in HGC-27 and MGC-803 cells; sh-1, 2, 3 and 4 are different interference plasmids. C and F: Show the overexpression of the THY-1 gene in AGS cells; lenti-THY-1 is the overexpression group, and lenti-NC is the control group.

used to downregulate THY-1 expression at the gene level was 28.4%, 68.4%, 85.7% and 53.4%, respectively, in MGC-803 cells compared with sh-nc cells (**Figure 2B**). The Western blot data were generally consistent with the PCR data (**Figure 2D** and **2E**). Based on the above results, we selected two sequences with the highest interference efficiency, sh-2 and sh-3, for stable transfection of HGC-27 and MGC-803 cells, which were then used in subsequent assays. The analysis of the overexpression of the THY-1 gene in the AGS cell line showed that in the overexpression group, THY-1 mRNA and protein expression was significantly elevated compared with the control and wild-type groups. In particular, the overexpression of THY-1 mRNA was 7621 times that of the control group and 10,944 times that of the wild-type group (**Figure 2C**). This change in mRNA expression was similar to the change in protein expression (**Figure 2F**).

Effect of THY-1 on the proliferative ability of gastric cancer cells

The effect of THY-1 on cell proliferation after the stable transfection of cells with interference plasmids, which led to the subsequent overexpression of the THY-1 gene, was evaluated by CCK-8 assay post-transfection. The results showed that after interference in the expression of the THY-1 gene, the OD values of HGC-27 and MGC-803 cells at 72 h were significantly lower than those of the control group (sh-nc group; $P < 0.01$ for both groups). In the following culture, the OD values of the interference group became increasingly lower than those of the blank group as time increased (**Figure 3A** and **3B**). This indicates that interference in the expression of the THY-1 gene inhibited the proliferation of HGC-27 and MGC-803 cells. When THY-1 was overexpressed in AGS cells, the OD value of the overexpression group was significantly higher than that of the control group at 48 h (**Figure 3C**). This indicates that THY-1 promoted the proliferation of AGS cells.

Effect of THY-1 on the cell cycle of gastric cancer cells

After THY-1 overexpression in AGS cells and THY-1 downregulation in HGC-27 cells, the cell cycle was assayed by flow cytometry in these two cell lines. In AGS cells, the average percentage of cells in S phase in the THY-1 overexpression group was 42.17 ± 1.78%, which was significantly higher than the corresponding percentage in the control group (27.87 ± 1.93%) ($P = 0.001$). Moreover, the percentage of cells in G0/G1 phase in the control group was significantly higher than that in the THY-1 overexpres-

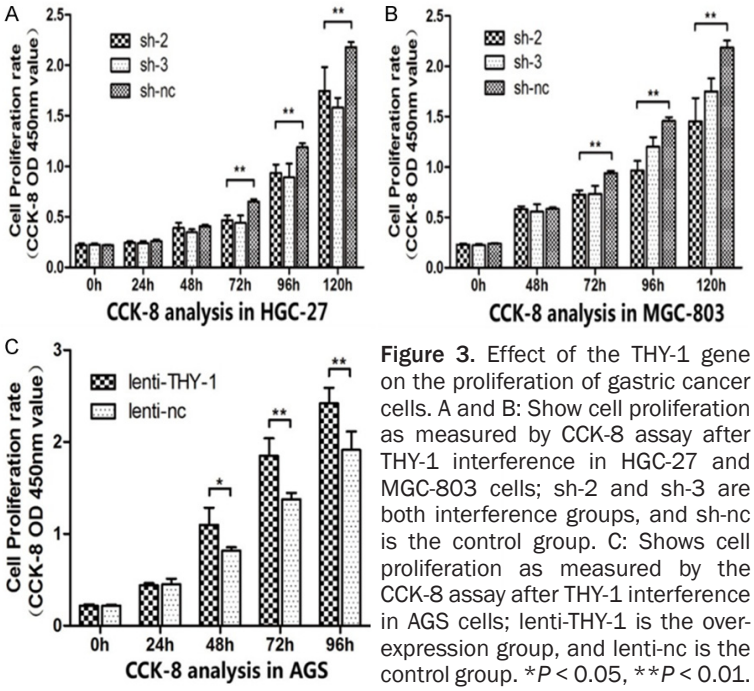

Figure 3. Effect of the THY-1 gene on the proliferation of gastric cancer cells. A and B: Show cell proliferation as measured by CCK-8 assay after THY-1 interference in HGC-27 and MGC-803 cells; sh-2 and sh-3 are both interference groups, and sh-nc is the control group. C: Shows cell proliferation as measured by the CCK-8 assay after THY-1 interference in AGS cells; lenti-THY-1 is the overexpression group, and lenti-nc is the control group. $*P < 0.05$, $**P < 0.01$.

sion group (41.20 ± 1.07% vs. 37.69 ± 0.65%) (P = 0.008). Thus, exogenous THY-1 reduced the number of AGS cells in G0/G1 phase arrest and promoted cell entry into S phase, which accelerated cell division and proliferation (**Figure 4A-C**). In HGC-27 cells, the average percentage of cells in S phase in the THY-1 interference group was 50.00 ± 0.19%, which was lower than that of the control group (54.05 ± 2.33%) (P = 0.04); the average percentage of cells in G0/G1 phase in the interference group was 36.48 ± 0.61%, which was significantly higher than that in the control group (24.80 ± 1.68%) (P < 0.001; **Figure 4D** and **4E**). These results suggest that after interference in the expression of the THY-1 gene, a significant number of HGC-27 cells entered G0/G1 phase arrest, and the percentage of cells entering S phase was reduced, thereby inhibiting cell proliferation (**Figure 4F**).

Effect of THY-1 on the ability of gastric cancer cells to avoid apoptosis

Here, we induced cell apoptosis using serum-free culture medium to investigate the effect of THY-1 on the ability of gastric cancer cells with a lack of nutrient supply to avoid apoptosis.

The effect of exogenous THY-1 on the ability of AGS cells to avoid apoptosis was evaluated by Annexin V/PI double staining after THY-1 overexpression in AGS cells (**Figure 5A** and **5B**). After 48 h of starvation, the average rate of apoptosis of cells in the overexpression group was 7.89 ± 1.08%, which was significantly lower than that of the control group, 11.90 ± 0.45% (P = 0.004; **Figure 5C**). This indicates that the ability of AGS cells to avoid apoptosis was significantly enhanced after overexpression of the THY-1 gene. The apoptosis assay was performed using the same method after interference in the expression of THY-1 in HGC-27 cells. The results showed that the average rate of apoptosis of cells in the interference group was 37.88 ± 5.47%. Compared with the rate of apoptosis in the control group (22.84 ± 1.50%, **Figure 6A** and **6B**), the percentage of apoptotic cells was increased significantly in cells in the interference group (P = 0.01; **Figure 6C**). This indicates that the ability of HGC-27 cells to avoid apoptosis significantly declined after THY-1 gene expression was downregulated.

THY-1 promoted the in vivo tumour formation ability of HGC-27 cells

None of the nude mice died during the experiment. Transplanted tumours were able to be seen with the naked eye on approximately day 6 in the control group, while obvious transplanted tumours appeared in the interference group on approximately day 9. We estimated the volume of the subcutaneous tumours in each group of nude mice every 3 days and generated the growth curve. The results showed that in the control group, the tumours began to grow rapidly 12 days after injection; the volume was 616 ± 287.30 mm^3 on day 18 and reached 1018.33 ± 521.48 mm^3 on day 21. In the interference group, the volume of the tumours was only 124.04 ± 53.26 mm^3 at day 18 and reached 213.72 ± 111.94 mm^3 at day 21 (**Figure 7C**). The nude mice were sacrificed at week 3, and the subcutaneous tumours were

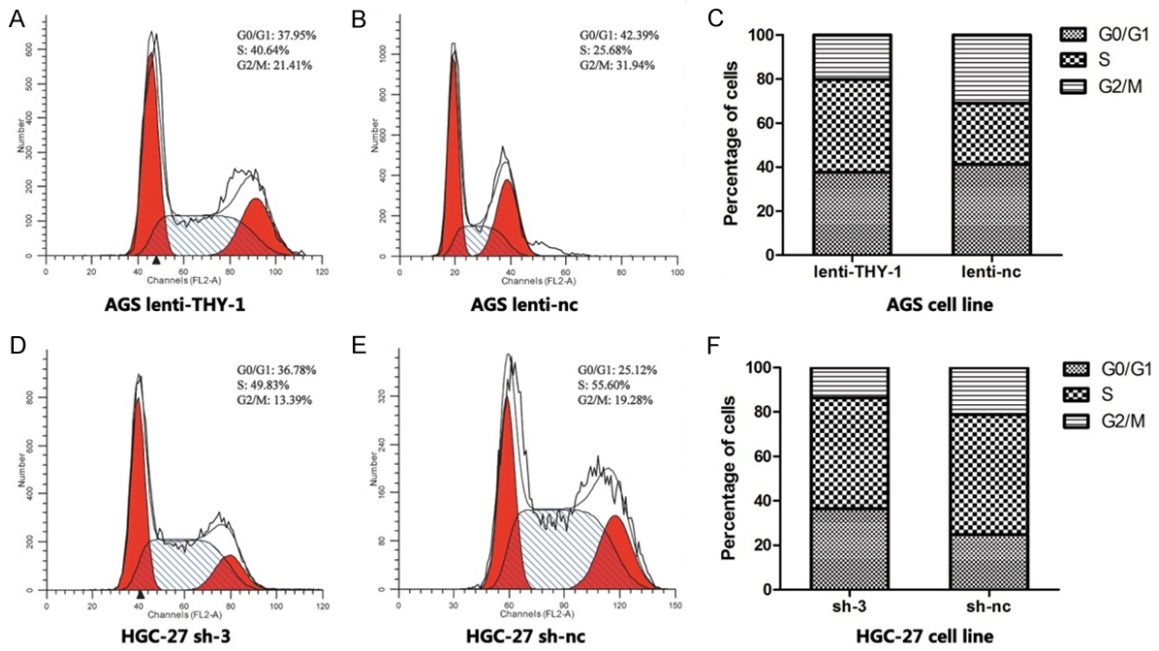

Figure 4. Effect of THY-1 on the cell cycle of gastric cancer cells. A and B: Illustrate the results of the cell cycle analysis by flow cytometry after THY-1 overexpression in AGS cells. C: Shows the statistical graph; lenti-THY-1 is the overexpression group, and lenti-nc is the control group; D and E: Illustrate the results of the cell cycle analysis by flow cytometry after THY-1 interference in HGC-27 cells. F: Shows the statistical graph; sh-3 is the interference group, and sh-nc is the control group.

dissected (**Figure 7A** and **7B**). The average weight of the subcutaneous tumours in the control group was 81.47 ± 41.72 mg, which was significantly different from that of 17.10 ± 9.00 mg in the interference group (P = 0.01; **Figure 7D**). Thus, the *in vivo* subcutaneous tumour formation ability of HGC-27 cells was strongly inhibited after THY-1 gene expression was downregulated.

Discussion

In this study, we first selected seven common human gastric cancer cell lines and one normal human gastric mucosal epithelial cell line. We then analysed the expression of THY-1 at the gene and protein levels by qRT-PCR and Western blot, respectively. The results showed that THY-1 mRNA and protein were highly expressed in HGC-27, SGC-7901 and MGC-803 cells, while the expression levels of the mRNA and protein were relatively low in the other gastric cancer cell lines, and almost no expression was observed in the normal gastric mucosal epithelial cell line GES-1. Among the gastric cancer cell lines with high THY-1 expression, HGC-27 cells were derived from an undifferentiated cell carcinoma, MGC-803 cells were derived from a poorly differentiated mucinous adenocarcinoma, and SGC-7901 cells were derived from a moderately differentiated adenocarcinoma. Among the gastric cancer cell lines with relatively low THY-1 expression, AGS cells were derived from a highly differentiated adenocarcinoma, while the remaining cell lines such as MKN-45 and BGC-823 were derived from a poorly differentiated adenocarcinoma. This indicates that THY-1 expression significantly differs among different gastric cancer cell lines and that the cell lines with high THY-1 expression tend to be poorly differentiated.

The growth and proliferative activities of the different gastric cancer cell groups were determined by CCK-8 assay. We found that the artificial interference in the expression of the THY-1 gene significantly reduced the proliferative ability of gastric cancer cells. The number of viable cells in the interference group was significantly lower than that in the control group at 72 h of culture with the same initial number of cells. This difference gradually increased over time, and the proliferative activity of the interference group declined by nearly 30% at day 5 com-

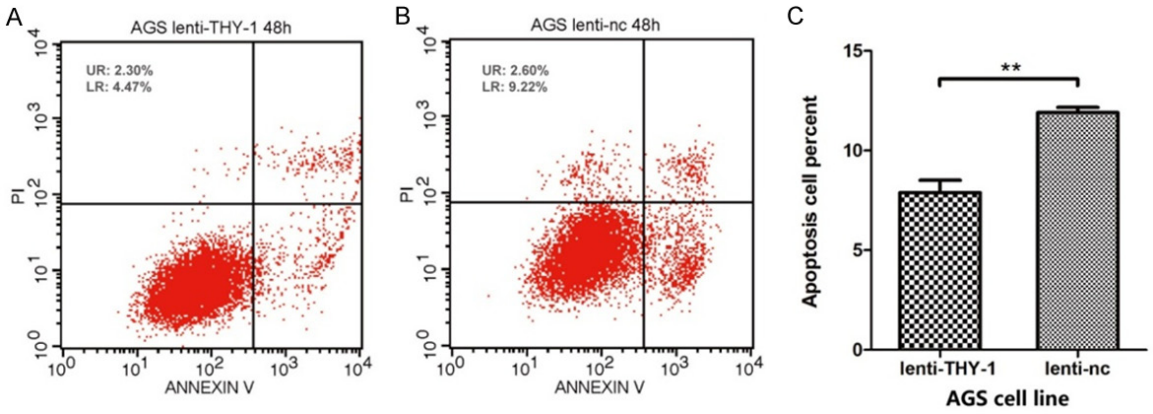

Figure 5. Overexpression of the THY-1 gene reduced apoptosis of AGS cells; lenti-THY-1 is the overexpression group, and lenti-nc is the control group; **, $P < 0.01$.

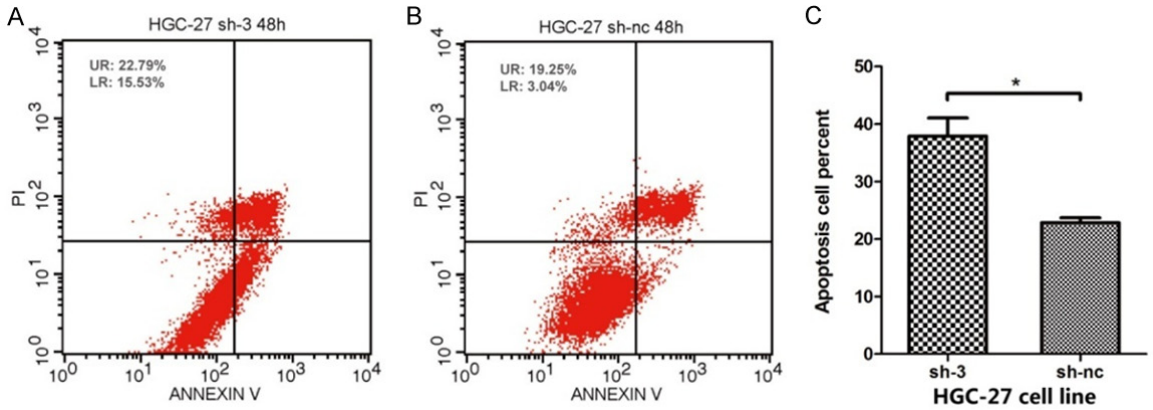

Figure 6. Interference in the expression of the THY-1 gene promoted apoptosis of HGC-27 cells; sh-3 is the interference group, and sh-nc is the control group. *, $P < 0.01$.

pared with the control group. This result was validated in the HGC-27 and MGC-803 cell lines, which indicates that the proliferative activity of gastric cancer cells was significantly inhibited after the downregulation of THY-1 expression. When THY-1 was overexpressed in AGS cells, we also found that exogenous THY-1 could promote the proliferation and growth of gastric cancer cells. Uncontrolled infinite proliferation is one of the characteristics that distinguish tumours from normal tissue. This phenomenon is often associated with cell cycle changes, and thus, we subsequently analysed cell cycle changes in each group. The results showed that after THY-1 overexpression through genetic recombination, exogenous THY-1 significantly reduced the number of AGS cells in G0/G1 phase arrest and promoted cell entry into S phase, which accelerated the division and proliferation of AGS cells. After interference in the expression of the THY-1 gene in HGC-27 cells, the interference group showed a significantly lower percentage of cells that entered S phase compared with the normal group; however, the percentage of cells arrested in G0/G1 phase was increased. This suggests that the downregulation of THY-1 resulted in the accumulation of gastric cancer cells in G1 phase and led to G1 phase arrest, which inhibited tumour overgrowth.

Under physiological conditions, cell apoptosis is mediated primarily through two signalling pathways (i.e., the membrane receptor pathway or the mitochondrial pathway). These pathways eventually activate the irreversible cascade amplification whereby the limited hydrolysis of caspases occurs, which triggers cell apoptosis [13]. In the present study, using Annexin V, we evaluated the effect of THY-1 on the ability of

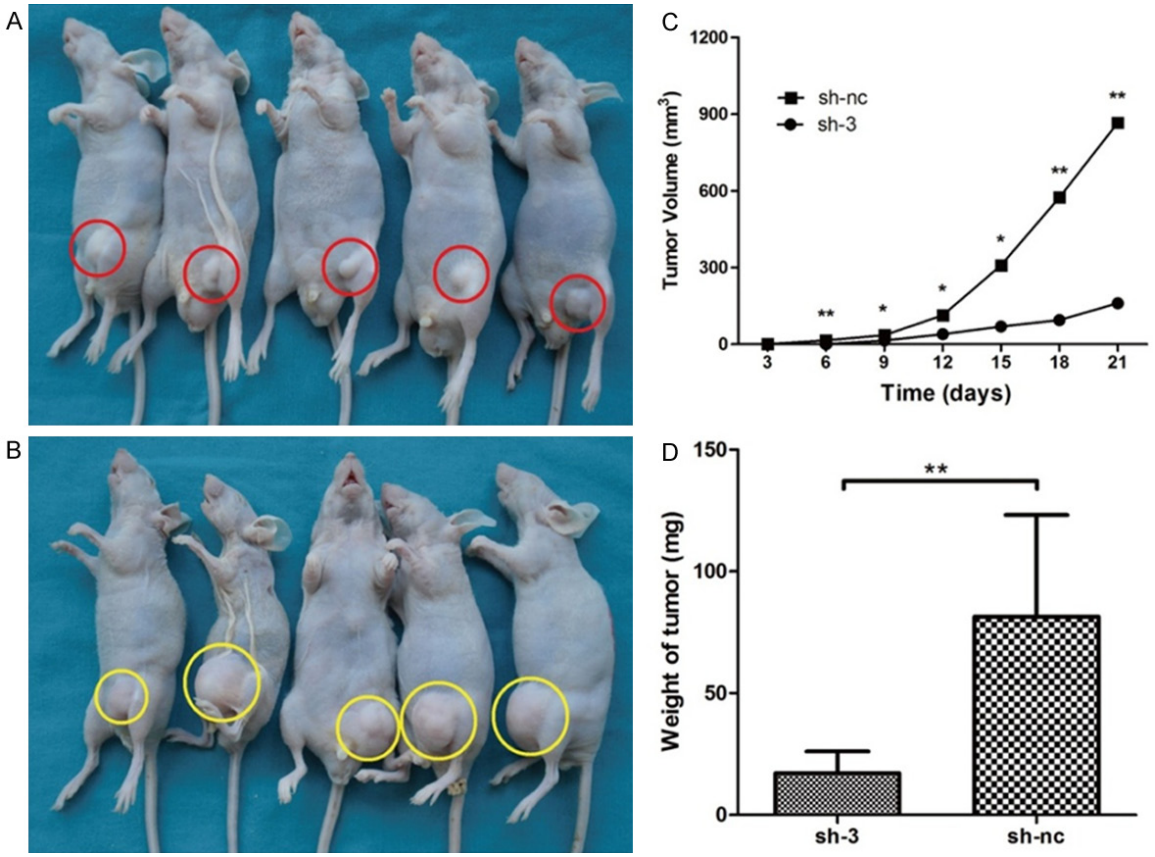

Figure 7. Interference in the expression of the THY-1 gene reduced the tumour formation ability of HGC-27 cells. A. Interference group; B. Normal group; C. Tumour growth curve, and D. Comparison of tumour weights; sh-3 is the interference group, and sh-nc is the control group; $^{*}P < 0.05$; $^{**}P < 0.01$.

gastric cancer cells to avoid apoptosis. The results showed that after overexpression of the THY-1 gene, the ability of AGS cells to avoid apoptosis in serum-free culture was significantly higher than that in the control group. In the interference assay, the ability of HGC-27 cells to avoid apoptosis was significantly inhibited after the downregulation of THY-1 expression. Moreover, the relationship between THY-1 and apoptosis has not been frequently reported. Sato et al. [14] found that a THY-1 cross-linked antibody could induce apoptosis in rat glomerular mesangial cells. Fujita et al. [15] also reported that cross-linked antibodies induced THY-1 polymerization and caused the activation of caspases and the downregulation of the anti-apoptotic proto-oncogene bcl-2, which led to the death of thymocytes and mesenchymal cells through apoptosis. To date, the effect of THY-1 on the apoptosis of tumour cells has not been reported. Thus, follow-up research needs to be performed to determine the mechanisms related to THY-1 and the ability of gastric cancer cells to avoid apoptosis. Nonetheless, it has been reported that THY-1 plays a role in the inhibition of the proliferation of tumour cells in some tumours. Abeysinghe et al. [16] found that THY-1 was expressed to varying degrees in non-tumourigenic human ovarian cancer cell lines. However, after interference in the expression of the THY-1 gene by stable transfection, the tumourigenicity of cells was restored to different extents. Moreover, after the subcutaneous injection of ovarian cancer cells and tumour formation in SCID mice, the tumour size and growth rate of THY-1-positive cells were significantly lower than the tumour size and growth rate of THY-1-negative cells [17, 18]. In nasopharyngeal carcinoma, THY-1 has also been reported to function as a tumour suppressor gene and is thought to inhibit the growth and development of tumour cells via the upregulation of TSP-1 and fibronectin [10, 19]. Thus, THY-1 plays varying roles in the prolifera-

tion of different cells, and these roles may depend on the cell type. Further in-depth research is needed to elucidate the mechanisms.

Finally, the effect of THY-1 expression changes *in vivo* on the tumourigenic ability of HGC-27 cells was investigated by subcutaneous tumour formation assay of gastric cancer cells in nude mice. The results showed that in the control group, subcutaneous tumour formation was observed in nude mice at day 6. A growth peak appeared at day 12, and a significant increase in tumour size was observed. In the interference group, subcutaneous tumours were initially formed at day 9, but tumour growth was markedly limited. This result is consistent with the results of a previous cell assay, which further provides evidence that interference in the expression of THY-1 in gastric cancer cells can effectively inhibit the development and progression of tumours. These data also provide experimental evidence for future use of THY-1 as a novel target in the treatment of gastric cancer.

Disclosure of conflict of interest

None.

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