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MicroRNA-101 is down-regulated in gastric cancer and involved in cell migration and invasion

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ABSTRACT

MicroRNAs (miRNAs) are short non-coding RNA molecules playing regulatory roles by repressing translation or cleaving RNA transcripts. Dysregulated expression of miRNAs is associated with several diseases, including cancer. In this study, we report that the expression of microRNA-101 (miR-101) is down-regulated in gastric cancer tissues and cells, and ectopic expression of miR-101 significantly inhibits cellular proliferation, migration and invasion of gastric cancer cells by targeting *EZH2*, *Cox-2*, *Mcl-1* and *Fos*. Our animal study also indicates that miR-101 could potentially suppress tumour growth *in vivo*. Collectively, these results suggest that miR-101 may function as a tumour suppressor in gastric cancer, as it has an inhibitory role not only in cellular proliferation, migration and invasion *in vitro*, but also in tumour growth *in vivo*.

1. Introduction

MicroRNAs (miRNAs) are a class of endogenous small non-coding regulatory RNAs, approximately 22 nucleotides in length, which are known to regulate gene expression by repressing translation or decreasing the stability of mRNAs.[1] It has been widely shown that miRNAs have important roles in controlling numerous biological processes, including cell differentiation, proliferation and apoptosis. Recent evidences indicated that miRNAs are closely involved with human cancers. miRNA genes are frequently located in cancer-associated genomic regions, such as fragile sites, minimal regions of loss of heterozygosity and minimal regions of amplification.[2] Altered miRNA expression profiles are reported in various types of cancers, including liver, colon and prostate cancer.[3–5] Moreover, recent studies also revealed that miRNAs can function as tumour suppressors or oncogenes. Tumour suppressive miRNAs, such as let-7, miR-143 and miR-145, are usually under-expressed in tumour tissues[6–8], while oncogenic miRNAs, such as miR-21, and miR-31, are usually over-expressed in tumour tissues and cell lines.[9] Loss or gain of function of specific miRNAs contributes to tumourigenesis and cancer progression.

Gastric cancer is one of the most common cancers in the world. It is considered as the second frequent cause of cancer-related death worldwide, with particularly high frequencies in East Asia. Although significant advances have been achieved since the Human Genomic Project finished, the molecular pathogenesis of gastric cancer still remains to be explored. Recent studies have confirmed the altered expression profile of miRNAs in gastric cancer.[10–12] Dysregulation of miRNAs, such as miR-21, miR-27a and miR-141, is reportedly involved in the gastric tumour growth, invasion, metastasis or multidrug resistance.[13–15] These data highlight the

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importance of miRNAs in gastric cancer development and provide insights into the mechanisms underlying tumourigenesis.

MicroRNA-101 (miR-101) is a tumour suppressive miRNA. Most of the studies indicated that miR-101 is significantly under-expressed in multiple kinds of cancers, including prostate, breast, liver, bladder and endometrial cancer, and displays a suppressive effect on cellular proliferation, migration and invasion.[16–20] However, little is known about the expression situation and biological role of miR-101 in gastric cancer. In the present study, we reported that miR-101 is significantly down-regulated in gastric cancer tissues and cells compared to the non-tumour gastric tissues and non-malignant gastric epithelial cells. Ectopic expression of miR-101 could inhibit proliferation, migration and invasion of gastric cancer cells *in vitro*, at least in part, by targeting *EZH2*, *Cox-2*, *Mcl-1* and *Fos*. Furthermore, we also found that miR-101 could inhibit gastric tumour growth in mice. These results suggest that miR-101 functions as a tumour suppressive miRNA in gastric cancer and is an important regulator of cellular proliferation, migration and invasion.

2. Materials and methods

2.1. Cell culture and tissues

Human gastric cancer cell lines BGC-823, SGC-7901, AGS, MKN-45 and non-malignant gastric epithelial cell line GES-1 were obtained from Shanghai Institute of Digestive Surgery (Shanghai, China), and cultured in RPMI1640 containing 10% foetal bovine serum (FBS), 50 U/ml penicillin and 50 μg/ml streptomycin. HEK 293 cells were obtained from Sir Run Run Shaw Hospital (Zhejiang, China), and were grown in DMEM containing 10% FBS, 50 U/ml penicillin and 50 μg/ml streptomycin. All cells were maintained at 37 °C under an atmosphere of 5% CO_2.

Gastric tumours and adjacent non-tumour tissues were obtained from patients with primary gastric adenocarcinoma at the Zhejiang Provincial People's Hospital, China, and the clinicopathologic characteristics of gastric cancer patients are summarised in Table 1. All patients provided informed consent for the use of their tissues before surgery. After surgical removal, the tissues were frozen immediately in liquid nitrogen and stored at –80 °C until used.

Table 1 – Summary of clinicopathologic characteristics of gastric cancer patients.

Clinical parameters	N (%)
Gender	
Men	19 (68)
women	9 (32)
Size	
<20	15 (54)
⩾20	13 (46)
Histologic differentiation	
Well	1 (4)
Moderately	14 (50)
Poorly	13 (46)
Lymphatic metastasis	
No	5 (18)
Yes	23 (82)
Distant metastasis	
No	24 (86)
Yes	4 (14)
TNM stage	
I	3 (11)
II	6 (21)
III	14 (50)
IV	5 (18)

2.2. RT-qPCR quantification of miR-101

RT-qPCR was performed to determine the expression of mature miR-101. Briefly, total RNA was extracted from tissues and cells, using Trizol (Invitrogen) according to the manufacturer's instructions. cDNA synthesis was carried out with the Superscript III cDNA synthesis kit (Invitrogen) using 1 μg of total RNA as the template and specific reverse primers under 16 °C, 30 min, 42 °C, 30 min and 85 °C, 5 min of reverse transcription. The specific reverse primer for RNU6-1 was 5′-CGCTTCACGAATTTGCGTGTCAT-3′, and the reverse primer for miR-101 was 5′-GTCGTATCCAGTGCGTGTCGTGGAGTCGG CAATTGCACTGGATACGACCTTCAGT-3′. The resulting cDNA was amplified by PCR using miRNA specific primers with SYBR Premix Ex Taq (Takara). Primers for RNU6-1 were 5′-GCTTCGGCAGCACATATACTAAAAT-3′ and 5′-CGCTTCACGAA TTTGCGTGTCAT-3′; primers for miR-101 were 5′-TGGGCTA CAGTACTGTGATA-3′ and 5′-TGCGTGTCGTGGAGTC-3′. PCR parameters were as follows: 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 20 s and 72 °C for 20 s. At the end of the PCR cycles, melting curve analysis was performed. The expression of miR-101 in tissues was compared to one non-tumour sample using $2^{-\Delta\Delta CT}$ method, and the expression of miR-101 in gastric cancer cells was compared to GES-1.

2.3. Adenovirus construction

Recombinant E1, E3-deleted adenovirus vectors were constructed using the Ad Max Cre-lox-based system (Microbix Biosystems Inc.). To generate adenovirus encoding miRNA-101 (Ad-miR-101), a 328-bp fragment carrying pri-miR-101 was amplified using human genomic DNA as a template, with primers 5′-GGAATTCTGCCTCCTCACGTCTC-3′ and 5′-GGAAGATCTATGACAGAGGTGCAGG-3′. The PCR products were then cloned into *EcoR I* and *Bgl II* restriction sites of the shuttle vector pDC316. The construct was confirmed by DNA sequencing (Invitrogen Biotechnology Co. Ltd.), then co-transfected with adenovirus genome plasmid pBH-GloxΔE1,3Cre into HEK 293 cells using liposome 2000 (Invitrogen). Transfected cells were maintained in DMEM containing 2% FBS. Approximately 10 days after transfection, when viral cytopathic effect was observed, the cells were lysed by freeze–thaw and then expanded according to the manufacturer's protocol. Then infectious virus was purified using Adenopure kit (Puresyn Inc.) according to the manufacturer's instructions. Ad-EGFP, a control virus, was similarly produced. The virus titre was quantified using a standard TCID50 assay.

The infection efficiency of recombinant adenovirus was determined by infecting gastric cancer cells with Ad-EGFP at various multiplicities of infection (MOI 25, 50, 100, 200). The miR-101 level of gastric cancer cells 24 h after Ad-miR-101 or Ad-EGFP infection was evaluated using RT-qPCR method described previously.

2.4. Cell proliferation assay

The effect of miR-101 on proliferation of gastric cancer cells was evaluated by the MTT assay. BGC-823, SGC-7901, AGS and MKN-45 cells were plated into 96-well plates in 1×10^3 cells/well in quintuplicate, and allowed to adhere overnight. Cells were then infected with Ad-miR-101 or Ad-EGFP as a control, at an appropriate MOI. Ten microlitres of MTT (5 mg/ml) (Sigma) were added to each well 72 h after infection, and the cells were incubated for a further 4 h. Media was then removed and 150 μl DMSO was added. Absorbance (A) at 570 nm and 630 nm was measured using a microplate reader. The relative cell proliferation (%) is shown by the following equation:

$$\text{Relative proliferation rate } (\%) = (A_{570\,\text{nm}} - A_{630\,\text{nm}}) \text{ of study group} / (A_{570\,\text{nm}} - A_{630\,\text{nm}}) \text{ of control group} \times 100\%.$$

2.5. In vitro cell migration and invasion assays

BGC-823, SGC-7901, AGS and MKN-45 were infected with Ad-mir-101 or Ad-EGFP as a control, at an appropriate MOI. Twenty-four hours after infection, the infected cells were harvested and subjected to the following assays.

For migration assays, the infected cells (1×10^5) were plated in the top chamber of Transwells (Millipore) with a membrane containing 8-mm diameter pores in 200 μl serum-free RPMI1640 in triplicate. The inserts were then placed into the bottom chamber wells of a 24-well plate containing RPMI1640 with 30% FBS as a chemo-attractant. After 24 h of incubation, cells remaining on the inserts' top layers were removed by cotton swab scrubbing; cells on the lower surface of the membrane were fixed in 100% methanol for 15 min, followed by staining with Giemsa solution. The cell numbers in five random fields (200×) were counted for each chamber, and the average value was calculated.

For invasion assays, the infected cells (4×10^5) were plated in the top chamber with Matrigel-coated membrane, whereas the bottom chambers were filled with conditioned medium. After 48 h-incubation, the number of migrated cells (lower side of the membrane) was counted as described above.

2.6. RT-qPCR quantification of miR-101 target genes

RT-qPCR was performed to determine the effect of miR-101 on its target genes, *EZH2*, *COX-2*, *Mcl-1* and *Fos*, in gastric cancer cells. Briefly, total RNA was extracted from null BGC-823, SGC-7901, AGS and MKN-45 cells or cells infected with Ad-miR-101 or Ad-EGFP, and used as template to synthesis cDNA with oligo dT primer. The resulting cDNA was amplified by PCR using gene specific primers with SYBR Premix Ex Taq (Takara). Primers for *EZH2* were 5′-GACCCTGACCTCTGTCTTACTT-3′ and 5′-GATGGTGCCAGCAATAGATG-3′; primers for *Cox-2* were 5′-CTGAATGTGCCATAAGACTG-3′ and 5′-CCACAGTGCTTGACACAGAAT-3′; primers for *Mcl-1* were 5′-TCAGCGACGGCGTAACAAACT-3′ and 5′-ACAAACCCATCCCAGCCTCTT-3′; primers for *Fos* were 5′-GACCTTATCTGTGCGTGAAAC-3′ and 5′-TTCCAATAATGAACCCAATAGAT-3′ and primers for *GAPDH* were 5′-TGAAGGTCGGAGTCAACGG-3′ and 5′-CTGGAAGAT GGTGATGGGATT-3′. PCR parameters were as follows: 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 56 °C for 20 s and 72 °C for 20 s. At the end of the PCR cycles, melting curve analyses were performed. The expression of miR-101 target genes was normalised to that of Ad-EGFP-infected BGC-823 cells using the $2^{-\Delta\Delta CT}$ method.

2.7. In vivo tumour model

Four to five-week-old female BALB/c athymic nude mice were purchased from Slac Laboratory Animal Co. Ltd. (Shanghai, China). Animal experiments were performed in accordance with the institutional guidelines of the university committee on the use and care of animals.

To evaluate the effects of miR-101 on gastric tumour progression, mice were inoculated subcutaneously in the right flank with 1×10^7 MKN45 cells. When tumours reached about 5 mm in diameter, mice were randomly divided into two groups ($n = 5$ each group): the Ad-mir-101 group and the Ad-EGFP group (control). Virus particles (1×10^9 PFU/100 μl) of Ad-miR-101 or Ad- EGFP were injected intratumourally on days 1, 4, 7, 10 and 13, with a total of five times. Tumour length and width were measured by caliper twice a week, and tumour volume was calculated as (length × width^2)/2. Mice were killed and tumours harvested 5 weeks after injection. The miR-101 level of tumours was also determined using RT-qPCR method described previously.

2.8. Statistical analysis

Statistical analysis was performed using Statistical Program for Social Sciences (SPSS) software 13.0 (SPSS Inc., Chicago, IL, USA). The paired-samples *t*-test was used to analyse the differences of miR-101 expression between tumour and non-tumour tissues. For *in vitro* and *in vivo* experiments, independent samples *t*-test was used to analyse the significant difference between the control and treatment groups. A *P* value of less than 0.05 was considered statistically significant.

3. Results

3.1. miR-101 is down-regulated in gastric tumour tissues and cell lines

The expression of miR-101 in gastric tumour tissues and corresponding non-tumour tissues was analysed using RT-qPCR and a total of 28 pairs of matched tissue specimens. Expression levels of miR-101 were much lower in gastric tumours (0.661 ± 0.396) than in non-tumour tissues (1.128 ± 0.697; $P < 0.01$; Fig. 1A). We also examined the miR-101 expression in gastric cancer cell lines BGC-823, SGC-7901, AGS and MKN-45 along with the non-malignant gastric epithelial cell

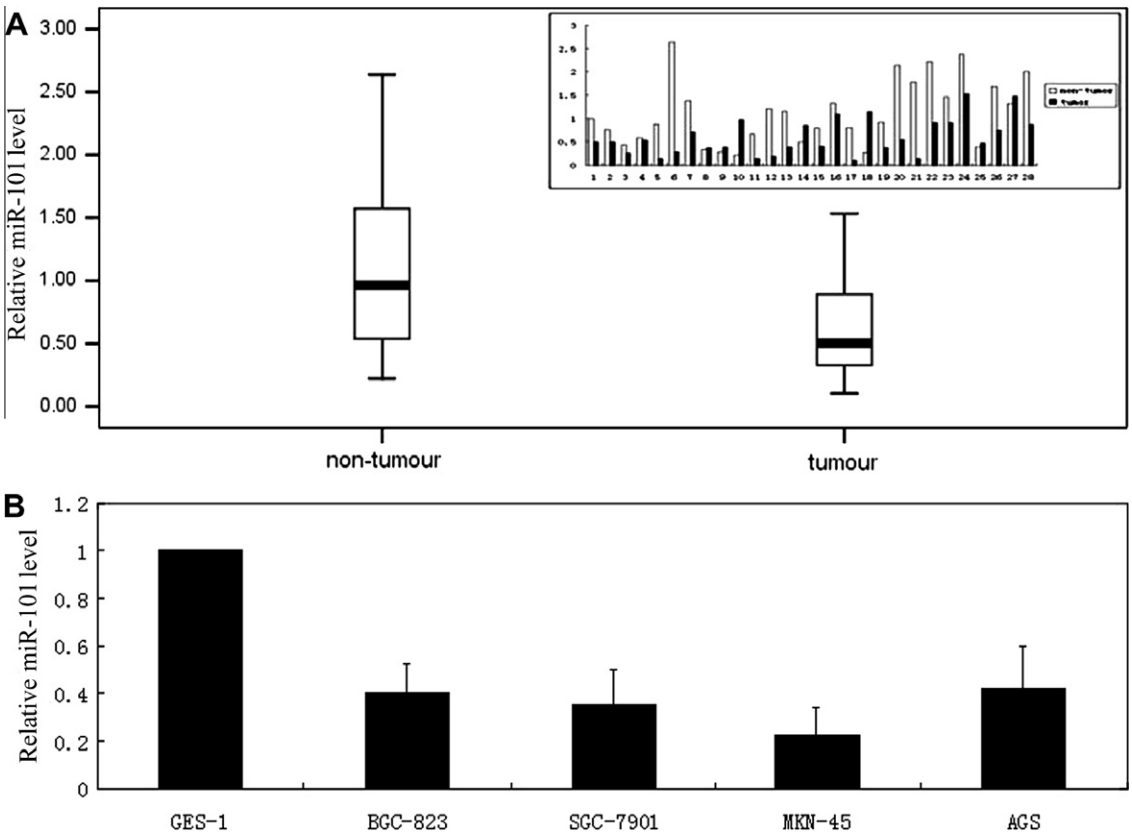

Fig. 1 – Down-regulated expression of miR-101 in gastric cancer tissues and cells. (A) Relative expression of miR-101 in the corresponding gastric tumours and non-tumour tissues compared to that in one non-tumour tissue sample. (B) Relative expression of miR-101 in gastric cancer cell lines compared to GES-1.

line GES-1. As shown in Fig. 1B, gastric cancer cell lines expressed lower levels of mir-101 compared with GES-1.

3.2. *miR-101 inhibited cell proliferation, migration and invasion in vitro*

To study the biological role of miR-101 in gastric cancer, we constructed a recombinant adenovirus Ad-miR-101 and a control virus Ad-EGFP. The infection efficiency of adenovirus on BGC-823, SGC-7901, AGS and MKN-45 all reached up to 90% without cytotoxicity at MOI 100, which was used as an appropriate MOI in the following studies. The level of miR-101 in gastric cancer cells infected with Ad-miR-101 or Ad-EGFP was also evaluated. As shown in Fig. 2, the miR-101 level of Ad-miR-101-infected cells was much higher than that of the Ad-EGFP-infected cells.

Then, we evaluated the effect of miR-101 on proliferation of human gastric cancer cell lines. Using MTT assay, we measured proliferation of gastric cancer cells infected with Ad-miR-101 or Ad-EGFP. As shown in Fig. 3, Ad-miR-101 significantly inhibited proliferation of MKN-45 cells. The relative proliferation rate in MKN-45 was 37.4% 72 h after Ad-miR-101 infection. However, Ad-miR-101 only had slight effect on

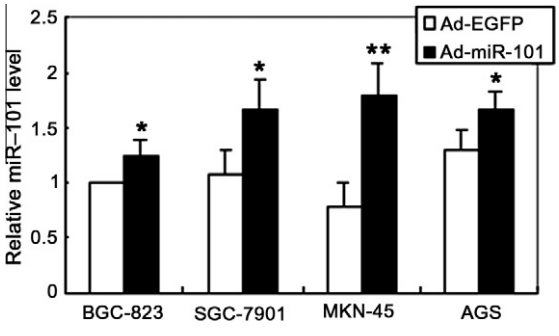

Fig. 2 – Relative expression of miR-101 in Ad-miR-101- or Ad-EGFP-infected gastric cancer cells compared to Ad-EGFP-infected BGC-823 cells.

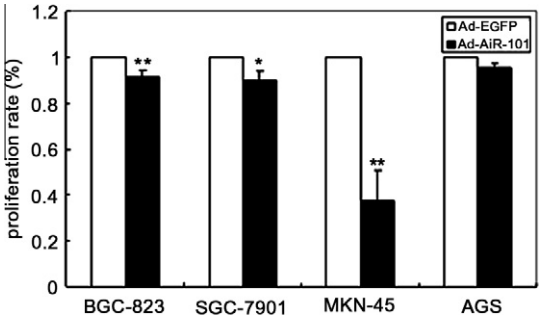

Fig. 3 – miR-101 inhibits cell proliferation of gastric cancer cells. Values are expressed as percentage of proliferation normalised to cells infected with Ad-EGFP (set to 100%) from three separate experiments. *P < 0.05, **P < 0.01.

proliferation of the other three gastric cancer cell lines; the relative proliferation rates in BGC-823, SGC-7901 and AGS were 91.3%, 89.7% and 95.3%, respectively.

We further analysed the effects of miR-101 on the migratory and invasive behaviour of gastric cell lines. As shown in Fig. 4A, gastric cancer cells infected with Ad-miR-101 displayed significantly lower transmembrane migration capacity compared with those infected with Ad-EGFP. The migration activity of Ad-miR-101-infected BGC-823, SGC-7901, MKN-45 and AGS was specifically reduced by approximately 22.5%, 50.4%, 72.1% and 51.3%, respectively (Fig. 4C). Moreover, the invasion assay also indicated that miR-101 substantially reduced the invasion ability of gastric cancer cells (Fig. 4B). As shown in Fig. 4D, Ad-miR-101 reduced the invasion of BGC-823, SGC-7901, MKN-45 and AGS cells by 23.6%, 56.6%, 76.1% and 48.2%, respectively. These findings suggest that miR-101 level seems to be closely associated with the invasion and migration of gastric cancer cell lines.

3.3. *miR-101 inhibited expression of EZH2, Cox-2, Mcl-1 and Fos in gastric cancer cells*

To explore the possible regulation pathway of miR-101 in gastric cancer cells, we analysed the effect of miR-101 on the

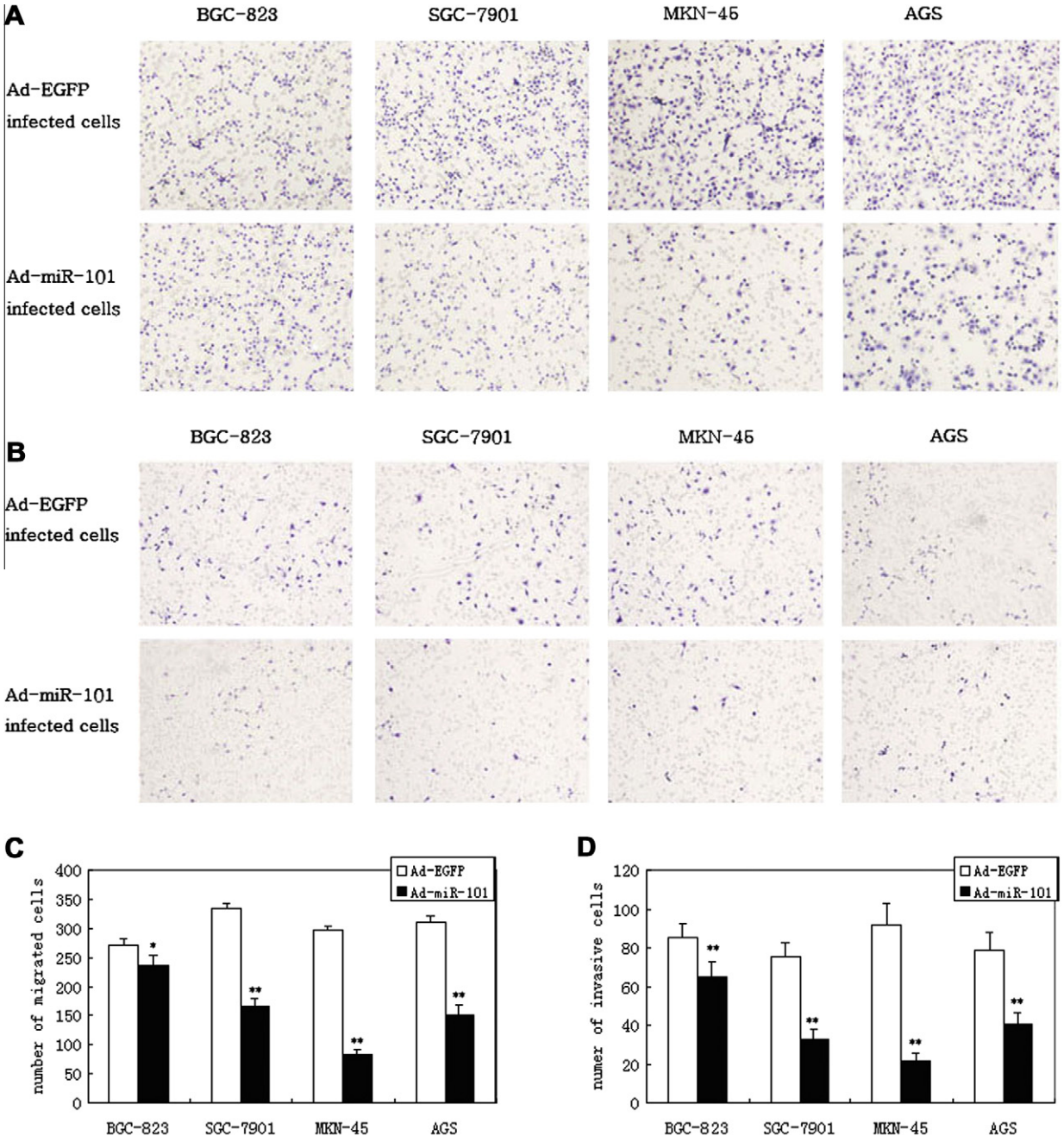

Fig. 4 – miR-101 inhibits cell migration and invasion. (A and B) Representative fields of migrated or invasive cells on membrane. (C and D) Average migrated or invasive cell number from three separate experiments. *P < 0.05, **P < 0.01.

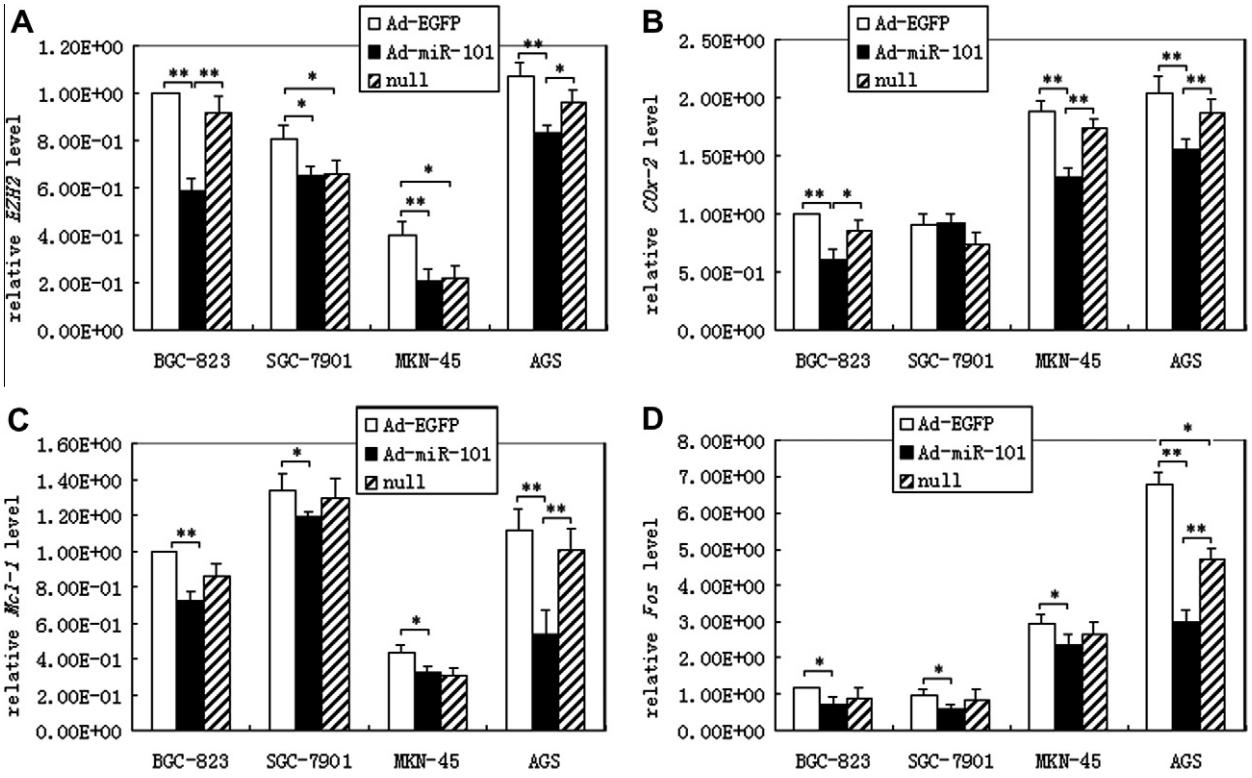

Fig. 5 – miR-101 inhibits expression of *EZH2*, *Cox-2*, *Mcl-1* and *Fos*. A-D, relative expression of *EZH2*, *Cox-2*, *Mcl-1*, *Fos* in Ad-miR-101, Ad-EGFP-infected or null gastric cancer cells compared to Ad-EGFP-infected BGC-823 cells from three separate experiments. *$P < 0.05$, **$P < 0.01$.

expression of *EZH2*, *Cox-2*, *Mcl-1* and *Fos*. RT-qPCR was performed to determine the mRNA level of these genes in null gastric cancer cells, Ad-miR-101- or Ad-EGFP-infected gastric cancer cells. As shown in Fig. 5, although infection of adenovirus up-regulated the expression of *EZH2*, *Cox-2*, *Mcl-1* and *Fos* in different degree in gastric cancer cells, the ectopic expression of miR-101 still showed an inhibition effect on the expression of these four genes. The mRNA levels of *EZH2*, *Cox-2*, *Mcl-1* and *Fos* in Ad-miR-101-infected BGC-823, SGC-7901, MKN-45 and AGS cells were much lower than the mRNA level of all the Ad-EGFP-infected cells, except the *Cox-2* in SGC-7901 cells.

3.4. *miR-101 inhibited tumour growth in vivo*

To explore whether miR-101 inhibits tumour growth *in vivo*, a gastric xenograft tumour model was established by subcutaneous injection of MKN-45 cells into the flanks of athymic nude mice. After tumour formation, the mice were randomised into two groups and injected intratumourally with Ad-miR-101 or Ad-EGFP with a total of five times. No mice showed notable toxic effect or body weight loss during experiment. Tumour volumes were measured twice a week. As shown in Fig. 6A, injection with Ad-miR-101 induced significant reduction in tumour growth compared to mice treated with Ad-EGFP at later time points, albeit to various extents. Tumours injected with Ad-miR-101 were much smaller in all animals, compared to those treated with Ad-EGFP ($333.56 \pm 46.71\ mm^3$ versus $806.41 \pm 51.83\ mm^3$, $P < 0.05$ Fig. 6B). The expression of miR-101 in Ad-miR-101-injected tumours was much higher than that in Ad-EGFP-injected tumours (1.572 ± 0.156 versus 1.141 ± 0.233, $P < 0.01$) (Fig. 6C). These data indicate that miR-101 can efficiently reduce tumour growth *in vivo*.

4. Discussion

Accumulating evidence indicates that miRNAs may contribute to cancer pathogenesis. Dysregulation of miRNAs is associated with the initiation and progression of cancer, since they may serve as tumour suppressors or oncogenes. In this study, we evaluated the expression of miR-101 in gastric tumour tissues and cell lines. We also explored the biological role and regulation mechanism of miR-101 in gastric cancer for the first time.

The expression of miR-101 in cancers is controversial. Most studies indicated that miR-101 is significantly under-expressed in different types of cancers, including prostate, breast, liver, bladder and endometrial cancer.[16–20] However, two studies found that miR-101 is over-expressed in HepG2 cells and prostate cancer tissues.[3,21] The discrepancies in miR-101 expression might be due to differences in sample origin, different analytical approaches or different technical platforms of the studies. However, it is also possible that miR-101 has a dual role in tumourigenicity. Thus far, several miRNAs with dual roles in cancer have been reported. For instances, miR-155 is up-regulated in various types of cancers, including lung and breast cancer, while the expression of miR-155 is significantly decreased in pancreatic tumour[22–24];

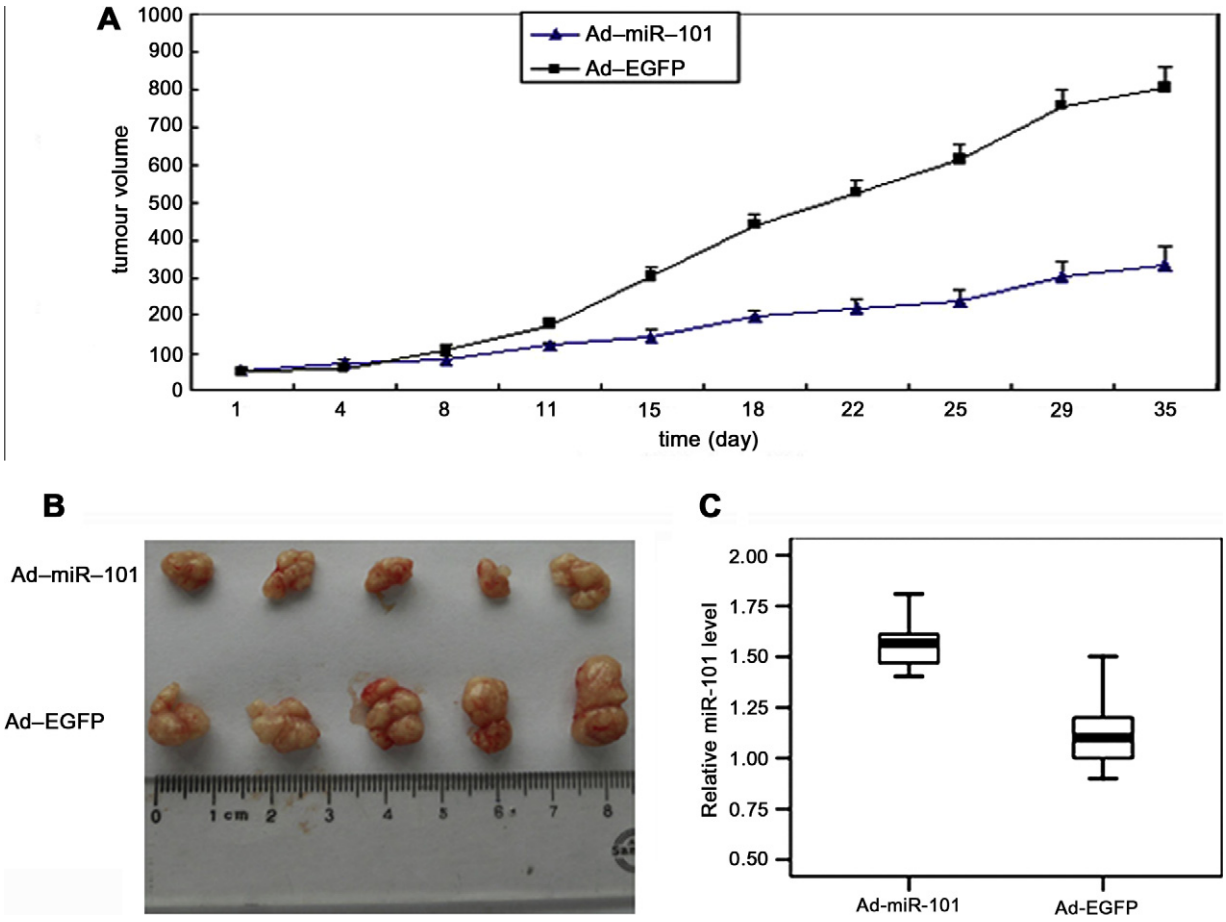

Fig. 6 – miR-101 inhibits tumour growth. (A) Growth curves of tumours which treated with intratumuoral injection of Ad-miR-101 or Ad-EGFP. (B) The average volume of tumours harvested. (C) Relative expression of miR-101 in Ad-miR-101 or Ad-EGFP-injected tumours compared to that in one Ad-EGFP-injected tumours.

miR-205 is up-regulated in lung and colon cancer, but down-regulated in breast cancer[12,25,26]; miR-31 is over-expressed in lung and colon, but under-expressed in gastric cancer.[27–29] Although genomic loss of miR-101 loci in gastric cancer has been reported by Varambally and his colleagues,[16] the expression level and the biological impact of miR-101 in gastric cancer are still unclear. Thus, we examined the expression of miR-101 in 28 matched gastric tumour tissues and non-tumour tissues, as well as four gastric cancer cell lines and the non-malignant gastric epithelial cell GES-1. Consistent with most previous studies, the expression of miR-101 is down-regulated in gastric tumours compared to non-tumour tissues. Besides, the expression of miR-101 is also decreased in four gastric cancer cell lines compared to GES-1. These results indicate that miR-101 may function as a tumour suppressor in gastric cancer.

As a tumour suppressive miRNA, miR-101 was reported to be able to suppress the cell proliferation and colony formative ability of endometrial, bladder, liver, breast and prostate cancer cells.[16–20] In addition, miR-101 could also inhibit tumour growth of prostate and liver cancer *in vivo*.[16,17] In the present study, we presumed that miR-101 could inhibit tumour growth and invasion of gastric cancer. As we expected, ectopic expression of miR-101 suppressed the cell proliferation, migration and invasion of four gastric cancer cell lines, particularly MKN-45, in which endogenous miR-101 is the lowest of the four. Intratumoural injection of Ad-miR-101 significantly inhibited MKN-45 tumour growth in nude mice. This study confirmed the tumour suppressive role of miR-101 in gastric cancer for the first time, and provided evidence for the potential usefulness of miR-101 in miRNA-based cancer therapy.

miR-101 has multiple predicted targets among which are many oncogenes. To date, four genes, including enhancer of zeste homologue 2 (*EZH2*), cyclooxygenase-2 (*Cox-2*), myeloid cell leukemia-1 (*Mcl-1*) and v-fos FBJ murine osteosarcoma viral oncogene homologue (*FOS*), have been identified as targets of miR-101 by luciferase reporter assay.[16–19,30] *EZH2* is a mammalian histone methyltransferase and functions in a multiprotein complex called Polycomb Repressive Complex 2. The primary activity of the *EZH2* protein complex is to tri-methylate histone H3 lysine 27 (H3K27) at target gene promoters, leading to epigenetic silencing. *EZH2* is broadly over-expressed in aggressive solid tumours and has properties of an oncogene, as over-expression promotes cell proliferation, colony formation, migration, invasion of cancer cells *in vitro* and xenograft tumour growth *in vivo*. *COX-2* is the rate-limiting enzyme in prostanoid biosynthesis, and is generally

considered to be a mediator of inflammation. Recent studies indicated that the over-expression of *Cox-2* in human cancer cells is associated with proliferative activity, inhibition of apoptosis, increased metastatic potential and angiogenesis. *Mcl-1* is an antiapoptotic member of *Bcl-2* family, which is commonly over-expressed in various types of cancers. Knockdown of *Mcl-1* has been well proven to sensitise human cancer cells to apoptosis. *Fos* is a key component of the activator protein-1 (AP-1) transcription factor. Over-expression of *Fos* is associated with tumourigenesis and cancer progression. *EZH2*, *COX-2*, *Mcl-1* and *Fos* are reportedly over-expressed in gastric cancer. In this study, we examined whether ectopic expression of miR-101 could reduce the expression of *EZH2*, *COX-2*, *Mcl-1* and *Fos* in gastric cancer cells. The mRNA levels of *EZH2*, *COX-2*, *Mcl-1* and *Fos* were reduced in BGC-823, SGC-7901, MKN-45 and AGS cells infected with Ad-miR-101, except the *Cox-2* mRNA level in SGC-7901. These results provided insight into regulation mechanism of miR-101 in gastric cancer. Down-regulation of miR-101 in gastric cancer cells resulted in enhanced expression of its target genes, which consequently favoured tumour progression. However, the down-regulation of miR-101 target genes did not correspond exactly to the biological behaviour change in four gastric cancer cells induced by ectopic expression of miR-101, indicating that the regulation pathway of miR-101 in gastric cancer was much more complicated than assumed. PicTar, miRanda and the TargetScan program predicted some other candidate target genes of miR-101, including oncogen *MYCN*, *STC1* and *DNMT3A*. The regulation mechanism of miR-101 still needs further study.

In summary, this study provides new insights into the role of miR-101 in human gastric cancers. It shows that miR-101 is down-regulated in gastric tumour tissues and gastric cancer lines, and is able to inhibit cell proliferation, migration and invasion of gastric cancer cells *in vitro* and reduce xenograft tumour growth *in vivo* by targeting EZH2, COX-2, Mcl-1 and Fos. These results suggest that miR-101 is a tumour suppressor in gastric cancer, and might serve as a therapeutic target in gastric cancer.

Conflict of interest statement

None declared.

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