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miR-22 suppresses the proliferation and invasion of gastric cancer cells by inhibiting CD151

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ABSTRACT

Gastric cancer (GC) is the second common cause of cancer-related death worldwide. microRNAs (miRNAs) play important roles in the carcinogenesis of GC. Here, we found that miR-22 was significantly decreased in GC tissue samples and cell lines. Ectopic overexpression of miR-22 remarkably suppressed cell proliferation and colony formation of GC cells. Moreover, overexpression of miR-22 significantly suppressed migration and invasion of GC cells. CD151 was found to be a target of miR-22. Furthermore, overexpression of CD151 significantly attenuated the tumor suppressive effect of miR-22. Taken together, miR-22 might suppress GC cells growth and motility partially by inhibiting CD151.

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1. Introduction

Gastric cancer (GC) is one of the most common cancers worldwide, especially in East Asia [1]. The carcinogenesis of GC is complicated, and it involves the dys-regulation of oncogenes and tumor suppressors [2,3]. Despite great advances in GC diagnosis and treatment, GC is still the second most frequent malignancy in global. The common molecular mechanism of GC remains poorly understood.

Increasing evidence suggests that microRNAs (miRNAs), which could regulate various target genes, including both oncogenes and tumor suppressor genes, contribute to the carcinogenesis of GC [4]. miRNAs are small noncoding RNAs (approximately 22 nucleotides), which negatively regulate gene expression by binding to the complimentary sequences in the 3′-untranslated region (3′-UTR) of target mRNA, inducing target mRNA degradation or translational suppression [5]. miRNAs involved in the development and progression of GC have been widely explored [6,7]. Many studies have identified a number of miRNAs aberrantly expressed in GC [8,9]. Among them, miR-22 was significantly decreased [10]. However, the detailed role of miR-22 in GC remains poorly understood.

In the present study, we found that miR-22 was substantially decreased in the GC tissue samples and cell lines, and ectopic overexpression of miR-22 significantly inhibited the proliferation, colony formation, migration and invasion of GC cells. CD151 was found to be a target of miR-22, and CD151 overexpression partially attenuated the tumor suppressive effect of miR-22 in GC cells.

2. Materials and methods

2.1. Tissue samples and cell lines

A total of 32 GC tissue samples and matched non-tumor tissue samples were collected in our department, and informed consent was obtained from all subjects. This work was approved by the Ethics Committee of Wuchang Hospital of Wuhan City. Human GC cell lines SGC7901, MKN28, and NUGC-3, and human gastric mucosa cell line GES-1 were obtained from the Chinese Academy of Sciences (Shanghai, China). Cells were maintained in DMEM supplemented with 10% fetal bovine serum at 37 °C with 5% CO_2.

2.2. RNA extraction and quantitative real time PCR (qRT-PCR)

Total RNA was extracted by TRIzol (Invitrogen, Carlsbad, CA, USA). RNA was reversely transcribed into cDNA, and SYBR Green Realtime PCR was used to detect gene expression on ABI 7900 (ABI, Foster City, CA, USA). miRNA was extracted using a microRNA extraction kit (Tiangen, Beijing, China). Primers used were as following: CD151 forward, 5′-CATCGCTGGTATCCTCG-3′ and reverse, 5′-CTCGCTGCCCACAAAG-3′; Primers for U6 and miR-22 were

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obtained from GeneCopoeia (Carlsbad, CA, USA). CD151 was normalized with GAPDH, and miR-22 was normalized with U6.

2.3. Plasmids

miR-22 and control mimics/inhibitors were obtained from RiboBio (Guangzhou, China). pEGFP-CD151 was generated using the following primers: forward, 5′-CCAAGCTTGATGGGTGAGT TCAACGAGA-3′ and reverse, 5′-GGGGTACCCAGTAGTGCTCCAGCTT-GA-3′. The PCR product was inserted into pEGFP-N1 within HindIII and KpnI restriction sites. The 3′-UTR of CD151 was amplified from human cDNA using the following primers: forward, 5′-CCCTCGAG-CACCTGAGGGTCATTGGG-3′ and reverse, 5′-TTGCGGCCGCGTATG TGGCAGGGATGGTT-3′. The PCR product was inserted into psi-CHECK2 vector with XhoI and NotI restriction sites (Promega, Madison, WI, USA). Mutation in the miR-22 binding motif of CD151 was performed using a fast mutation kit (NEB, Ipswich, Canada).

2.4. MTT cell proliferation assay

Cells were seeded in 96-well plates (2×10^3/per well) 24 h after transfection. MTT (Roche Applied Science, Foster City, CA, USA) was added to each well and cells were further cultured for 4 h at 37 °C. The reaction was stopped by 150 μl DMSO and optical density at 490 nm was detected on a microplate reader.

2.5. Colony formation assay

500 of each transfected cells were plated in six-wells and cultured for 14 days without any disturbance. Cells were stained with 0.5% crystal violet for 1 h at 37 °C. Visible colonies were counted in four different fields and the mean value was calculated.

2.6. Cell migration and invasion assays

For migration, 5×10^4 transfected cells were seeded into the upper chamber (Millipore, Billerica, MA, USA). For invasion, 5×10^4 transfected cells were seeded into the upper chamber precoated with Matrigel. DMEM containing 10% FBS was used as a chemoattractant, and added to the lower chamber. After 24 h incubation, membranes were stained with 0.5% crystal violet for 20 min. Invaded cells on the lower membrane were counted under a microscope (Olympus, Tokyo, Japan).

2.7. Luciferase reporter assays

HEK293 cells were co-transfected with miR-22 or control mimics and wild type (WT) or the mutated 3′-UTR (Mut) of CD151. 48 h later, cells were collected and luciferase activity was assayed using the dual-luciferase assay system (Promega, Wisconsin, WI, USA).

2.8. Western blotting

Cells were washed twice and lysed in lysis buffer. Equal amount proteins were separated by 10% SDS–PAGE gel and transferred to PVDF membranes (Bio-Rad, Hercules, CA, USA). Membranes were probed with primary antibodies at 4 °C overnight followed by incubation with HRP-conjugated secondary antibodies and detected by ECL.

2.9. Statistical analysis

Data were expressed as the mean ± SD. Statistical significance was analyzed using One-way ANOVA or two-tail Student's *t*-test with SPSS 16.0. $P < 0.05$ was considered statistically significant.

3. Results

3.1. miR-22 was decreased in GC tissue samples and cell lines

miR-22 was significantly decreased in GC patient tissue samples compared with matched non-tumor tissue samples (Fig. 1A). miR-22 was also remarkably decreased in three GC cell lines, SGC7901, MKN28, and NUGC-3, compared with that of human gastric mucosa cell line, GES-1 (Fig. 1B).

3.2. miR-22 suppressed GC cell growth and motility in vitro

SGC7901 cells were transfected with miR-22 or control mimic. Ectopic overexpression of miR-22 significantly suppressed proliferation of SGC7901 cells (Fig. 2A). Similarly, Forced expression of miR-22 significantly suppressed colony formation of SGC791 cells (Fig. 2B). The effect of miR-22 mimics was confirmed by qRT-PCR (Fig. 2C).

miR-22 or control mimic was transfected into SGC7901 cells and migration and invasion assays were performed *in vitro*. miR-22 significantly suppressed *in vitro* migration and invasion capabilities of SGC7901 cells (Fig. 2D and E).

3.3. CD151 was a direct target of miR-22

TargetScan 6.2 was used to screen the target of miR-22. CD151 was predicted to be a target of miR-22 (Fig. 3A). Luciferase activity assay found that miR-22 significantly suppressed the WT but not the Mut 3′-UTR luciferase activity in HEK293 cells (Fig. 3B). Furthermore, overexpression of miR-22 significantly inhibited CD151 protein levels in SGC7901 cells (Fig. 3C).

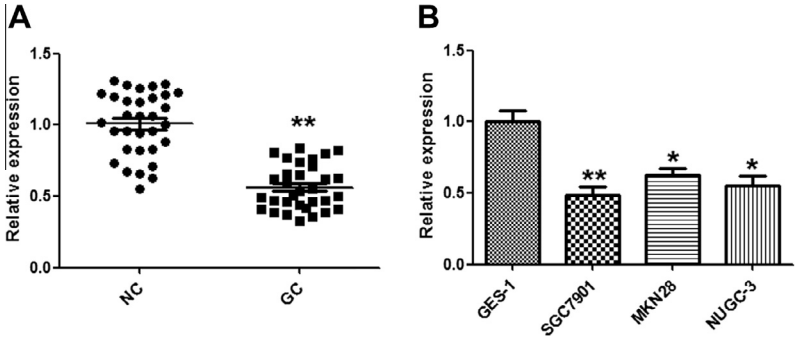

Fig. 1. miR-22 was decreased in GC tissue samples and cell lines. (A) Expression of miR-22 in 32 GC tissue samples and matched non-tumor tissue samples was detected by qRT-PCR. (B) Expression of miR-22 in three GC cell lines, SGC7901, MKN28, and NUGC-3, and GES-1 was detected by qRT-PCR. $^*P < 0.05$, $^{**}P < 0.01$ compared with the control.

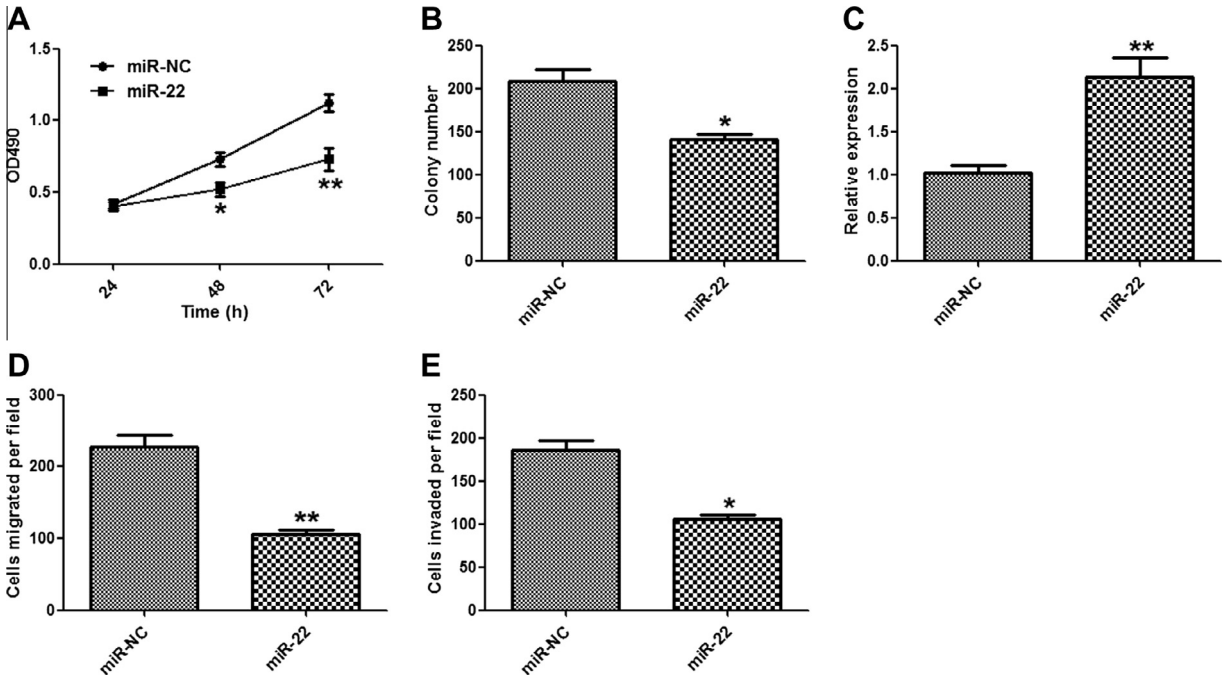

Fig. 2. miR-22 suppressed GC cell growth *in vitro*. (A) Cell proliferation of SGC7901 cells transfected with miR-22 or control mimic was detected by MTT assay. (B) The Colony number of SGC7901 cells transfected with miR-22 or control mimic was calculated. (C) Expression of miR-22 in SGC7901 cells transfected with miR-22 or control mimic was detected by qRT-PCR. (D) *In vitro* migration assay of SGC7901 cells transfected with miR-22 or control mimic. (E) *In vitro* invasion assay of SGC7901 cells transfected with miR-22 or control mimic. Data were drawn from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control.

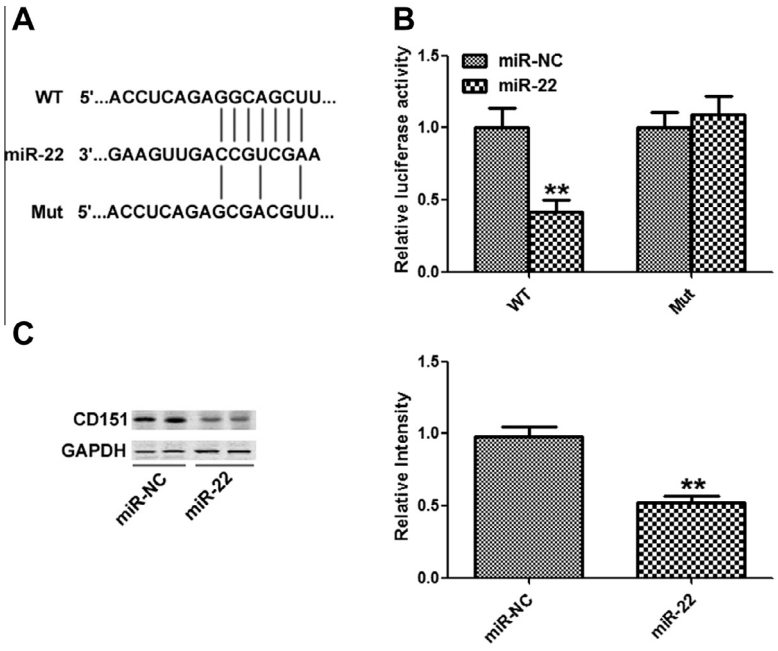

Fig. 3. CD151 was a direct target of miR-22. (A) The potential miR-22 binding sequence of CD151 3′-UTR and the mutant. (B) HEK293 cells were co-transfected with miR-22 or control mimic with WT or Mut CD51 3′-UTR. Luciferase activity was assayed. (C) Protein level in SGC7901 cells transfected with miR-22 or control mimic was detected by Western blotting. Data were drawn from three independent experiments. $^{**}P < 0.01$ compared with the control.

3.4. miR-22 suppressed GC cell growth and motility by inhibiting CD151

We further investigated whether overexpression of CD151 could reverse tumor suppressive effect of miR-22. MTT assay (Fig. 4A), *in vitro* migration and invasion (Fig. 4B and C) showed that supplement of CD151 remarkably attenuated tumor suppressive effect of miR-22. The effect of pEGFP-CD151 was confirmed by qRT-PCR (Fig. 4D).

4. Discussion

Documented evidence has revealed that miRNAs act as a novel class of oncogenic and tumor suppressive genes [11]. Recently,

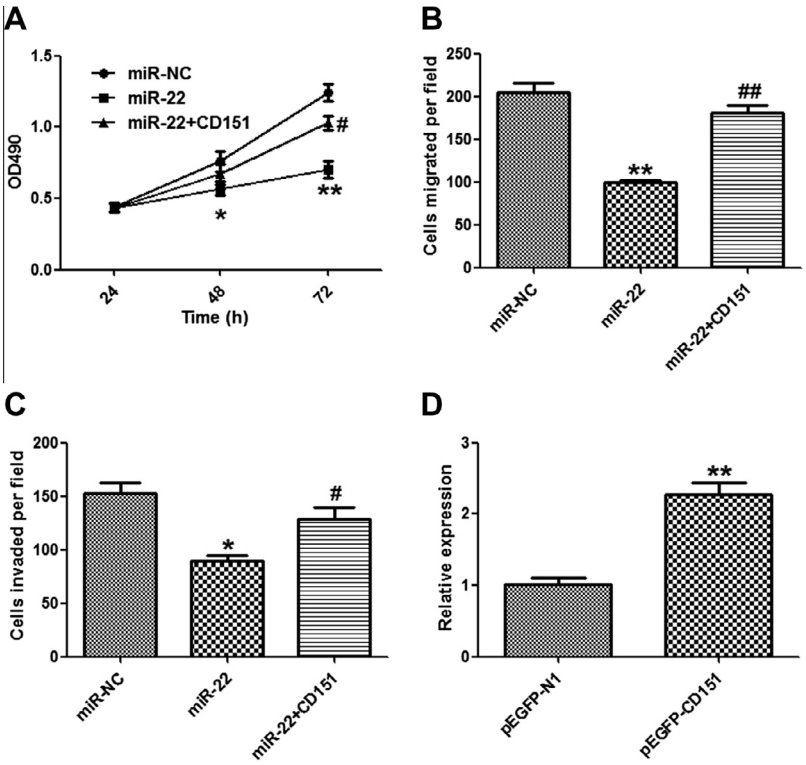

Fig. 4. miR-22 suppressed GC cell growth and motility by inhibiting CD151. (A) SGC7901 cells were co-transfected with miR-22 and pEGFP-CD151 or pEGFP-N1. MTT assay was used to assay proliferation. (B) *In vitro* migration assay of SGC7901 cells co-transfected with miR-22 and pEGFP-CD151 or pEGFP-N1. (C) *In vitro* invasion assay of SGC7901 cells co-transfected with miR-22 and pEGFP-CD151 or pEGFP-N1. (D) Expression of CD151 in SGC7901 transfected with pEGFP-CD151 or pEGFP-N1. Data were drawn from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with miR-22 group.

numerous studies have reported the aberrant expression of miRNAs in a wide range of human cancers, and the aberrant expression of miRNAs contributed to carcinogenesis by inhibiting the expression of their target genes. The direct link between miRNA function and carcinogenesis is supported by studies detecting the expression of miRNAs in clinical samples [12,13]. Here, we found that the expression of miR-22 was significantly decreased in GC tissue samples and cell lines, consistent with the report of Guo et al. [14]. We demonstrated that ectopic overexpression of miR-22 could suppress proliferation, colony formation, migration and invasion of GC cells by negatively regulating CD151 expression post-transcriptionally via binding to 3′-UTR of CD151. Moreover, overexpression of CD151 could partially attenuate the tumor suppressive effect of miR-22. These data suggest that miR-22 might be useful as a novel potential therapeutic strategy for GC.

miR-22 has been found to be elevated in some cancers. For instance, Li et al. reported that in colon cancer cells miR-22 was weakly expressed, and overexpression of miR-22 suppressed colon cancer cell migration and invasion via inhibiting the expression of matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9), and T-cell lymphoma invasion and metastasis 1 (TIAM1) [15]. In ovarian cancer, miR-22 performed inhibitory effects on cell migration and invasion [16]. Zhang et al. reported that in hepatocellular carcinoma (HCC), low miR-22 expression was predictive of poor survival in HCC patients, and miR-22 had an anti-proliferative effect on HCC cells [17]. In lung cancer, miR-22 was significantly decreased. Ling et al. reported that miR-22 suppressed lung cancer cell progression by targeting ErB3 [18]. Guo et al. also found that miR-22 suppressed GC cell progression by targeting transcription factor Sp1 [14]. Our work expanded the function of miR-22 in lung cancer.

CD151, a member of the tetraspanin family, was associated with regulation of migration of both normal and cancer cells via regulating cell surface microdomain formation [19]. CD151 has been found to be elevated in several cancers [20–22]. Overexpression of CD151 predicted poor prognosis [23]. CD151 was involved in the regulation of cancer cell motility and metastasis both *in vitro* and *in vivo* [20,24]. CD151 could be regulated post-transcriptionally by several miRNAs, including miR-124, miR-506 and so on [20,25]. In this study, we found that CD151 overexpression partially attenuated the suppressive effect of miR-22.

5. Conclusion

Collectively, this study showed that miR-22 was decreased in GC tissue samples and cell lines, and forced expression of miR-22 substantially suppressed cell proliferation, colony formation, migration, and invasion of GC cells. CD151 was identified as a target of miR-22, and overexpression of CD151 partially attenuated the suppressive effect of miR-22, suggesting that miR-22 might be a novel therapeutic strategy for the treatment of GC.

Conflict of interest

We declare that we have no conflict of interest.

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