MiR-1202 promotes cell migration and tumor metastasis in gastric cancer cells

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Abstract

Introduction : Tumor metastasis significantly impacts the survival rate of gastric cancer (GC) patients. Increasing evidence has suggested that numerous microRNAs (miRNAs) are associated with tumor metastasis and could be potential candidate cancer biomarkers or therapeutic targets. Materials and Methods : Early GC cases were collected and divided into two groups according to the lymphatic metastasis (LM) situation. The microarray analysis was carried out to screen out differentially expressed miRNAs in the two groups, which were further evaluated by quantitative reverse transcription polymerase chain reaction (qRT-PCR). The roles of miR-1202 on GC cells were determined through cell growth assay, cell migration assay in vitro and pulmonary metastasis assay in the mouse model. Bioinformatics analysis was used to explore the potential mechanisms of miR-1202-mediated biological effects. Results : MiR-1202 was first identified to be the most differentially expressed miRNA in GC patients with metastasis and those without metastasis. The overexpression of miR-1202 promoted GC cell migration in vivo whereas the knockdown of miR-1202 suppressed this process. However, miR-1202 did not affect GC cell growth or cell cycle distribution. The overexpression of miR-1202 promoted pulmonary metastasis and colonization after the tail-vein injection of GC cells. Pathways related to cell adhesion, collagen fibril organization, and positive regulation of mitogen-activated protein kinase (MAPK) cascade might be involved in miR-1202-mediated biological effects. Conclusion : Our results demonstrated a positive role of miR-1202 in regulating GC cell metastasis. MiR-1202 might be developed as a novel biomarker and a potential therapeutic target for GC metastasis.

Background

GC is a common malignant tumor of the digestive system worldwide. The incidence of GC ranks fourth in cancers, and its mortality rate ranks second worldwide [1]. In the recent years, the incidence of GC has steadily declined mainly benefiting from improvements in sanitation, nutritional standards, and *Helicobacter pylori* treatment rate. However, due to its pathological complexity and diversity, the early diagnosis and survival rate of GC are still not ideal. In China, the incidence and mortality of GC are the second highest in all the cancers [2]. Because of the lack of specific clinical pathological features in early-stage GC, patients diagnosed with GC are mostly already in the advanced stage and accompanied by metastasis. If it could be predicted whether GC will metastasize in early stage, specific treatments would be taken so as to improve the survival rate of GC patients [1,3-5]. Therefore, the identification of specific biomarkers related to GC metastasis has a promising prospect in clinical therapy.

In the large amounts of RNAs produced by the genome, only the open reading frame of mRNAs is “coding sequence”, while the rest mRNAs and other untranslated transcripts are called non-coding RNAs (ncRNAs). NcRNAs include “housekeeping” RNAs, like ribosomal RNA (rRNA), transfer RNA (tRNA), small cytoplasmic RNA (scRNA), among others. On the basis of the lengths, ncRNAs can be divided into long ncRNAs and short ncRNAs. MiRNAs are small RNAs with about 22 nucleotides in length, encoded by endogenous genes. MiRNAs are important to gene expression regulation in plants, animals, and fungi, participating in post-transcriptional regulation of protein-coding genes via translational repression, mRNA

cleavage, and other mechanisms. A single miRNA can downregulate several target genes by guiding argonaute proteins to its target sites, which are generally located in the 3'UTR of mRNAs [6]. Emerging evidence demonstrates that miRNAs play an important regulatory role in cancer. Generally, miRNAs associated with cancer are divided into two major types, namely tumor suppressive miRNAs and oncogenic miRNAs. Usually, the tumor suppressive miRNAs prevent tumorigenesis and tumor development by targeting and downregulating the expression of oncogenes, normally exhibit downregulated expression profiles in tumor. In contrast, the oncogenic miRNAs' expressions are often increased in tumor and can inhibit tumor suppressor genes, directly or indirectly.

Recent studies have found that a number of miRNAs are involved in GC development. These miRNAs play diverse roles in cell apoptosis, proliferation, and metastasis of GC. For instance, miR-21, which is overexpressed in almost all the solid tumors, promotes tumor proliferation, migration, and invasion by targeting *PTEN* in GC [7,8]. Exosomal miR-21 induced mesothelial-to-mesenchymal transition and promoted cancer peritoneal metastasis by targeting *SMAD7* [9]. MiR-375 was found to inhibit cell proliferation by targeting *JAK2* and was frequently downregulated in GC [10].

MiR-1202 was first reported in major depressive disorder (MDD). The expression of miR-1202 is low in the blood of MDD patients [11-13]. However, little is known about the biological function of miR-1202 during GC development. Here, we reported that miR-1202 was significantly upregulated in early-stage GC patients with metastasis compared with those without metastasis. To investigate the effect of miR-1202 on GC cells, lentivirus was utilized to establish stable cell lines. Nevertheless, miR-1202 did not affect cell cycle distribution or cell growth rate of GC cells. Cell migration assay *in vitro* and tumor metastasis assay *in vivo* demonstrated a positive regulatory role of miR-1202 in GC metastasis. Based on the data acquired from Oncomine, Gene Expression Omnibus (GEO), and 9 online miRNA targets prediction tools, we performed bioinformatics analysis to further explore the underlying mechanism of miR-1202's role in GC metastasis.

Methods

Clinical samples

The paraffin-embedded tumor specimens from 199 early GC cases were collected from Zhongshan Hospital affiliated to Fudan University (Shanghai, China). These specimens were obtained by open or laparoscopic surgery. According to the neoplasm staging and LM situation by the pathology results, we divided specimens into two groups, 30 cases in the with LM group, and 169 cases in the non-lymphatic metastasis (NLM) group. This study was approved by the Ethics Committee of Zhongshan Hospital affiliated to Fudan University.

Cell culture and reagents

GC cell lines used in this study include AGS and SGC-7901. AGS was cultured in F-12 medium (Gibco, USA) with 10% fetal bovine serum (FBS, Gibco, USA) containing 100 U/mL penicillin and streptomycin (Gibco, USA). SGC-7901 was cultured in RPMI-1640 (Gibco, USA) with 10% FBS containing 100 U/mL penicillin and streptomycin. All cells were cultured at 37℃ with 5% CO_2 humidified atmosphere.

Lentivirus infection

Recombinant plasmids carrying either pre-miR-1202 sequence or short hairpin (shRNA) targeting mature miR-1202 were subcloned at Shanghai Genechem Co., Ltd. (China). The lentiviral particles and the control lentivirus were further packaged in parallel. Cells were seeded into a 6-well plate (1×10^5 cells/well) and lentivirus infection was performed when the cells reached ~80% confluence. The multiplicity of infection (MOI) of SGC-7901 and AGS cells was 10.

RNA isolation

Total RNA was extracted from tissues or cells with Trizol reagent according to the manufacturer's instructions (Invitrogen, USA).

Microarray analysis

The total RNA from GC tissues was used for miRNA chip analysis at Genechem. The threshold we used to screen differentially expressed miRNAs with statistical significance was fold change ≥ 1.5 and *P*-value ≤ 0.05.

Quantitative reverse transcription PCR (qRT-PCR)

A total of 1 μg RNA was used for reverse transcription in the reaction mixture containing oligo (dT) primer, reverse transcriptase (Takara, Japan), U6 and miR-1202 stem-loop RT primers (Ribobio, China). The SYBR Green Supermix kit (Takara, Japan) was used in the qRT-PCR assay according to the manufacturer's instructions. PCR reaction was carried out in triplicates for each gene, while blank controls were performed in parallel to rule out contamination. The miR-1202 gene expression was normalized to U6. The relative expression level of miR-1202 and others was calculated by the formula $2^{-\Delta\Delta Ct}$ [14]. Cycle parameters were 95℃ for a 3 min hot start, and 50 cycles of amplification: 95℃ for 10 s, 58℃ for 15 s and 72℃ for 20 s. Melting curve analysis was used to confirm the specificity of the PCR product.

Transwell migration assay

Cells ($0.8\text{-}1 \times 10^5$) were plated on 24-well PET inserts (8.0 μm pore size, Falcon). Cells passing through the filter were fixed, stained, and counted after being cultured at 37℃ for 16 h.

Tumor xenograft and metastasis assays *in vivo*

This work was approved by the Comments, Provisos or Reservations of Animal Ethics Committee of Zhongshan Hospital, Fudan University. Animal experiments were carried out in accordance with the guidelines for the care and use of laboratory animals of Shanghai municipality, China. The protocol was approved by the Science and Technology Commission of Shanghai Municipality (Permit Number: SYXK 2015-0006). All efforts were made to minimize suffering.

Three-week-old female BALB/c nude mice were purchased from the Shanghai Laboratory Animal Company (SLAC, China) and maintained in individually ventilated cages (IVC) under a 12 h:12 h light–dark schedule, 20-22°C temperature and 40-60% humidity with free access to food and water. Cells were harvested and washed with RPMI-1640 medium and resuspended in sterile phosphate buffer saline (PBS) (2.5×10^6 cells/ml). Cells (5×10^5 cells in 200 μl) were further injected subcutaneously under the skin of anterior oxter of mice or through the lateral tail vein. Five mice were used in each group.

For the xenograft growth of subcutaneous animal assay, the tumor size was measured with a caliper every three days. The tumor volume was calculated using the following formula: length × width^2 × 0.5. When the average tumor volume reached approximately 1000 mm^3, all efforts were made to minimize suffering and mice were euthanized by carbon dioxide in a closed cage. The tumors were then excised and weighed. For *in vivo* metastasis assay, after 2.5 months' injection through the tail vein, the mice were euthanized, and the lung tissues were dissected and sectioned. Metastatic tumors were detected by hematoxylin and eosin (H&E) staining.

Bioinformatics analyses

To predict the putative target genes of miR-1202, the following databases were used: microrna.org (http://www.microrna.org), microT-CDS (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=microT_CDS/index), miRDB (http://www.mirdb.org), miRTarbase (http://mirtarbase.mbc.nctu.edu.tw/php/index.php), miRWalk 3.0 (http://mirwalk.umm.uni-heidelberg.de), PolymiRTs 3.0 (http://compbio.uthsc.edu/miRSNP/), Tarbase v8 (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=tarbasev8%2Findex/), Targetminer (https://www.isical.ac.in/~bioinfo_miu/targetminer20.htm), and TargetScan 7.2 (http://www.targetscan.org/vert_72/). Genes overlapping in at least three databases were selected. Differently expressed genes related to GC were also identified by using Oncomine (https://www.oncomine.org/resource/main.html) and GEO database (www.ncbi.nlm.nih.gov/geo/). Based on the interaction of miRNA-related and GC-related genes mentioned above, gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis were conducted by using the online tool (https://david.ncifcrf.gov/).

Statistical analysis

A two-tailed Student's *t*-test was applied to evaluate group-level differences. We considered two groups with a *P*-value < 0.05 (*) to be different and with a *P*-value < 0.01 (**) to be statistically significant.

Results

MiR-1202 was up-regulated in tissues of GC patients with metastasis

We first carried out miRNA microarray analysis on three GC tissues (clinical phase I) with metastasis and three tissues without metastasis. As shown in Fig. 1A, 19 miRNAs exhibited significantly different expression levels between metastatic group and non-metastatic group, including miR-23b, miR-24, miR-1225-5p, miR-1207-5p, miR-4281, miR-1202, miR-150, miR-30c, miR-130a, miR-4270, miR-142-3p, miR-132, miR-486-5p, miR-4291, miR-766, miR-3651, miR-140-5p, miR-139-5p, and miR-582-5p.

To further confirm this finding, other 20 GC tissues with metastasis and 20 GC tissues without metastasis were utilized in the qRT-PCR experiment. It was shown that 10 out of the 19 miRNAs were differentially expressed in the two groups with miR-1202 being the most significant (Fig. 1B). Therefore, we focused our attention on the functional study of miR-1202 in GC cells in the following work.

MiR-1202 played a regulatory role in GC cell migration

To assess the biological function of miR-1202 on regulating cell growth and migration, SGC-7901 cells were infected with lentivirus expressing pre-miR-1202 (LV-miR-1202) and control (LV-miR-Ctl) lentivirus at a MOI of 10. The infection efficiency was confirmed by fluorescence microscopy. More than 80% of cells had green fluorescence, indicating that the infection efficiency was high enough for the following experiments (Fig. 2A).

Flow cytometry analysis was first performed and it was found that there was no statistical difference in cell cycle distribution (supplementary Figure 1A). A similar result was gained from a cell growth curve in cell proliferation assay (supplementary Figure 1B).

Based on the differential expression pattern of miR-1202 between metastatic GC tissues and non-metastatic GC tissues, we further assessed the potential function of miR-1202 on cell migration using the transwell assay. Fig. 2B-C shows that, compared with those of the control group, SGC-7901 cells infected with LV-miR-1202 showed an increased migration ability. Furthermore, we performed miR-1202 inhibition experiments to confirm the potential role of miR-1202 in GC cells migration. The target sequence of the lentiviral vector of miR-1202-inhibitor (LV-anti-miR-1202) complemented mature miR-1202 and thus inhibited its function. The efficiency of AGS cells infected with LV-anti-miR-1202 was also confirmed by fluorescence microscopy (Fig. 2D). Consistently, the knockdown of miR-1202 did not significantly affect cell cycle and cell growth rate (Supplementary Figure 2A and 2B), but suppressed the cell migration ability of GC cells (Fig. 2E-F). These results suggested that miR-1202 was able to regulate cell migration ability *in vitro* in GC cells.

Overexpression of miR-1202 promoted distal pulmonary metastasis in nude mice

To further investigate the effect of miR-1202 *in vivo*, we selected SGC-7901 cells to perform the tumor xenograft and lung metastasis on BALB/c nude mice. The body weight and overall health status of the nude mice were assessed before mice were randomly divided into two groups. Equal numbers of cells overexpressing miR-1202 and control cells were injected subcutaneously into the mice and the tumor size was measured every three days. As displayed in Fig. 3A, the volumes of the tumors formed by SGC-7901 cells overexpressing miR-1202 and the control cell did not show statistical difference during the experimental period. Consistent with the tumor volumes, weights of tumors in the two groups were not statistically different, either (Fig. 3B-C).

The pulmonary metastasis assay was carried out to investigate the role of miR-1202 in tumor metastasis. Two groups of SGC-7901 cells were injected into nude mice through the lateral tail vein. After 2.5 months, the lungs of mice were isolated. While no metastatic tumor was observed in the lungs of all the control mice, four of the five mice injected with cells overexpressing miR-1202 had multiple visible lung metastases. The representative photos were shown in Fig. 3D. The results of H&E staining further confirmed lung metastases in the miR-1202 group (Fig. 3E). Collectively, the results suggested that miR-1202 had no effect on primary GC cell growth but promoted distal pulmonary metastasis *in vivo*.

Identification of miR-1202 target genes and bioinformatics analysis

The information about the regulatory target of miR-1202 is limited, we, therefore, utilized 9 miRNA target prediction databases, such as Oncomine database and GEO database, in order to get a better understanding of miR-1202 mechanism. As shown in Fig. 4A-B, 139 potential target genes of miR-1202 were selected. According to the KEGG pathway analysis and GO enrichment in DAVID, 4 KEGG pathways, 39 GO terms of biological processes (BP), 13 GO terms of cellular components (CC), and 14 GO terms of molecular function (MF) were identified. Four KEGG pathways and the top 10 GO annotations of each section were listed in Fig. 4C. According to the KEGG pathway results, 17 target genes were likely involved in the cytokine-cytokine receptor interaction, CAMs (cell adhesion molecules), valine, leucine and isoleucine degradation, and glutathione metabolism. In the GO enrichment, the target genes accumulated in the BP of cell adhesion, collagen fibril organization, and positive regulation of mitogen-activated protein kinase (MAPK) cascade, in the CC of the external side of the plasma membrane, melanosome, apical plasma membrane, and in the MF of peroxidase activity, signal transducer activity, and fatty-acyl-CoA binding.

Discussion

The occurrence and development of GC are a multi-stage process influenced by numerous environmental and genetic factors. Environmental factors include unhealthy eating habits, excessive smoking and drinking, obesity, occupational exposure, *Helicobacter pylori* infection, and Epstein-Barr virus infection [1]. The influence of genetic factors on tumorigenesis includes the accumulation of gene mutations,

epigenetic alteration such as chromatin remodeling, histone modification, and ncRNAs action. Recently, the role of ncRNAs in GC development has gained more and more attention.

In our study, we first performed a miRNA expression profiling of metastatic and non-metastatic early GC samples. And it was found that miR-1202 was the most significantly differentially expressed miRNA associated with GC metastatic capability. MiR-1202 is a primate-specific miRNA [11]. Although a certain level of miR-1202 can be detected in various human tissues, its expression is significantly enriched in the human brain. Previous studies concentrating on miR-1202 and MDD suggested that miR-1202 could be used as a potential biomarker of MDD with high sensitivity and specificity [15].

Several previous studies also reported the expression pattern of miR-1202 in different cancers but the role of miR-1202 during cancer development is only beginning to be explored. Some studies have suggested that miR-1202 is highly expressed in cancer tissues and may act as an oncogenic miRNA. For instance, Chen H. et al. investigated the expression level of miR-1202 in endometrial tissue from endometrial carcinoma (EC) and atypical endometrial hyperplasia (AEH) patients. A high level of miR-1202 was found in EC compared with that of AEH [16]. In contrast, miR-1202 was downregulated in clear cell papillary renal cell carcinoma (CCPRCC) and cervical cancer [17,18]. Some other studies have suggested that miR-1202 might be associated with cancer metastasis. For instance, compared with that of papillary thyroid carcinoma (PTC) patients without lymph node metastasis (LNM), a higher expression level of miR-1202 was found in PTC patients with LNM [19]. Tokuhisa M. et al. found that miR-1202 was expressed in exosomes of malignant ascites samples, and it might serve as a biomarker of peritoneal dissemination [20]. Du B. et al. explored the role of miR-1202 in hepatocellular carcinoma (HCC), they found that elevated expression of miR-1202 weakened HCC cells migration and invasion abilities [21]. In glioma cells, it was found that miR-1202 inhibited cell proliferation and induced endoplasmic reticulum stress and apoptosis [22]. In summary, previous studies of miR-1202 in cancers found that miR-1202 played different roles in different cancer types and different clinical stages, which might be partially attributable to cancer heterogeneity.

After we revealed a correlation between miR-1202 expression and GC metastasis, cell experiments *in vitro* and animal experiments *in vivo* were used to further analyze the role of miR-1202 in GC. Our in vitro cell culture results showed that the overexpression of miR-1202 promoted GC cell migration but knockdown of miR-1202 suppressed cell migration. Our *in vivo* animal model results confirmed the function of miR-1202 in GC metastasis. The overexpression of miR-1202 in GC cells had no impact on primary tumor growth but promoted distal pulmonary metastasis.

To explore the molecular mechanism underlying miR-1202 functions, we performed a comprehensive bioinformatics analysis. We chose 139 genes on the basis of 9 miRNA-related databases, Oncomine, and GEO database for further analysis. According to the results of KEGG pathway and GO enrichment, several pathways and GO terms are potentially essential to GC metastasis. Certain hub target genes including *NCAM1* are probably involved in the metastasis of GC. *NCAM1* encodes a cell adhesion protein that is a member of the immunoglobulin superfamily. In 2015, Shi Y. et al. found a decreased level of *NCAM1* in

GC tissues, and NCAM1 protein is negatively associated with GC invasion [23]. In another research, match pairs of peritoneal tissues and peritoneal metastasis in GC patients were collected for whole-exome sequencing to characterize the mutation spectrum of GC with peritoneal metastasis and thus provide a basis for finding new biomarkers and treatment targets. The result showed that *NCAM1* was the only mutated gene in metastatic cells and was expected to become a treatment target [24]. Our future study will try to explore the regulatory relationship between *NCAM1* and miR-1202, as well as the mechanism of miR-1202-mediated GC metastasis.

Yang X. et al. have reported that the combined diagnosis of miR-1202 and other miRNAs is of higher value in the early diagnosis of cervical cancer [18]. Our study first reported a potential relationship between miR-1202 and GC metastasis. MiR-1202 may affect metastatic steps by post-transcriptional regulation of multiple target genes, providing GC cell with a greater metastatic ability. Our results suggested that miR-1202 might be used to develop a GC metastasis prediction model. Further studies with larger clinical sample sizes and further association study of miR-1202 expression with clinicopathological features are warranted to fully elucidate the role of miR-1202 in cancer.

Conclusions

Our present work identified miR-1202 as the most differentially expressed miRNA between gastric cancer (GC) patients with metastasis and GC patients without metastasis. We further demonstrated a positive role of miR-1202 in regulating GC cell metastasis. The present work brings a novel insight into role of miR-1202 during GC development. Moreover, miR-1202 might be developed as a novel biomarker and a potential therapeutic target for GC.

Abbreviations

AEH, atypical endometrial hyperplasia

BP, biological processes

CAMs, cell adhesion molecules

CC, cellular components

CCPRCC, clear cell papillary renal cell carcinoma

GEO, Gene Expression Omnibus

GC, gastric cancer

GO, gene ontology

HCC, hepatocellular carcinoma

H&E, hematoxylin and eosin

IVC, individually ventilated cages

KEGG, kyoto encyclopedia of genes and genomes

LM, lymphatic metastasis

LNM, lymph node metastasis

MDD, major depressive disorder

MF, molecular function

miRNA, microRNA

MOI, multiplicity of infection

NLM, non-lymphatic metastasis

PTC, papillary thyroid carcinoma

qRT-PCR, quantitative real-time PCR

rRNA, ribosomal RNA

scRNA, cytoplasmic small RNA

tRNA, transfer RNA

Declarations

Ethics approval and consent to participate

Declaration of Helsinki and approved by the institutional review boards of the centers participating in this study. All patients gave their signed informed consent for this study.

Consent for publication

Not applicable

Availability of data and material

The datasets used during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that no conflict of interest exists.

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Authors' contributions

Conceived and designed the experiments: J. Hu, Y. Wang. Performed the experiments: J. Hu, L. Shang, W. Xie. Analyzed the data: L. Shang, Y. Wang, Q. Li, M. Xu, W. Niu, H. Lou, Y. Wang. Wrote the paper: L. Shang, Y. Wang, J. Hu.

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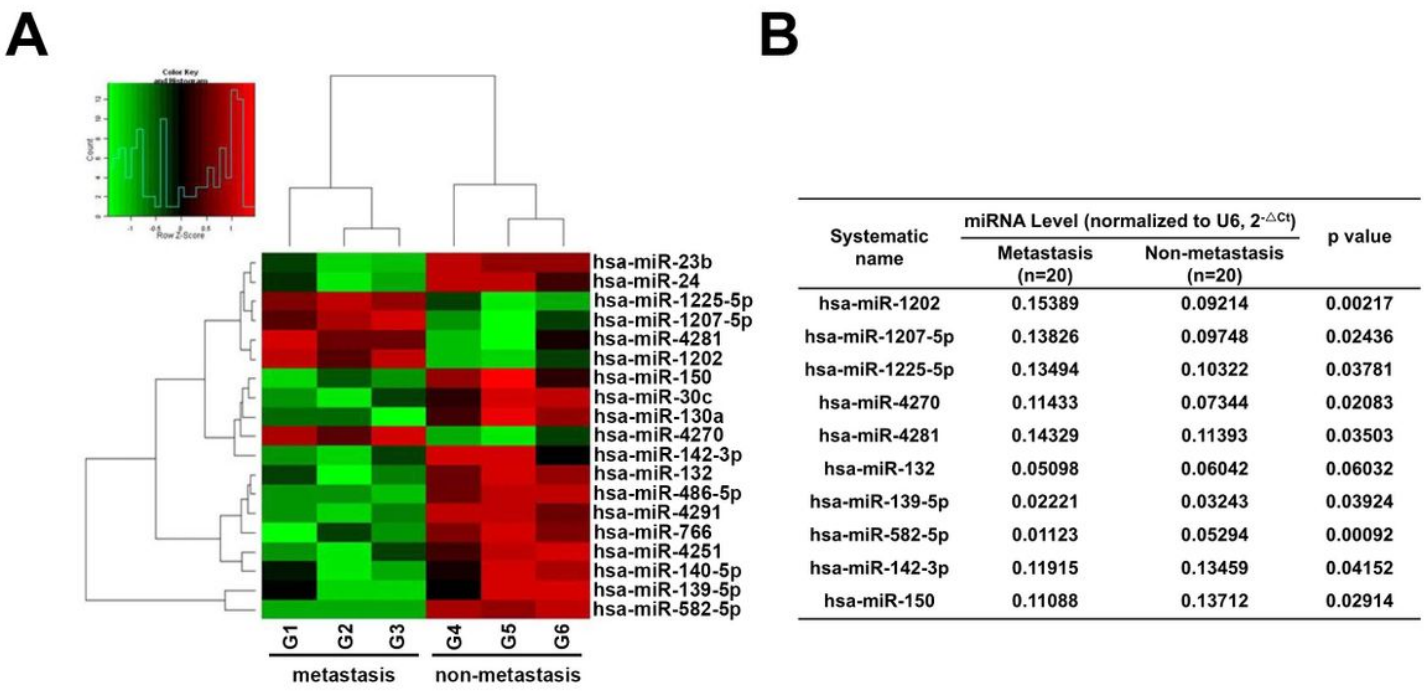
Figures

Figure 1



Systematic name	miRNA Level (normalized to U6, $2^{-\triangle Ct}$)		p value
	Metastasis (n=20)	Non-metastasis (n=20)	
hsa-miR-1202	0.15389	0.09214	0.00217
hsa-miR-1207-5p	0.13826	0.09748	0.02436
hsa-miR-1225-5p	0.13494	0.10322	0.03781
hsa-miR-4270	0.11433	0.07344	0.02083
hsa-miR-4281	0.14329	0.11393	0.03503
hsa-miR-132	0.05098	0.06042	0.06032
hsa-miR-139-5p	0.02221	0.03243	0.03924
hsa-miR-582-5p	0.01123	0.05294	0.00092
hsa-miR-142-3p	0.11915	0.13459	0.04152
hsa-miR-150	0.11088	0.13712	0.02914

Figure 1

Differentially expressed miRNAs in tissue samples of GC patients. (A) Hierarchical cluster analysis of miRNA microarray data. Cluster analysis was used to show the expression levels of the 19 differentially expressed miRNAs in the tissue samples of metastatic GC patients and non-metastatic GC patients. Each

column represented a single tissue sample, and each row represented a miRNA. The top-left legend (red to green) depicts a range of log expression values. Black coloring indicates the mean expression level. Distance metric, Pearson centered, Linkage rule, average. (B) Validation for differentially expressed miRNAs using quantitative real-time PCR. U6 was used as an endogenous control. The means of miRNAs' level normalized to U6 with the p-value being shown.

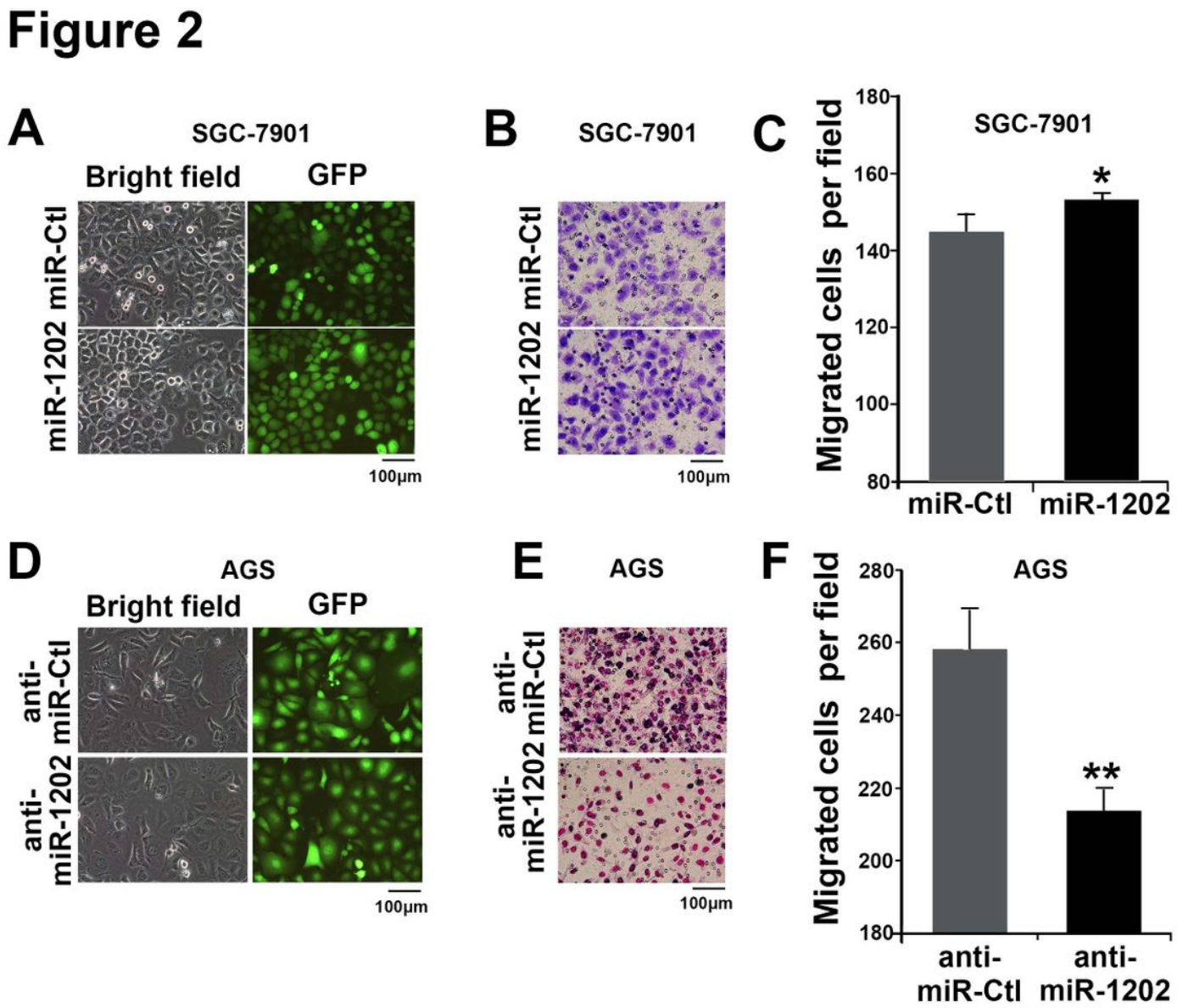


Figure 2

MiR-1202 promoted migration of GC cells. (A) Representative images of SGC-7901 cells infected with LV-miR-Ctl and LV-miR-1202 respectively. (B) Representative images of cell migration assay were shown (magnification, × 200). (C) Statistical analysis of cell migration assay in SGC-7901. Values are indicated as mean ± SD, n = 6. (D) Representative images of AGS cells infected with control lentivirus (LV-anti-miR-Ctl) and LV-anti-miR-1202 respectively. (E) Representative images of cell migration assay in AGS cells were shown (magnification, × 200). (F) Statistical analysis of cell migration assay in AGS cells. Values are indicated as mean ± SD, n = 3.

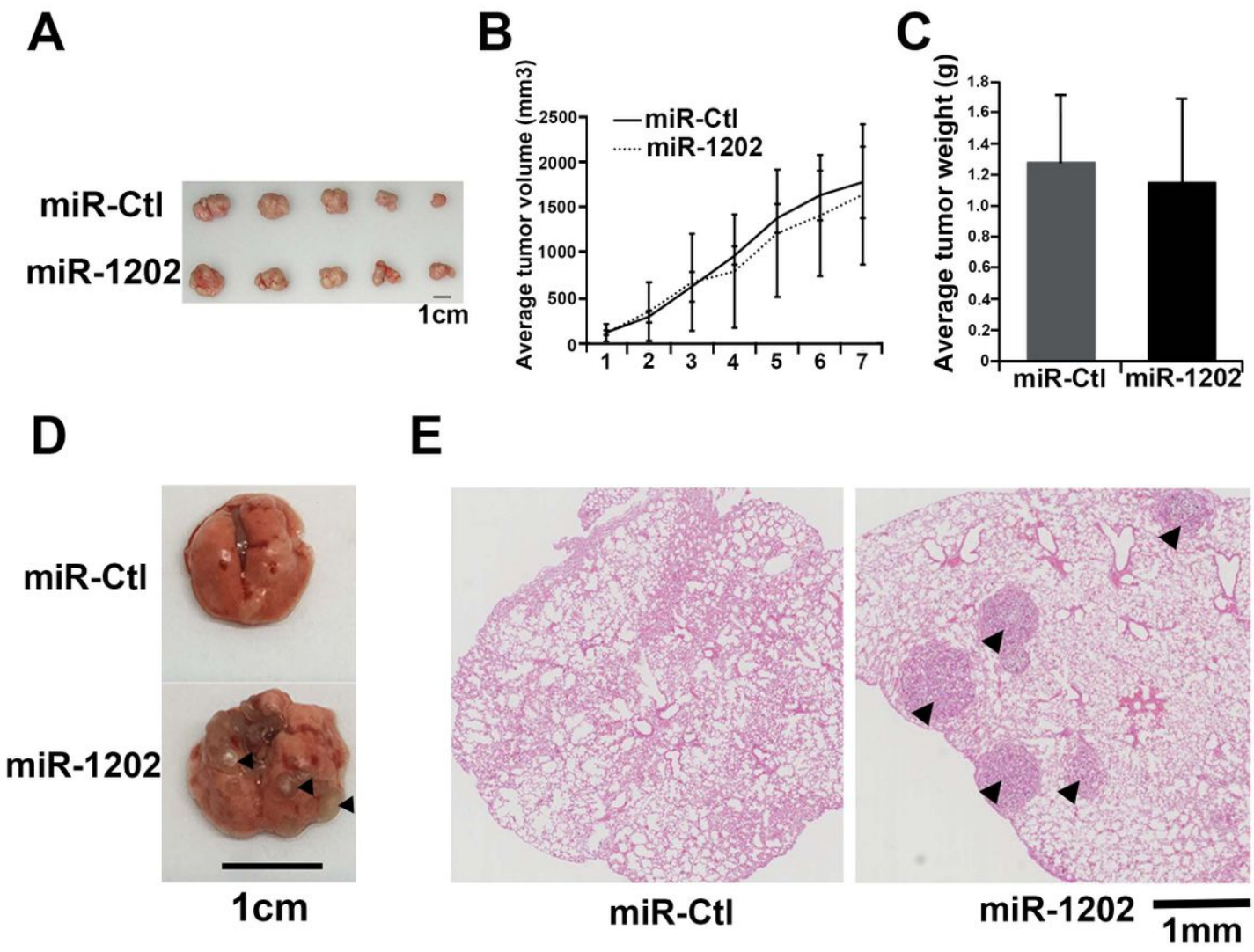
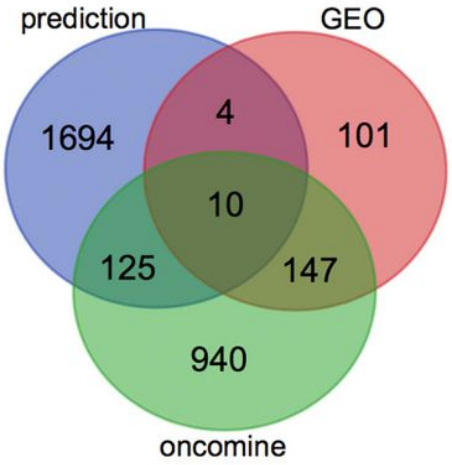


Figure 3

Effects of miR-1202 on tumor growth and metastasis in vivo. (A) Photographs of tumors dissected from nude mice engrafted with control SGC-7901 cells and SGC-7901 cells overexpressing miR-1202 respectively. Measurements of tumor size (B) and tumor weight (C) in nude mice. Values are indicated as mean ± SD, n = 5. (D) Representative photographs of lungs from mice infected with control SGC-7901 cells and SGC-7901 cells overexpressing miR-1202. Scale bar, 1 cm. Pulmonary metastases are indicated by arrows. (E) Representative pictures of H&E staining of metastatic lungs. Scale bar, 1 mm.

Figure 4

A



B

Validated genes of miR-1202 from overlap of 9 prediction websites, GEO and Oncomine.

IGF2BP3	TFR2	CTNNB1	C5orf30	SOX21	NEK1	EHD3
CDH3	LIF	TGIF2	ST5	BTG1	NICN1	SH2B3
CNTD1	KDM2A	TOX	SNX22	FAS	ADRB2	GSTM3
SULF1	NCAM1	CSRNP1	RAP2A	ABCA1	RBPMS2	EDARADD
CLDN1	ACADL	C21orf2	NR2C1	RAP1GAP	GREM1	TMOD1
SLC26A7	PJA2	ZFP1	PTGFRN	TSC22D3	TXNIP	ITGA11
BCAS1	KIF14	ADAMTS6	ACACB	PIGR	SLC7A8	NFKBIE
SLC26A9	CAMSAP1	TNFRSF25	TCF20	INTS8	RAB17	SGSM2
ACER2	RPS21	CD109	RORC	EXOSC10	DUOX1	GPX3
HAPLN1	NFE2L2	ACER3	FAM46C	JAG1	PRPF40A	NAA25
FCRL5	ANKRD52	FAM102A	RNF8	RUNX1	COL3A1	COL5A1
DUOX2	IDS	SH3PXD2B	GSTM5	PLXNC1	DPT	HKDC1
SLC1A2	ALDH6A1	MMP14	ADAMTSL1	DUSP26	ST3GAL2	BCAT1
HOXC6	CTSB	ITGBL1	DDX56	LTBP2	FAM101A	CASP2
G3BP1	ASAP1	NDST3	AFF3	SH3KBP1	CCR1	TAOK1
SEMA4D	CNGA2	BTBD1	CCDC25	DBT	NOD1	E2F3
B3GAT1	ANTXR1	SMARCA4	MALT1	LMOD1	TPP1	FGD6
GDPD5	ACADVL	EML4	HMGB3	ZBTB16	FNDC5	MTMR11
GHR	NEGR1	DIO2	PROM2	MAGIX	CXCL12	CDH5
PLCL2	CBS	PPP1CB	LAMP1	SARM1	SLC20A1	

C

Category	Term	Count	%	PValue
KEGG_PATHWAY	ptr04060:Cytokine-cytokine receptor interaction	6	0.02664	0.04470
	ptr04514:Cell adhesion molecules (CAMs)	5	0.02220	0.05294
	ptr00280:Valine, leucine and isoleucine degradation	3	0.01332	0.07509
	ptr00480:Glutathione metabolism	3	0.01332	0.08070
GOTERM_BP_DIRECT	GO:0043410~positive regulation of MAPK cascade	5	0.02220	0.00089
	GO:0048814~regulation of dendrite morphogenesis	3	0.01332	0.00748
	GO:0045453~bone resorption	3	0.01332	0.00958
	GO:0042391~regulation of membrane potential	4	0.01776	0.00964
	GO:0002415~immunoglobulin transcytosis in epithelial cells mediated by polymeric immunoglobulin receptor	2	0.00888	0.01753
	GO:0035116~embryonic hindlimb morphogenesis	3	0.01332	0.01868
	GO:0001974~blood vessel remodeling	3	0.01332	0.02019
	GO:0030199~collagen fibril organization	3	0.01332	0.02501
	GO:0008652~cellular amino acid biosynthetic process	2	0.00888	0.02618
	GO:0070458~cellular detoxification of nitrogen compound	2	0.00888	0.02618
GOTERM_CC_DIRECT	GO:0005887~integral component of plasma membrane	14	0.06216	0.00442
	GO:0009897~external side of plasma membrane	6	0.02664	0.00754
	GO:0055038~recycling endosome membrane	3	0.01332	0.00983
	GO:0009986~cell surface	8	0.03552	0.01762
	GO:0042470~melanosome	3	0.01332	0.01937
	GO:0016324~apical plasma membrane	5	0.02220	0.02442
	GO:0016323~basolateral plasma membrane	4	0.01776	0.02940
	GO:0005578~proteinaceous extracellular matrix	5	0.02220	0.03052
	GO:0005730~nucleolus	10	0.04440	0.03776
	GO:0030173~integral component of Golgi membrane	3	0.01332	0.04591
GOTERM_MF_DIRECT	GO:0004871~signal transducer activity	5	0.02220	0.02107
	GO:0004364~glutathione transferase activity	3	0.01332	0.02224
	GO:0005201~extracellular matrix structural constituent	3	0.01332	0.03866
	GO:0016174~NAD(P)H oxidase activity	2	0.00888	0.04376
	GO:0005223~intracellular cGMP activated cation channel activity	2	0.00888	0.04376
	GO:0005523~tropomyosin binding	2	0.00888	0.06073
	GO:0043295~glutathione binding	2	0.00888	0.06073
	GO:0005222~intracellular cAMP activated cation channel activity	2	0.00888	0.06073
	GO:0004601~peroxidase activity	2	0.00888	0.07740

Figure 4

Bioinformatics analysis of miR-1202 target genes. (A) Venn diagram for identifying 139 promising target genes of miR-1202 in GC. (B) Overlapping genes from 9 prediction websites, GEO, and Oncomine. (C) Functional annotation of miR-1202 target genes by KEGG pathway and GO enrichment analysis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- NC3RsARRIVEGuidelinesChecklistmiR1202.pdf