Int J Clin Exp Pathol 2016;9(5):5313-5319
www.ijcep.com /ISSN:1936-2625/IJCEP0023232

Original Article

MiR-486-5p inhibits metastasis by targeting neuropilin-2 in gastric cancer

Haifeng Lian*, Ranran Zhang*, Qiong Niu, Jian Wang, Kun Li, Fangkang Liu, Weiping Mu, Chengxia Liu

*Department of Gastroenterology, The Affiliated Hospital of Binzhou Medical University, Binzhou, Shandong Province, China. *Equal contributors.*

Received January 4, 2016; Accepted March 18, 2016; Epub May 1, 2016; Published May 15, 2016

Abstract: Previous studies have revealed that aberrant expressions of miRNAs participate in almost every step of carcinogenesis. Despite of large numbers of miRNAs disclosed, the function of miR-486-5p in the metastasis of gastric cancer is not elucidated so far. In the current study, we collected 65 gastric cancer samples and adjacent tissues to detect the miR-486-5p expression using qPCR, and applied luciferase reporter assay to verify the effect of miR-486-5p on the transcriptional activity of the Neuropilin-2 (NRP2) mRNA 3'UTR. Xenograft model was performed to test the effect of miR-486-5p on gastric cancer cell proliferation and metastatic ability in nude mice. Immunohistochemistry were used to confirm the relationship between NRP2 and lymphatic vessel density in these gastric cancer samples. Our results found that expression of MiR-486-5p was obviously decreased in the gastric cancer samples compared with the adjacent samples (P<0.01). Ectopic overexpression of miR-486-5p significantly inhibited the transcriptional activity and expression level of NRP2, as detected by luciferase and western blot, respectively. Furthermore, the gastric cancer cell SGC-7901 transfected with miR-486-5p showed obvious low tumorigenesis ability compared with the non-target control in the nude mice xenograft model (p<0.01). In xenograft tissues the expression of NRP2 had a positive correlation with the LYVE-1 expression, suggesting NRP2 promote lymphangiogenesis. Thus, our findings demonstrated that miR-486-5p function as a tumor suppressor in gastric cancer cell proliferation and lymphangiogenesis via targeting NRP2, and suggesting miR-486-5p a new potential target for developing diagnostics and therapy strategy in gastric cancers.

Keywords: miR-486-5p, NRP2, proliferation, metastasis

Introduction

Gastric cancer (GC) is the fourth most common malignancies worldwide. The incidence is much higher in male and in developing countries, including East Asian and Eastern European nations [1]. Despite advances in surgical and chemotherapies for GC, the current treatments available to patients with advanced GC are very limited because of the distant metastasis [2, 3]. Therefore, better understanding of the molecular mechanisms that involved in gastric cancer metastasis is essential for the development of novel therapeutic strategies.

Metastasis is a major feature of malignant tumors and cause of cancer-related deaths [4]. The main metastatic routes of gastric cancer include direct invasion, vascular metastasis, lymphatic metastasis and enterocoelia metastasis [5]. Lymphatic metastasis is an increasingly important prediction of optimizing surgical treatment and as a prognostic factor [6]. The two established ways of tumor cells entry into the lymphatic system are invasion of existing lymphatic ducts and induction of lymphangiogenesis], and the latter is considered as the major way [5]. Thus, studies of lymphangiogenesis have a promising impact on the treatment [7].

Neuropilin-2 (NRP2) plays an important role in lymphangiogenesis in cancer. Previous study demonstrated that NRP2 expression in breast cancer correlates with lymph node metastasis and poor prognosis [8]. Moreover, NRP2 activation can promote colorectal carcinoma lymphangiogenesis via activating integrin α9 β1/FAK/Erk [9]. Hironao Yasuoka also declared that anti-NRP2 blocked lymph node metastasis

in papillary thyroid carcinoma [10]. However, the relationship between NRP2 with lymphangiogenesis in gastric cancer is not clear so far.

Increasing evidence has demonstrated that miRNAs take part in the regulation of cancer pathological processes, especially tumor metastasis [11-13]. To date, several deregulated miRNAs have been demonstrated to be involved in GC cell migration, invasion and metastasis, such as miR-1, miR-181a, miR-449a, miR-223, miR-30 and miR-194 [1, 14-17]. A series of studies have reported that miR-486-5p is frequently decreased in many cancers, including lung cancer, breast cancer and myeloid leukemia of Down Syndrome, and functions as a tumor suppressor by inhibiting tumor cell growth, invasion, metastasis, and tumorigenesis [18-20]. Based on our recent microarray analysis, we discovered that miR-486-5p was significantly downregulated in GC tissues. However, the effect and mechanisms of miR-486-5p deregulation involved in GC development and progression remain unknown. Therefore, in this study, we aim to investigate the roles and functions of miR-486-5p in gastric carcinogenesis.

Materials and methods

Cell culture

The gastric cancer cell line SGC-7901 was purchased from ATCC (Manassas, VA, USA) and maintained in RPMI-1640 (Hyclone Waltham, MA, USA) supplemented with 10% fetal bovine serum (Hyclone Waltham, MA, USA) in a humidified atmosphere of 5% CO_2 at 37°C.

Clinical samples

Sixty-five freshly-frozen biopsy GC and adjacent normal epithelium tissue samples (2 cm from the cancer location) were collected from the pathology archives of the Affiliated Hospital of Binzhou Medical University. The protocol of this study was approved by the Institutional Ethical Review Committee of Binzhou Medical University and written informed consent was obtained from each patient for the use of their tissue samples.

Plasmid transfection

SGC-7901 cells were transfected using Lipofectamine 2000 (Invitrogen, CA, USA) the day after cell seeding in accordance with the manufacturer's instructions. The miR-486-5p overexpression vector (GenePharma, Shanghai, China) and its control mimic (GenePharma, Shanghai, China) were used at a final concentration of 500 ng/μl. After 72 h post -transfection, quantitative real-time polymerase chain reaction (qRT-PCR) were used to verify transfection efficiency.

RNA isolation and quantitative RT-PCR

Total RNA was isolated from GC cell lines and clinical samples using TRizol reagent (Invitrogen, CA, USA). The isolated RNA was reversely transcribed and amplified using the TaqMan miRNA assays RT-PCR kit (TaKaRa, Tokyo, Japan) according to the manufacturer's protocol. Quantitative real-time PCR was performed by using an Applied RT-3000 real-time PCR System. the primers for miR-486-5p and U6 internal control were synthesized by RiboBio company (RiboBio, Guangzhou, China). The mRNA expression levels were determined using the $2^{-\Delta\Delta Ct}$ method.

Luciferase reporter assay

SGC-7901 cells were seeded in 24-well tissue culture plates the day before transfection. The cells were then co-transfected with pMIR-GLO-Vector (GenePharma, Shanghai, China) and miR-486-5p mimics (GenePharma, Shanghai, China) or non-target control mimics. After 72 h of transfection, the lysates were harvested and the luciferase activities were measured using the Dual Luciferase Reporter Assay kit (BeyotimeBiotechnology, Beijing, China).

Tumorigenicity assay in nude mice

Female BALB/c nude mice aging 4 to 6 weeks were purchased from HFK BIOSCIENCE CO.LTD (Beijing, China). 1×10^7 SGC-7901 cells were suspended in 200 μl RPMI-1640, and then injected subcutaneously into the dorsal sites of the nude mice. Tumor formation was monitored about seven days. Some mice were peritumoral injected of miR-486-5p overexpression mimics every 3 days for 2 weeks. PBS or mocks were injected into some animals as negative controls. The tumor volume (mm^3) was measured every 3 days and was calculated using the following formula: volume = $0.5\times L\times W^2$ (in millimeters), where L represented the length of

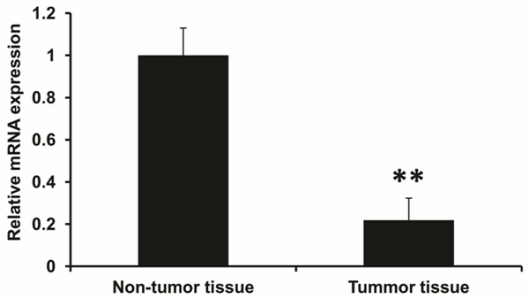

Figure 1. miR-486-5p expression in gastric cancer. Relative expression of miR-486-5p in 65 gastric cancer tissues compared with their pair-matched adjacent non-tumor tissues. U6 snRNA was used as the endogenous control. The data represented at least three independent experiments and were shown as the MEAN±SD. **P<0.01.

the tumor and W represented the width of the tumor. The mice were sacrificed and the tumors were collected after 3 weeks, half of which was fixed in 4% formalin and embedded in formaldehyde and the remainder was snap-frozen in liquid nitrogen for histological studies.

Western blotting

Total protein was extracted and quantified with Pierce BCA Protein Assay Kit (Thermo, Waltham, MA USA). Protein samples were separated with 6% SDS-PAGE and transferred to PVDF membranes (Beyotime Biotechnology, Beijing, China). Then, membranes were blocked with 7% fat-free milk, and incubated with rabbit Polyclonal anti-NRP2 antibody (1:1000; Abcam, Cambridge, MA, USA) followed by secondary antibody (Santa Cruz, CA, USA). The signals were determined using an enhanced chemiluminescence, and the anti-GAPDH antibody (Santa Cruz, USA) was used as a loading control.

Immunohistochemistry

Xenograft were fixed for 24 h at 4°C in 4% formaldehyde, dehydrated and embedded in paraffin, sectioned (thickness, 4 um) for immunohistochemical analysis of NRP2 and lymphatic microvessel density (LMD). LYVE-1 (abcam , Cambridge, MA, USA) was used to monitor the numbers of lymphatic microvessels in the tumors. Briefly, slides were washed and incubated with 3.0% H_2O_2 for 20 min to quench endogenous peroxid activities and then were blocked with 1% bovine serum albumin in PBS for 40 min at 37°C. A monoclonal antibody against NRP2 (1:200) and LYVE-1 (1:100) were used as the primary antibody for detecting protein expression. Immunodetection was performed by incubation with a specific biotinylated secondary antibody followed by use of the kit (ZSGB-BIO, Beijing, China). Slides were examined under a light microscope (Olympus, Tokyo, Japan) with a 200 magnification.

Statistical analysis

All data are presented as mean ± SD. Statistical significance of the data was using the Statistical Package of SPSS version 13.0 for Windows (SPSS Inc, IL, USA). Differences between groups were considered statistically significant at p values <0.05.

Results

Expression of miR-486-5p is decreased in gastric cancer tissues

To investigate the role of miR-486-5p in gastric cancers, miR-486-5p expression in the GC patients' samples and adjacent normal tissues were first evaluated. Compared to the normal adjacent tissues, we found that miR-486-5p expression was obviously reduced in GC tissues (P<0.01) (**Figure 1**).

NRP2 is a target of miR-486-5p

The function of miRNA primarily relies on its target genes. To investigate whether NRP2 is a target of miR-486-5p in gastric cancer cells, the SGC-7901 cells were transfected with miR-486-5p expressing plasmid (the vector only as negative control). The transfection efficiency was verified by RT-PCR (**Figure 2A**). At 72 h post-transfection, the expression of NRP2 was determined by western blotting. The results showed that NRP2 significantly decreased in the miR-486-5p overexpression group (**Figure 2B**). To assess whether NRP2 was a direct target of miR-486-5p, the cells were co-transfected with NRP2 mRNA 3'-UTR vector and miR-486-5p expressing plasmid (vector only as the negative control). After 72 h of transfection, the lysates were harvested and the luciferase activities were measured. We found that transfection of vector-miR-486-5p significantly suppressed the luciferase activity of the vector-NRP2 (**Figure 2C**, **2D**). Taken together, these

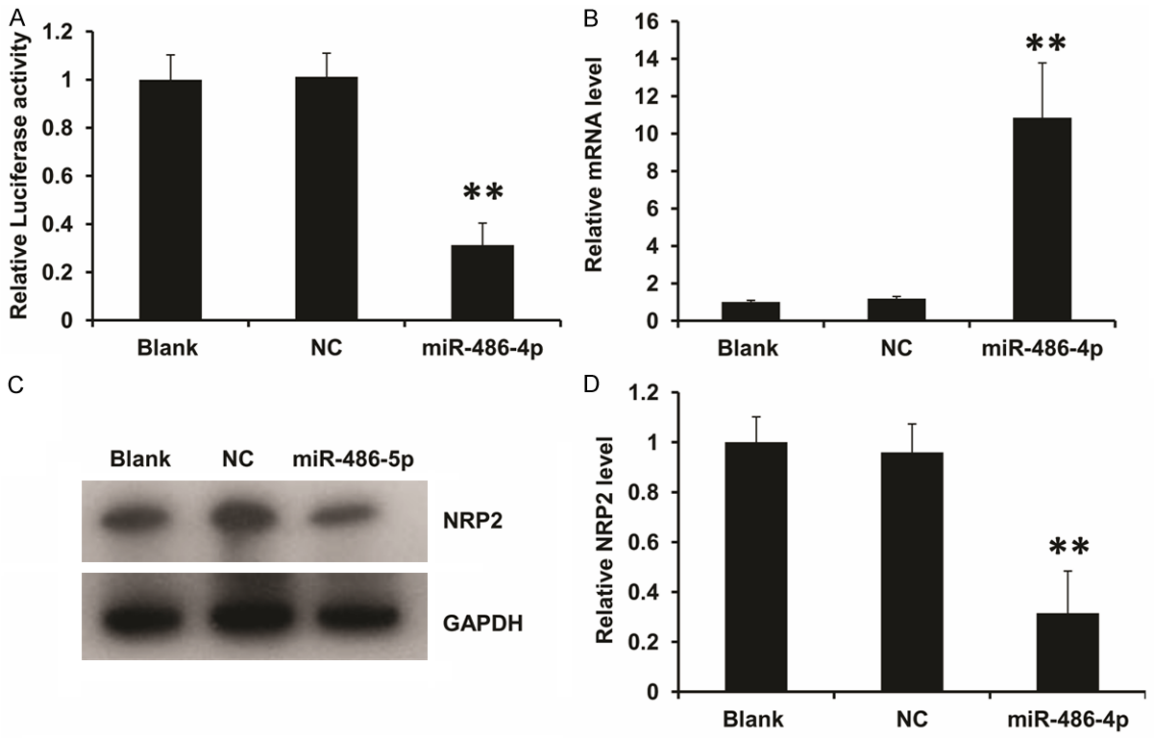

Figure 2. NRP2 was a target of miR-486-5p. A. Effect of miR-486-5p on NRP2 mRNA transcriptional activity in SGC-7901 was determined by a lucif-reporter assay. The data were normalized by determining the rat firefly and Renilla luciferase activities measured at 72 h post-transfection. B. Expression of miR-486-5p in SGC-7901 cells transfected with miR-486-5p mimics were detected by qRT-PCR. C. NRP2 protein level was significantly decreased in miR-486-5p-transfected cells. D. Quantitative analysis of the NRP2 protein level using a gray scan analysis. The data represented at least three independent experiments and were shown as the MEAN ± SD. **P<0.01.

findings demonstrated that miR-486-5p could negatively regulate the expression of NRP2 by directly targeting the NRP2 mRNA 3'UTR.

MiR-486-5p suppresses gastric tumor growth in vivo

To explore the function of miR-486-5p in gastric cancer cell proliferation, a SGC-7901 cell line stably overexpressing miR-486-5p was established using plasmid transfection, and then used to conduct an experimental xenograft through injecting it into the dorsal sites of nude mice. Three weeks later, the mice were sacrificed and the size and mass of xenograft was quantified. The total weight and size of tumors generated from the miR-486-5p expressing SGC-7901 cell were significantly lower that tumors generated from the SGC-7901 cells transfected with vector control. (**Figure 3A**, **3B**).

To determine whether miR-486-5p affects gastric cancer cell proliferation by targeting NRP2 in vivo, we investigated the NRP2 expression level in the tumor cells. NRP2 expression was significantly decreased in miR-486-5p-transfected tumors compared with its levels in the control group (**Figure 3C**, **3D**). These results suggested that overexpression of miR-486-5p inhibited tumor growth by targeting NRP2 in vivo.

MiR-486-5p suppresses gastric tumor lymphangiogenesis via targeting NRP2 in vivo

To determine the in vivo significance of our in vitro observations, NRP2 expression levels of xenograft derived from miR-486-5p overexpression SGC-7901 cells were significantly lower than those from the xenograft derived from blank and negative control SW620 cells (**Figure 4A**). Correspondingly, NRP2 expression levels were found to be positively correlated with the lymphatic vessel density of the xenograft (**Figure 4B**, **4C**). These findings suggested miR-486-5p could attenuate the lymphangiogenesis of GC in vivo.

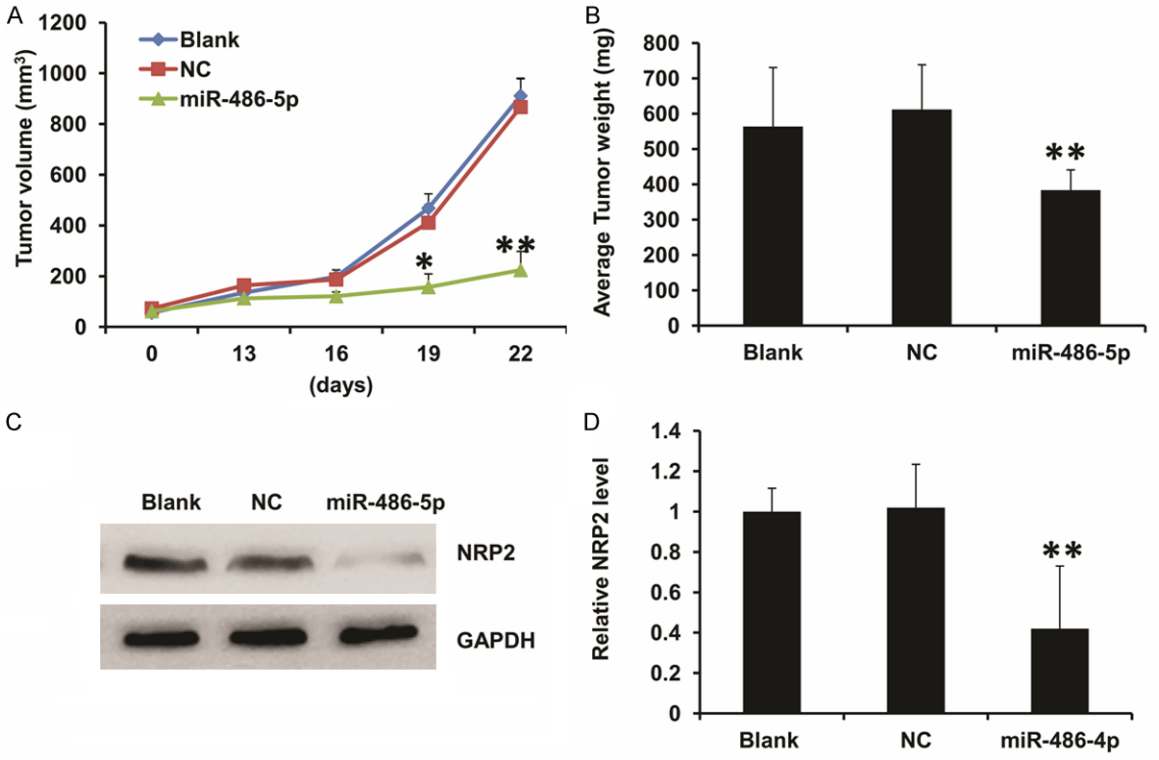

Figure 3. miR-486-5p inhibited xenograft tumor growth by targeting NRP2 in vivo. A. Groups of nude mice were implanted subcutaneously with SGC-7901/PBS cells, SGC-7901/mimic-control cells or SGC-7901/miR-486-5p cells, Tumor volumes were recorded at the indicated times. B. The tumor mass was determined when the mice were sacrificed. C. NRP2 expression in tumors was examined by Western blot. D. Quantitative analysis of the NRP2 protein level using a gray scan analysis. The data represented at least three independent experiments and were shown as the MEAN ± SD. *P<0.05, **P<0.01.

Discussion

miRNAs are a class of small non-coding RNAs (approximately 22 nt) that bind to multiple target mRNAs to control gene expression post-transcriptionally by inhibiting translation [21]. Recently, miRNAs are increasingly become hotspots for their multiple biological functions. A single miRNA can influence the expression of hundreds of target genes, and miRNAs have been reported as key regulatory molecules in various diseases, including cancer [22]. Among them, reduced miR-486-5p expression is a frequent molecular event in human malignances. Furthermore, Evidence showed that miR-486-5p overexpression inhibited progression and metastasis via targeting protumorigenic ARHGAP5 in lung cancer [18]. Moreover, miR-486-5p expression in gastric carcinoma is relation to clinicopathological features and prognosis [23]. However, its relation to progression and metastasis in gastric cancer are unclear. In this study, we first demonstrated that miR-486-5p expression was lower in GC tissues compared with adjacent normal tissues by qRT-PCR, and Down-regulation of miR-486-5p promoted lymphatic metastasis.

Neuropilin-2 is a co-receptor for vascular endothelial growth factor-D (VEGF-D) that is expressed on the surface of endothelial cells [10]. Recently, Nrp2 was shown to play a role in breast cancer cells migration as well as in the induction of tumor growth and invasion of pancreatic adenocarcinoma cells [24, 25]. Moreover, Neuropilin-2 (NRP2) as a cell surface receptor involved in tumor-associated angiogenesis and lymphangiogenesis, has recently been shown to be expressed in melanoma [26]. Juan-juan Ou et al also indicated that NRP2 activation can promotes colorectal cancer lymphangiogenesis via activating integrin α9β1/FAK/Erk signaling independent of VEGF-C/VEGFR3 pathway [18]. However, the relationship between NRP2 and gastric cancer tumor growth and lymphangiogenesis remain unclari-

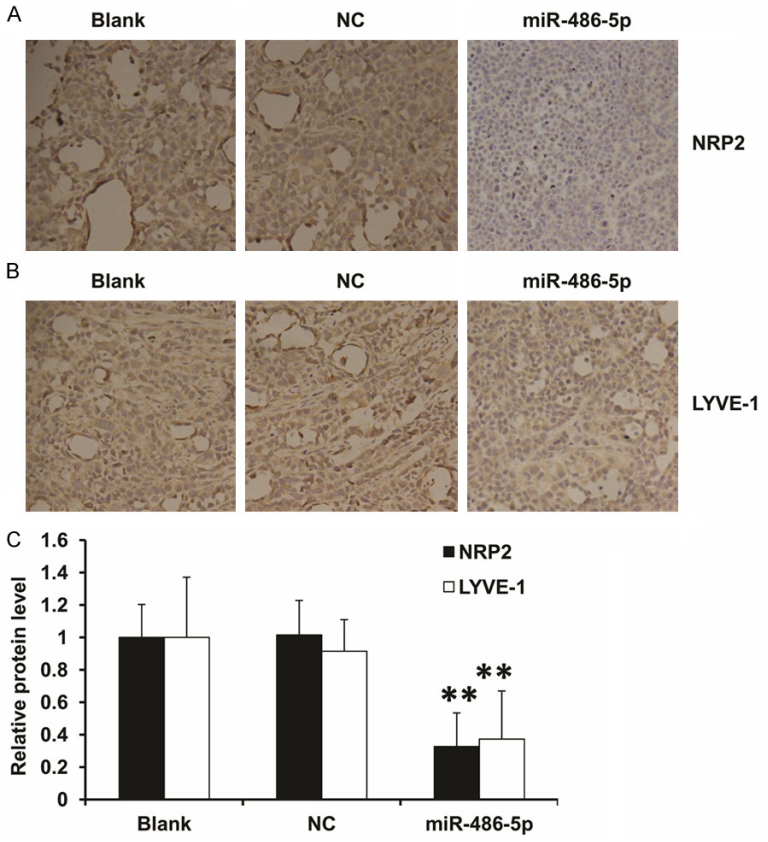

Figure 4. miR-486-5p suppressed NRP2 expression and lymphangiogenesis in vivo. (A) NRP2 (magnification, x400) and (B) LYVE-1 (magnification, x400) expressions in the xenograft tissues generated from the wild type SGC-7901 cells (blank), or the SGC-7901 cells transfected with non-target control (NC) and miR-486-5p, respectively. (C) Representative Immunohistochemistry staining and (D) statistical analysis of NRP2 and LYVE-1 positive lymphatic vessels in the xenografts derived from miR-486-5p overexpression SGC-7901 and control cells. The data represented at least three independent experiments and were shown as the MEAN ± SD. **P<0.01.

fied. In our study, we first suggested that NRP2 expression levels are directly targeted by the miR-486-5p in gastric cancer cells. Then, our study for the first time revealed miR-486-5p expression levels are significantly correlated with the expression level of NRP2 in vitro and vivo. Most importantly, the density of lymphatic microvessels and tumor mass of xenografts derived from miR-486-5p overexpression SGC-7901 cells were significantly lower than those from the xenografts derived from control SGC-7901 cells. Our findings for the first time revealed that miR-486-5p mediates GC angiogenesis and lymphangiogenesis via regulating NRP2 manner.

In summary, our present study showed that miR-486-5p expression was decrease in GC tissues. We have identified that miR-486-5p attenuated the proliferation and lymphangiogenesis of GC cells in vivo by directly targeting NRP2. So, miR-486-5p may provide a potential therapeutic target of metastatic GC in the future. We hope that our investigation can facilitate further exploration of the molecular mechanisms of miR-486-5p in GC.

Acknowledgements

This work was partly supported by the Natural Science Foundation of Shandong Province (No. ZR2014HQ072; ZR2015LH083); the Science and Technology Development Plan of Medicine and Health Care in Shandong Province (No. 2014WS0490); Science and Technology Development Plans of BinZhou (No. 2014-ZC0116).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Chengxia Liu, Department of Gastroenterology, The Affiliated Hospital of Binzhou Medical University, 661 Huanghe 2nd Rd, Binzhou 256603, Shandong, China. Tel: +86-13854309199; E-mail: phdlcx@163.com@163.com

References

[1] Han C, Zhou Y, An Q, Li F, Li D, Zhang X, Yu Z, Zheng L, Duan Z and Kan Q. MicroRNA-1 (miR-1) inhibits gastric cancer cell proliferation and migration by targeting MET. Tumour Biol 2015; 36: 6715-6723.

[2] Chen W, Zheng R, Zhang S, Zhao P, Li G, Wu L and He J. Report of incidence and mortality in China cancer registries, 2009. Chin J Cancer Res 2013; 25: 10-21.

[3] Bang YJ, Kim YW, Yang HK, Chung HC, Park YK, Lee KH, Lee KW, Kim YH, Noh SI, Cho JY, Mok YJ, Ji J, Yeh TS, Button P, Sirzen F and Noh SH.

Adjuvant capecitabine and oxaliplatin for gastric cancer after D2 gastrectomy (CLASSIC): a phase 3 open-label, randomised controlled trial. Lancet 2012; 379: 315-321.
[4] Gupta GP and Massague J. Cancer metastasis: building a framework. Cell 2006; 127: 679-695.
[5] Liu C, Lu P, Lu Y, Xu H, Wang S and Chen J. Clinical implications of metastatic lymph node ratio in gastric cancer. BMC Cancer 2007; 7: 200.
[6] Go Y, Tanaka H, Tokumoto M, Sakurai K, Toyokawa T, Kubo N, Muguruma K, Maeda K, Ohira M and Hirakawa K. Tumor-Associated Macrophages Extend Along Lymphatic Flow in the Pre-metastatic Lymph Nodes of Human Gastric Cancer. Ann Surg Oncol 2016; 23 Suppl 2: 230-5.
[7] Chen H, Guan R, Lei Y, Chen J, Ge Q, Zhang X, Dou R, Liu H, Qi X, Zhou X and Chen C. Lymphangiogenesis in gastric cancer regulated through Akt/mTOR-VEGF-C/VEGF-D axis. BMC Cancer 2015; 15: 103.
[8] Yasuoka H, Kodama R, Tsujimoto M, Yoshidome K, Akamatsu H, Nakahara M, Inagaki M, Sanke T and Nakamura Y. Neuropilin-2 expression in breast cancer: correlation with lymph node metastasis, poor prognosis, and regulation of CXCR4 expression. BMC Cancer 2009; 9: 220.
[9] Ou JJ, Wei X, Peng Y, Zha L, Zhou RB, Shi H, Zhou Q and Liang HJ. Neuropilin-2 mediates lymphangiogenesis of colorectal carcinoma via a VEGFC/VEGFR3 independent signaling. Cancer Lett 2015; 358: 200-209.
[10] Yasuoka H, Kodama R, Hirokawa M, Takamura Y, Miyauchi A, Inagaki M, Sanke T and Nakamura Y. Neuropilin-2 expression in papillary thyroid carcinoma: correlation with VEGF-D expression, lymph node metastasis, and VEGF-D-induced aggressive cancer cell phenotype. J Clin Endocrinol Metab 2011; 96: E1857-1861.
[11] Ma L, Teruya-Feldstein J and Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature 2007; 449: 682-688.
[12] Valastyan S, Reinhardt F, Benaich N, Calogrias D, Szasz AM, Wang ZC, Brock JE, Richardson AL and Weinberg RA. A pleiotropically acting microRNA, miR-31, inhibits breast cancer metastasis. Cell 2009; 137: 1032-1046.
[13] Tavazoie SF, Alarcon C, Oskarsson T, Padua D, Wang Q, Bos PD, Gerald WL and Massague J. Endogenous human microRNAs that suppress breast cancer metastasis. Nature 2008; 451: 147-152.
[14] Lin F, Li Y, Yan S, Liu S, Qian W, Shen D, Lin Q and Mao W. MicroRNA-181a inhibits tumor proliferation, invasiveness, and metastasis and is downregulated in gastric cancer. Oncol Res 2015; 22: 75-84.
[15] Li X, Li H, Zhang R and Liu J. MicroRNA-449a inhibits proliferation and induces apoptosis by directly repressing E2F3 in gastric cancer. Cell Physiol Biochem 2015; 35: 2033-2042.
[16] Zhou X, Jin W, Jia H, Yan J and Zhang G. MiR-223 promotes the cisplatin resistance of human gastric cancer cells via regulating cell cycle by targeting FBXW7. J Exp Clin Cancer Res 2015; 34: 28.
[17] Sousa JF, Nam KT, Petersen CP, Lee HJ, Yang HK, Kim WH and Goldenring JR. miR-30-HNF4gamma and miR-194-NR2F2 regulatory networks contribute to the upregulation of metaplasia markers in the stomach. Gut 2015; [Epub ahead of print].
[18] Wang J, Tian X, Han R, Zhang X, Wang X, Shen H, Xue L, Liu Y, Yan X, Shen J, Mannoor K, Deepak J, Donahue JM, Stass SA, Xing L and Jiang F. Downregulation of miR-486-5p contributes to tumor progression and metastasis by targeting protumorigenic ARHGAP5 in lung cancer. Oncogene 2014; 33: 1181-1189.
[19] Zhang G, Liu Z, Cui G, Wang X and Yang Z. MicroRNA-486-5p targeting PIM-1 suppresses cell proliferation in breast cancer cells. Tumour Biol 2014; 35: 11137-11145.
[20] Shaham L, Vendramini E, Ge Y, Goren Y, Birger Y, Tijssen MR, McNulty M, Geron I, Schwartzman O, Goldberg L, Chou ST, Pitman H, Weiss MJ, Michaeli S, Sredni B, Gottgens B, Crispino JD, Taub JW and Izraeli S. MicroRNA-486-5p is an erythroid oncomiR of the myeloid leukemias of Down syndrome. Blood 2015; 125: 1292-1301.
[21] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
[22] van Kouwenhove M, Kedde M and Agami R. MicroRNA regulation by RNA-binding proteins and its implications for cancer. Nat Rev Cancer 2011; 11: 644-656.
[23] Chen H, Ren C, Han C, Wang D, Chen Y and Fu D. Expression and prognostic value of miR-486-5p in patients with gastric adenocarcinoma. PLoS One 2015; 10: e0119384.
[24] Timoshenko AV, Rastogi S and Lala PK. Migration-promoting role of VEGF-C and VEGF-C binding receptors in human breast cancer cells. Br J Cancer 2007; 97: 1090-1098.
[25] Dallas NA, Gray MJ, Xia L, Fan F, van Buren G 2nd, Gaur P, Samuel S, Lim SJ, Arumugam T, Ramachandran V, Wang H and Ellis LM. Neuropilin-2-mediated tumor growth and angiogenesis in pancreatic adenocarcinoma. Clin Cancer Res 2008; 14: 8052-8060.
[26] Rossi M, Tuck J, Kim OJ, Panova I, Symanowski JT, Mahalingam M, Riker AI, Alani RM and Ryu B. Neuropilin-2 gene expression correlates with malignant progression in cutaneous melanoma. Br J Dermatol 2014; 171: 403-408.