MicroRNA-488 inhibits ovarian cancer cell metastasis through regulating CCNG1 and p53 expression

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Abstract. – **OBJECTIVE:** The roles of microRNAs (miRNAs) have been widely exploited in cancer. MiRNAs have become a potential breakthrough in cancer diagnosis and treatment. Here, the regulatory mechanism of microRNA-488 (miR-488) was investigated in ovarian cancer (OC).

PATIENTS AND METHODS: The expression levels of miR-488 and CCNG1 (Cyclin G1) were detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) and Western blot assays. Transwell assay and epithelial-mesenchymal transition (EMT) markers were used to clarify the effect of miR-488 on cell metastasis. The dual-luciferase reporter assay was used to verify the relation between miR-488 and CCNG1.

RESULTS: The expression of miR-488 was reduced in OC, which was associated with poor clinical outcomes and prognosis in OC patients. MiR-488 inhibited cell metastasis in OC by blocking EMT and promoting tumor suppressor p53 expression. In addition, CCNG1 was confirmed as a direct target of miR-488. Upregulation of CCNG1 impaired the inhibitory effect of miR-488 in OC.

CONCLUSIONS: MiR-488 serves as a tumor inhibitor in OC by suppressing cell metastasis, indicating that miR-488 has a great potential in the diagnosis and treatment of OC.

Key Words:
   Ovarian cancer, MiR-488, CCNG1, p53, Cell metastasis.

Introduction

Ovarian cancer (OC) accounts for 2.4% to 6.5% of common malignant tumors in women, ranking third in female reproductive system carcinomas, followed by cervical cancer and endometrial cancer[1]. Epithelial cancer is the most common in OC and its mortality is the first in various gynecological tumors, posing a serious threat to women's lives[2]. The reason for the high mortality rate is that the growth site of OC is concealed and it is difficult to find it early[3]. In addition, the symptoms of early OC are not significant, and there is still a lack of simple and practical diagnostic methods[4]. Most OC patients have advanced in the first diagnosis, with a 5-year survival rate of approximately 25-50%. Cell metastasis is the main cause of poor prognosis in patients with OC[5]. There is an urgent need to investigate the molecular mechanism of cell metastasis to find effective biomarkers for early diagnosis of OC patients.

As a research hotspot, microRNA (miRNA) has been widely explored in the pathogenesis of human cancer. MiRNAs play an important role in gene regulation and tumorigenesis[6]. Some miRNAs have been reported to have the potential to diagnose and treat OC. Of note, miR-423 served as a diagnostic indicator and inhibited the proliferation and invasion of OC cells[7]. MiR-532 was a prognostic marker that suppressed cell proliferation and invasion by targeting TWIST1 in epithelial OC[8]. In contrast, miR-590 was found to promote tumor growth and metastasis in OC *via* the novel FOXA2-versican pathway[9]. Among the numerous miRNAs, an abnormal regulation of miR-488 has been found in various cancers and it is actively involved in the pathogenesis of human cancers. Downregulation of miR-488 has been identified in glioma, malignant melanoma, and prostate carcinoma[10-12]. Moreover, it was reported[13] that a low miR-488 expression was associated with tumor node metastasis (TNM) stage in patients with gastric cancer and acted as a tumor suppressor. However, aberrant expression and biological functions of miR-488 have not been reported in OC, which still needs to be investigated.

Cyclin G1 (CCNG1) has been identified as a new member of the cyclin family that shares

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homology with c-src[14]. CCNG1 has contrast tissue specificity and can regulate cell cycle[15]. In addition, CCNG1 was reported to be upregulated in nasopharyngeal carcinoma and was correlated with tumor progression[16]. Besides that, CCNG1 is a transcriptional target of p53 tumor suppressor protein and negatively regulates p53 expression[17]. It was found that CCNG1 induced expansion of liver tumor-initiating cells by Sox2 *via* protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling[18]. Moreover, miR-122/CCNG1 interaction has been identified to modulate p53 activity in hepatocellular carcinoma cells[19]. In particular, miR-1271 inhibited OC tumor growth by targeting CCNG1[20]. However, as far as we know, the interaction between CCNG1 and miR-488 in OC has not been elucidated.

Therefore, the relation between miR-488 and CCNG1 and their effect on OC cell metastasis were investigated. At the same time, the association between miR-488 and clinical outcomes or prognosis in OC patients was analyzed. In addition, it was explored how miR-488 regulates p53 expression. Our results will provide new ideas for the therapeutic targets of human OC.

Patients and Methods

Clinical Tissues

Experiment tissues were obtained from 58 patients with OC at the Beijing Jishuitan Hospital. All OC patients enrolled in this research did not receive any other treatment prior to surgery. Before the study began, participants provided written informed consent and the Human Ethics Committee of Beijing Jishuitan Hospital approved the investigation.

Cell Culture and Transfection

Human OC cell lines A2780, OVCAR3, SKOV3, and normal human ovarian epithelium IOSE80 cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). These cells were incubated at 37°C with 5% CO_2 in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (HyClone; South Logan, UT, USA) with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA). Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was used to transfer miR-488 mimics or inhibitors, CCNG1 plasmid and unexpressed sequence (NC, Generay Biotech, Shanghai, China) to SKOV3 cells, respectively.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The total RNA was extracted from tissue samples or cell lines using TRIzol reagent (Jining Shiye, Shanghai, China). The complementary deoxyribose nucleic acid (cDNA) was obtained using the iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA, USA). The temperature conditions of the reverse transcription were as follows: 37°C for 15 min and 85°C for 5 sec. The mixture of the qRT-PCR standard reaction system was placed on ABI7300 real-time PCR machine (Applied Biosystems, Foster City, CA, USA) using the ABI Power SYBR Green PCR Master Mix (Unique, Beijing, China). The thermocycling conditions for PCR amplification were as follows: 5 min at 95°C, followed by 38 cycles of 95°C for 30 sec, and 60°C for 45 sec. U6 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as controls for miR-488 and CCNG1, which were quantified by the $2^{-\Delta\Delta ct}$ method. The primers used in our work were as follows: miR-488, forward primer: 5'-TTATTGCGATGTGTTCCT-TATG-3', reverse primer: 5'-ATGCATGCCAC-GGGCATATACACT-3'; U6, forward primer: 5'-CTCGCTTCGGCAGCACA-3', reverse primer: 5'-AACGCTTCACGAATTTGCGT-3'; CCNG1 forward primer: 5'-GTTACCGCTGAG-GAGCTGCAGTC-3', reverse primer: 5'-GCAG-CCATCCTGGATGGATTCAG-3'; GAPDH forward, 5'-ACATCGCTCAGACACCATG-3', reverse, 5'-TGTAGTTGAGGTCAATGAAGGG-3'.

Transwell Assay

The upper transwell chamber (8-μm pore size membranes) surface of the bottom membrane was coated with Matrigel (BD, Franklin Lakes, NJ, USA). 3×10^4 SKOV3 cells were added to the transwell chamber. Then, a medium containing 20% FBS (600 μL) was added to the 24-well plate in the lower chamber. After 24 h of routine incubation, the cells were fixed and stained. Fields of x200 magnification of each insert were randomly selected and counted under a light microscope (Olympus, Tokyo, Japan). Transwell cell migration assay was performed without Matrigel and the other procedure was essentially consistent with the transwell invasion assay.

Western Blot Analysis

Radioimmunoprecipitation assay (RIPA) buffer (Beyotime, Shanghai, China) was used to lyse the protein samples. Equal amounts of protein were loaded for 10% sodium dodecyl sulfate-poly-

acrylamide gel electrophoresis (SDS-PAGE) on polyacrylamide gels, followed by transfer to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were blocked in 5% dried skimmed milk for 2 h at room temperature. Next, the protein was incubated with the corresponding primary antibodies overnight at 4°C, including E-cadherin, N-cadherin, Vimentin, p53, CCNG1, and GAPDH. After washing, PVDF membranes were incubated with the diluted secondary antibodies for 1 h at room temperature. Finally, proteins were detected using the enhanced chemiluminescence (ECL) protein detection kit (ECL; Millipore, Billerica, MA, USA). The signals from target proteins were normalized to those of GAPDH.

Dual-Luciferase Reporter Gene Assay

The 3′-untranslated region (3′-UTR) of wild-type or mutant CCNG1 was inserted into the pRL-SV40 *Renilla* plasmid (Promega, Madison, WI, USA). The luciferase vector and miR-488 mimics were then co-transfected into SKOV3 cells for 6 h. After 48 h of routine incubation, the luciferase activity of SKOV3 cells was observed by a dual-luciferase reporter assay system (Promega, Madison, WI, USA).

Statistical Analysis

Data were analyzed by Statistical Product and Service Solutions (SPSS) 13.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6 (GraphPad Software La Jolla, CA, USA). Comparisons between multiple groups were calculated using Chi-squared Test or One-way analysis of variance (ANOVA) test followed by Post-Hoc Test (Least Significant Difference). Survival differences were calculated by Kaplan-Meier analysis with the log-rank test. Data are shown as mean ± SD (standard deviation). When $p<0.05$, the difference is considered significant.

Results

MiR-488 was Downregulated in OC Tissues

The mRNA expression of miR-488 was observed in OC tissues. The qRT-PCR assay showed that miR-488 was downregulated in OC tissues compared to normal tissues (Figure 1A). Furthermore, the imbalance in miR-488 expression was closely related to FIGO stage or lymph node metastasis in OC patients (Table I). In addition, OC patients with low expression of miR-488 had shorter overall survival, suggesting that the downregulation of miR-488 predicts poor prognosis in OC patients (Figure 1B). These results suggest that miR-488 may be involved in OC tumorigenesis and regulate the prognosis of OC patients.

MiR-488 Inhibited Cell Metastasis in OC

The expression levels of miR-488 were examined in A2780, OVCAR3, SKOV3, and IOSE80 cell lines. Expression of miR-488 was significantly lower in A2780, OVCAR3, and SKOV3 cell lines than in IOSE80 cells (Figure 2A). To confirm the role of miR-488 in OC progression, miR-488 mimics or inhibitor was transfected into
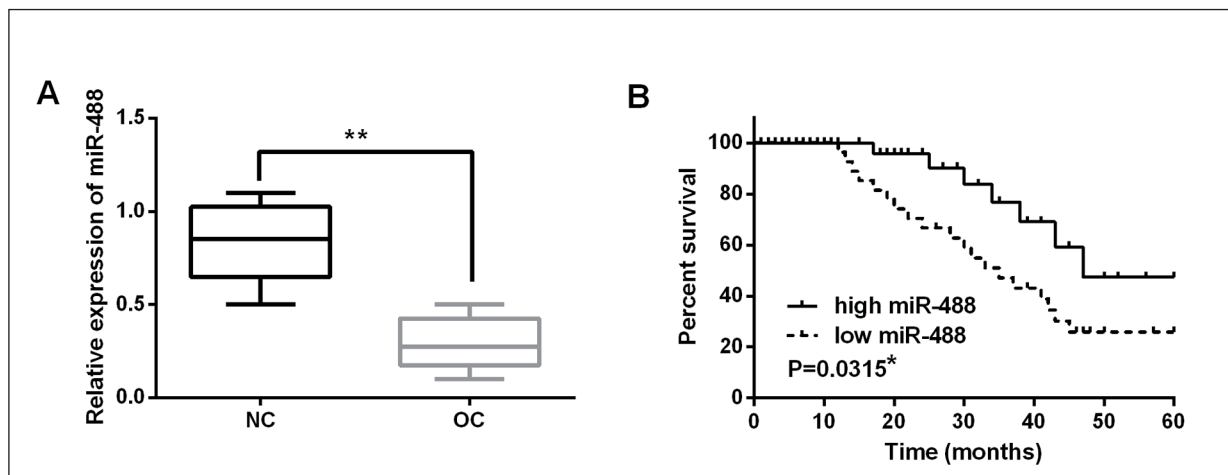

Figure 1. MiR-488 was downregulated in OC tissues. **A,** mRNA expressions of miR-488 were observed in OC tissues. **B,** Low miR-488 expression predicted poor prognosis in OC patients. *$p<0.05$, **$p<0.01$.

Table I. The relationship between miR-488 expression and clinic-pathological characteristics of OC patients.

Characteristics	Cases (n = 58)	MiR-488 High (n = 18)	MiR-488 Low (n = 40)	*p*-value
Age (years)				0.125
≥ 60	27	9	18	
< 60	31	9	22	
FIGO stage				0.006*
I+II	35	11	24	
III+IV	23	7	16	
Lymph node metastasis				0.015*
No	38	12	26	
Yes	20	6	14	
Differentiation				0.372
Well	26	7	19	
Moderate-poor	32	11	21	

Statistical analyses were performed by the χ^2-test. *$p < 0.05$ was considered significant.
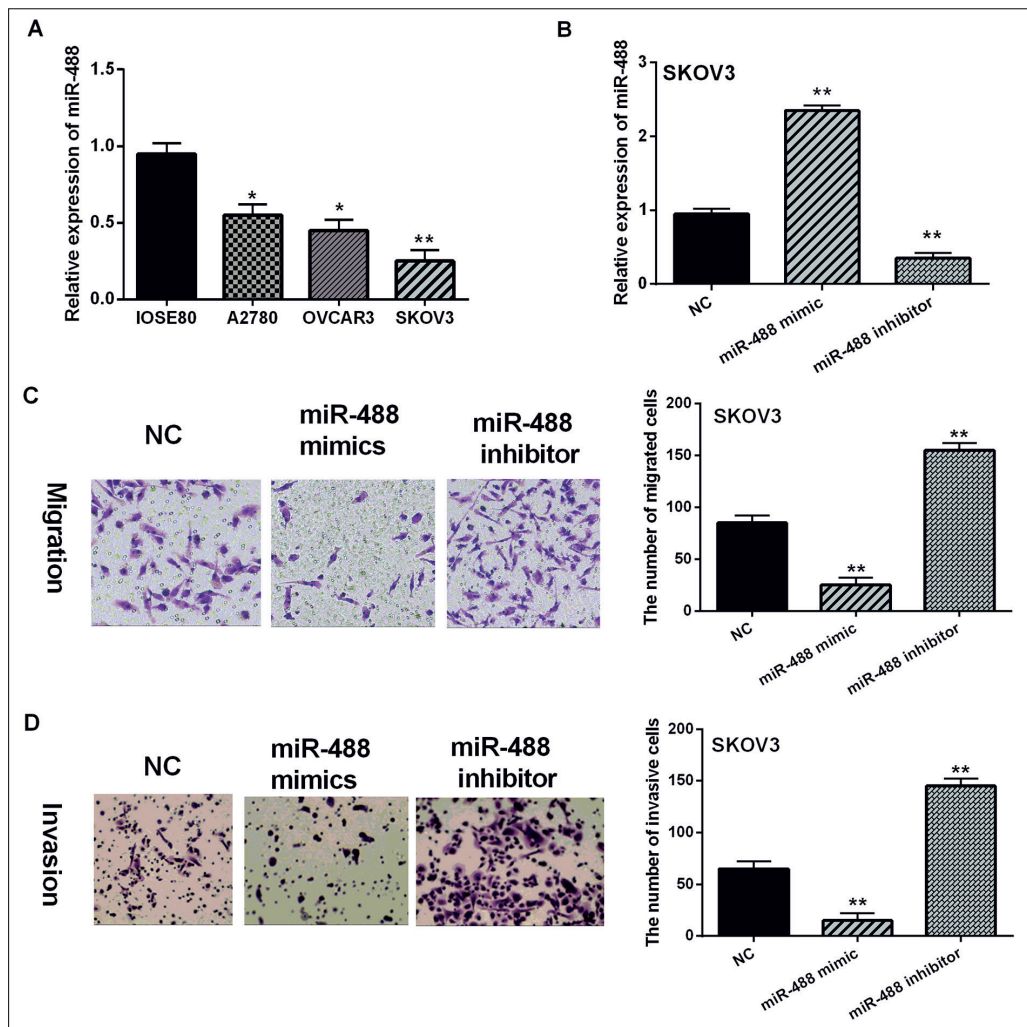

Figure 2. MiR-488 inhibited cell metastasis in OC. **A,** Expression of miR-488 was observed in A2780, OVCAR3, SKOV3 and IOSE80 cell lines. **B,** MiR-488 mimics or inhibitor regulated its expression in SKOV3 cells. **C, D,** Cell migration and invasion were regulated by miR-488 mimics or inhibitor in SKOV3 cells (magnification × 200). *$p<0.05$, **$p<0.01$.

SKOV3 cells. The expression of miR-488 was promoted by its mimics but was suppressed by its inhibitor (Figure 2B). Functionally, the transwell assay showed that miR-488 mimic inhibited SKOV3 cell migration. In contrast, miR-488 inhibitor promoted migration of SKOV3 cells (Figure 2C). Furthermore, the overexpression of miR-488 suppressed cell invasion in SKOV3 cells, while the knockdown of miR-488 promoted cell invasion (Figure 2D). Taken together, miR-488 has an inhibitory effect on cell migration and invasion in OC.

MiR-488 Inhibited EMT and Regulated p53 Expression in OC

To further confirm the effect of miR-488 on OC cell metastasis, we investigated how miR-488 regulates EMT. Western blot analysis showed that miR-488 mimic promoted E-cadherin expression and suppressed N-cadherin, and Vimentin expressions in SKOV3 cells (Figure 3). In contrast, miR-488 inhibitor reduced E-cadherin expression and enhanced expression levels of N-cadherin and Vimentin (Figure 3). In addition, p53 has been identified as a tumor suppressor in the previous studies. Here, the effect of miR-488 on p53 expression was also investigated in OC. We found that miR-488 mimics enhanced the protein expression of p53. MiR-488 inhibitor reduced the protein level of p53 (Figure 3). Briefly, miR-488 inhibits cell metastasis by blocking EMT and promoting the p53 expression.
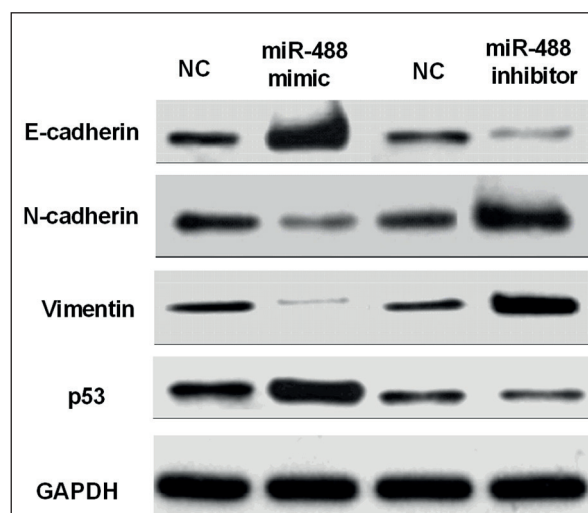

Figure 3. MiR-488 inhibited EMT and regulated p53 expression in OC. The protein expressions of E-cadherin, N-cadherin, Vimentin, and p53 were regulated by miR-488 mimics or inhibitor in SKOV3 cells.

CCNG1 is a Direct Target of MiR-488

The TargetScan (http://www.targetscan.org/) database was used to identify targets for miR-488 to explain its regulatory mechanism in OC. Among these target genes, we selected CCNG1 as a target gene for miR-488 in OC since it has a specific role in human cancers. It predicts that miR-488 has a binding site to the 3'-UTR of CCNG1 (Figure 4A). The luciferase reporter assay indicated that miR-488 mimics reduced the luciferase activity of Wt-CCNG1, but it had no effect on Mut-CCNG1 luciferase activity (Figure 4B). Furthermore, miR-488 was negatively correlated with CCNG1 in OC tissues (R^2=0.9045; Figure 4C). In addition, the CCNG1 expression was assessed in SKOV3 cells with miR-488 mimics or inhibitor. The mRNA and protein expression of CCNG1 was reduced by miR-488 mimics and promoted by miR-488 inhibitor in SKOV3 cells (Figure 4D, 4E). Collectively, miR-488 directly targets CCNG1 and reverse regulates its expression in OC.

Upregulation of CCNG1 Impaired the Inhibitory Effect of MiR-488 in OC

The mRNA expression of CCNG1 was then detected in OC tissues. Upregulation of CCNG1 was identified in OC tissues compared to normal tissues (Figure 5A). Furthermore, the upregulation of CCNG1 was found to be associated with a shorter overall survival in OC patients (Figure 5B). It was concluded that CCNG1 may be involved in OC progression. Next, miR-488 mimics and the CCNG1 vector were co-transfected into SKOV3 cells to elucidate their interaction in OC. We found that the reduction of CCNG1 expression induced by miR-488 mimics was recovered by CCNG1 vector (Figure 5C, 5D). Functionally, the upregulation of CCNG1 attenuated miR-488-mediated inhibition of cell migration and invasion (Figure 5E, 5F). These findings revealed that miR-488 inhibits OC cell metastasis by targeting CCNG1.

Discussion

Many miRNAs can serve as carcinogenic or anti-cancer factors in OC progression[21], suggesting that miRNAs may provide usability for OC prediction. For example, miR-135a was downregulated and served as a tumor suppressor in OC by targeting CCR2. In this work, the downregulation of miR-488 was also identified in OC, and
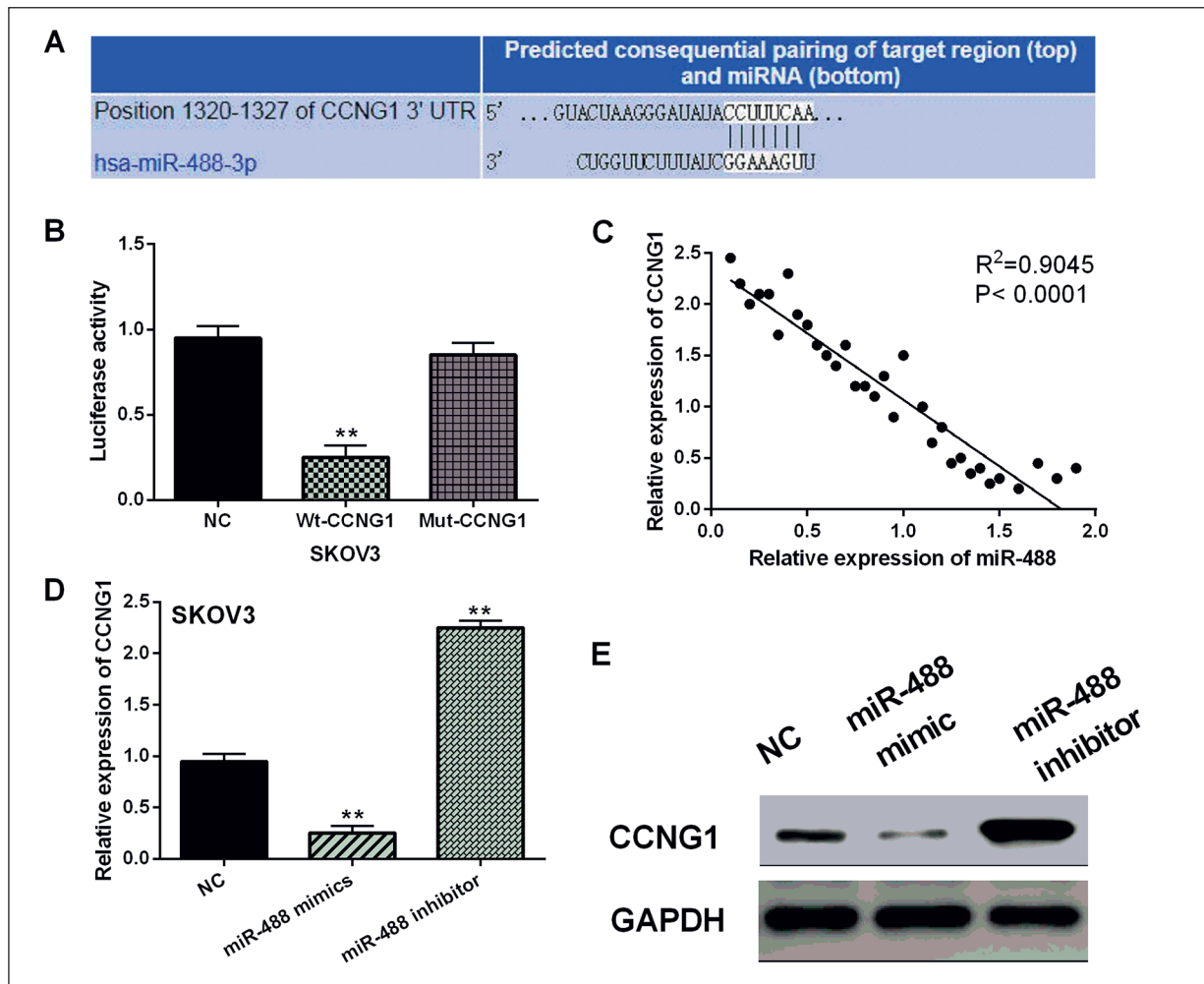
Figure 4. CCNG1 is a direct target of miR-488. **A,** Binding site between CCNG1 and miR-488 **B,** Luciferase reporter assay **C,** MiR-488 was negatively correlated with CCNG1 expression in OC tissues. **D, E,** MiR-488 mimics or inhibitor regulated CCNG1 expression in SKOV3 cells. *******p*<0.01.

miR-488 was an inhibitory miRNA in OC. In addition, it was reported that miR-203 inhibited OC tumor metastasis and EMT by targeting BIRC5[22]. Similarly, the overexpression of miR-488 was also found to suppress cell metastasis and EMT in OC. Besides that, miR-532 was associated with clinic pathological variables and poor prognosis in EOC patients[8]. We also found that miR-488 was associated with FIGO stage, lymph node metastasis, and poor prognosis in patients with OC. As far as we know, we are the first to propose that miR-488 has an inhibitory effect on OC progression by inhibiting cell metastasis.

The same effect of miR-488 has been identified in other malignancies. The downregulation of miR-488 has been found in non-small cell lung cancer and colorectal cancer[23,24]. Function-ally, Shi et al[25] reported that miR-488 inhibited tongue squamous carcinoma cell invasion and EMT *via* directly targeting ATF3. Inhibition of cell migration and invasion induced by miR-488 has also been identified in ductal adenocarcinoma and renal cell carcinoma[26,27]. Notably, Yang et al[28] demonstrated that miR-488 inhibited the chemoresistance of OC cells by targeting Six1 and mitochondrial function. The above findings are consistent with our results and demonstrate the accuracy of our experimental results. It was again indicated that miR-488 is a tumor inhibitor in OC development.

To further elucidate the regulatory mechanism of miR-488 in OC, CCNG1, as a direct target of miR-488, was investigated. The upregulation of CCNG1 was identified in OC tissues, which was
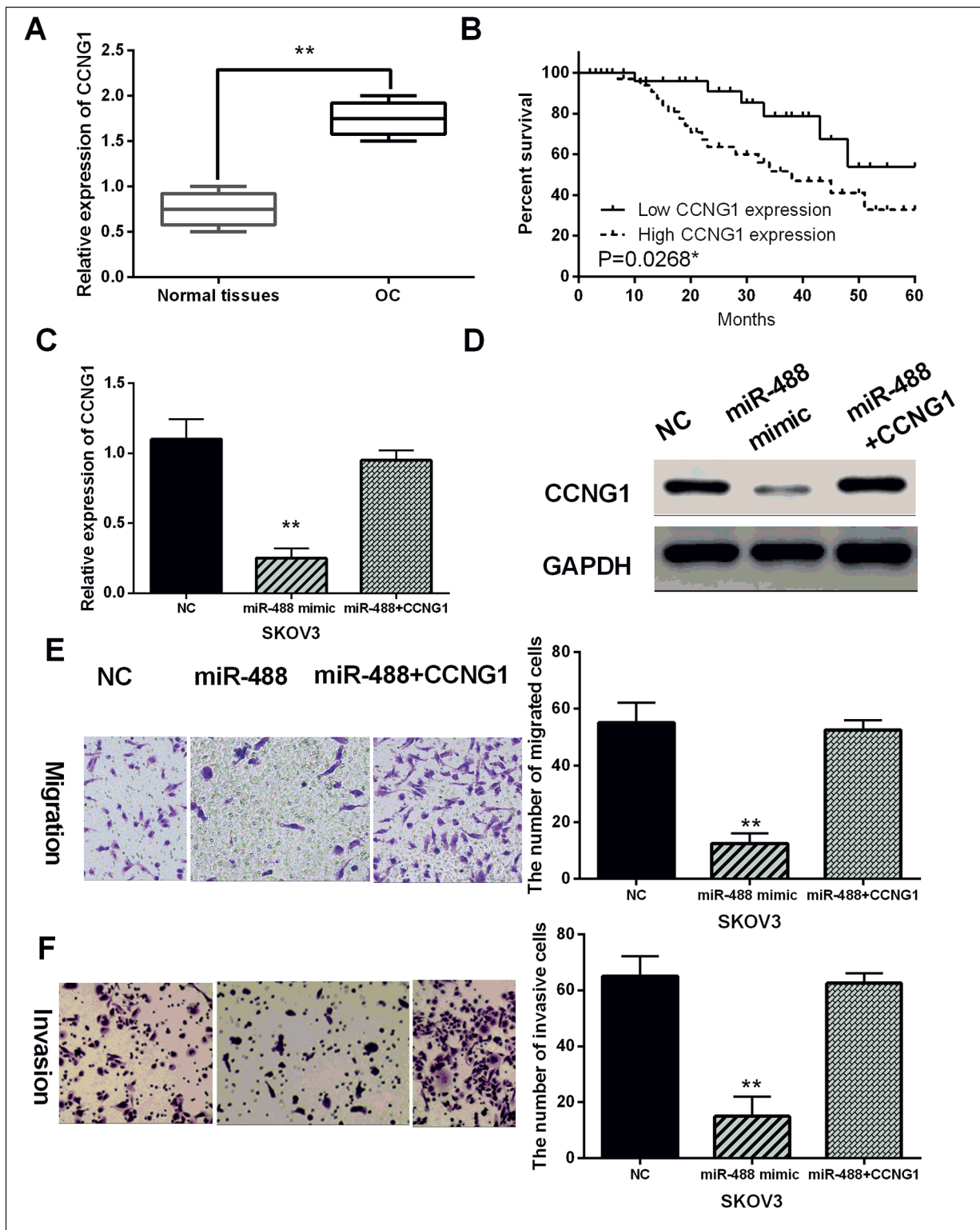
Figure 5. Upregulation of CCNG1 impaired the inhibitory effect of miR-488 in OC. **A,** mRNA expression of CCNG1 was measured in OC tissues. **B,** Upregulation of CCNG1 was associated with shorter overall survival in OC patients. **C, D,** CCNG1 expression was measured in SKOV3 cells with CCNG1 vector and miR-488 mimics. **E, F,** Cell migration and invasion were identified in SKOV3 cells with CCNG1 vector and miR-488 mimics (magnification × 200). *p<0.05, **p<0.01.

correlated with poor prognosis in OC patients. The increased expression of CCNG1 was also detected in cervical carcinoma and lung carcinoma[29,30]. In addition, a negative correlation between miR-488 and CCNG1 expression was observed in OC tissues. Han et al[31] reported that miR-23b directly targets CCNG1 and negatively regulates its expression in lung carcinoma. In this study, the upregulation of CCNG1 was found to impair the inhibitory effect of miR-488 on OC cell metastasis. Similarly, the reversal effect of CCNG1 was also identified in esophageal squamous cell carcinoma[32]. Besides that, Fornari et al[19] showed that miR-122/CCNG1 interaction modulated p53 activity to involve in human hepatocellular carcinoma development. CCNG1 is a p53 downstream effector and their interaction can regulate cell growth[33]. Therefore, we investigated how miR-488 regulates p53 expression. Results suggested that the overexpression of miR-488 promoted p53 expression in OC cells. Furthermore, p53 has been confirmed as a tumor suppressor in many malignant tumors, including OC[34]. Also, Yan et al[35] found that miR-23b targets CCNG1 and suppressed the occurrence and development of OC. Combining these findings, we conclude that miR-488 inhibits OC cell metastasis through the downregulation of CCNG1 and promoting p53 expression.

Conclusions

We showed that the expression of miR-488 was decreased in OC. This downregulation of miR-488 was associated with poor clinical outcomes and prognosis in patients with OC. Functionally, miR-488 inhibited OC cell metastasis by the downregulation of CCNG1 and promoting p53 expression. These findings will help us better understand the pathogenesis of OC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Liu B, Nash J, Runowicz C, Swede H, Stevens R, Li Z. Ovarian cancer immunotherapy: opportunities, progresses and challenges. J Hematol Oncol 2010; 3: 7.

2) Mezzanzanica D. Ovarian cancer: a molecularly insidious disease. Chin J Cancer 2015; 34: 1-3.

3) Chien JR, Aletti G, Bell DA, Keeney GL, Shridhar V, Hartmann LC. Molecular pathogenesis and therapeutic targets in epithelial ovarian cancer. J Cell Biochem 2007; 102: 1117-1129.

4) Chu ZP, Dai J, Jia LG, Li J, Zhang Y, Zhang ZY, Yan P. Increased expression of long noncoding RNA HMMR-AS1 in epithelial ovarian cancer: an independent prognostic factor. Eur Rev Med Pharmacol Sci 2018; 22: 8145-8150.

5) Lee YJ, Chung YS, Lee JY, Nam EJ, Kim SW, Kim S, Kim YT. Impact of increased utilization of neoadjuvant chemotherapy on survival in patients with advanced ovarian cancer: experience from a comprehensive cancer center. J Gynecol Oncol 2018; 29: e63.

6) He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 2004; 5: 522-531.

7) Tang X, Zeng X, Huang Y, Chen S, Lin F, Yang G, Yang N. MiR-423-5p serves as a diagnostic indicator and inhibits the proliferation and invasion of ovarian cancer. Exp Ther Med 2018; 15: 4723-4730.

8) Wei H, Tang QL, Zhang K, Sun JJ, Ding RF. MiR-532-5p is a prognostic marker and suppresses cells proliferation and invasion by targeting TWIST1 in epithelial ovarian cancer. Eur Rev Med Pharmacol Sci 2018; 22: 5842-5850.

9) Salem M, O'Brien JA, Bernaudo S, Shawer H, Ye G, Brkic J, Amleh A, Vanderhyden BC, Refky B, Yang BB, Krylov SN, Peng C. MiR-590-3p promotes ovarian cancer growth and metastasis via a novel FOXA2-versican pathway. Cancer Res 2018; 78: 4175-4190.

10) Xue W, Chen J, Liu X, Gong W, Zheng J, Guo X, Liu Y, Liu L, Ma J, Wang P, Li Z, Xue Y. PVT1 regulates the malignant behaviors of human glioma cells by targeting miR-190a-5p and miR-488-3p. Biochim Biophys Acta Mol Basis Dis 2018; 1864: 1783-1794.

11) Li N, Ma Y, Ma L, Guan Y, Ma L, Yang D. MicroRNA-488-3p sensitizes malignant melanoma cells to cisplatin by targeting PRKDC. Cell Biol Int 2017; 41: 622-629.

12) Sikand K, Slaibi JE, Singh R, Slane SD, Shukla GC. MiR 488* inhibits androgen receptor expression in prostate carcinoma cells. Int J Cancer 2011; 129: 810-819.

13) Zhao Y, Lu G, Ke X, Lu X, Wang X, Li H, Ren M, He S. MiR-488 acts as a tumor suppressor gene in gastric cancer. Tumour Biol 2016; 37: 8691-8698.

14) Tamura K, Kanaoka Y, Jinno S, Nagata A, Ogiso Y, Shimizu K, Hayakawa T, Nojima H, Okayama H. Cyclin G: a new mammalian cyclin with homology to fission yeast Cig1. Oncogene 1993; 8: 2113-2118.

15) Horne MC, Goolsby GL, Donaldson KL, Tran D, Neubauer M, Wahl AF. Cyclin G1 and cyclin G2 comprise a new family of cyclins with contrasting tissue-specific and cell cycle-regulated expression. J Biol Chem 1996; 271: 6050-6061.

16) Ye XX, Liu CB, Chen JY, Tao BH, Zhi-Yi C. The expression of cyclin G in nasopharyngeal carcino-

ma and its significance. Clin Exp Med 2012; 12: 21-24.

17) Окамото K, Beach D. Cyclin G is a transcriptional target of the p53 tumor suppressor protein. EMBO J 1994; 13: 4816-4822.

18) Wen W, Han T, Chen C, Huang L, Sun W, Wang X, Chen SZ, Xiang DM, Tang L, Cao D, Feng GS, Wu MC, Ding J, Wang HY. Cyclin G1 expands liver tumor-initiating cells by Sox2 induction via Akt/mTOR signaling. Mol Cancer Ther 2013; 12: 1796-1804.

19) Fornari F, Gramantieri L, Giovannini C, Veronese A, Ferracin M, Sabbioni S, Calin GA, Grazi GL, Croce CM, Tavolari S, Chieco P, Negrini M, Bolondi L. MiR-122/cyclin G1 interaction modulates p53 activity and affects doxorubicin sensitivity of human hepatocarcinoma cells. Cancer Res 2009; 69: 5761-5767.

20) Liu X, Ma L, Rao Q, Mao Y, Xin Y, Xu H, Li C, Wang X. MiR-1271 inhibits ovarian cancer growth by targeting cyclin G1. Med Sci Monit 2015; 21: 3152-3158.

21) Duan S, Dong X, Hai J, Jiang J, Wang W, Yang J, Zhang W, Chen C. MicroRNA-135a-3p is down-regulated and serves as a tumour suppressor in ovarian cancer by targeting CCR2. Biomed Pharmacother 2018; 107: 712-720.

22) Wang B, Li X, Zhao G, Yan H, Dong P, Watari H, Sims M, Li W, Pfeffer LM, Guo Y, Yue J. MiR-203 inhibits ovarian tumor metastasis by targeting BIRC5 and attenuating the TGFβ pathway. J Exp Clin Cancer Res 2018; 37: 235.

23) Fang C, Chen YX, Wu NY, Yin JY, Li XP, Huang HS, Zhang W, Zhou HH, Liu ZQ. MiR-488 inhibits proliferation and cisplatin sensibility in non-small-cell lung cancer (NSCLC) cells by activating the eIF3a-mediated NER signaling pathway. Sci Rep 2017; 7: 40384.

24) Wang YB, Shi Q, Li G, Zheng JH, Lin J, Qiu W. MicroRNA-488 inhibits progression of colorectal cancer via inhibition of the mitogen-activated protein kinase pathway by targeting claudin-2. Am J Physiol Cell Physiol 2019; 316: C33-C47.

25) Shi B, Yan W, Liu G, Guo Y. MicroRNA-488 inhibits tongue squamous carcinoma cell invasion and EMT by directly targeting ATF3. Cell Mol Biol Lett 2018; 23: 28.

26) Yu DL, Zhang T, Wu K, Li Y, Wang J, Chen J, Li XQ, Peng XG, Wang JN, Tan LG. MicroRNA-448 suppresses metastasis of pancreatic ductal adenocarcinoma through targeting JAK1/STAT3 pathway. Oncol Rep 2017; 38: 1075-1082.

27) Wei X, Yu L, Kong X. MiR-488 inhibits cell growth and metastasis in renal cell carcinoma by targeting HMGN5. Onco Targets Ther 2018; 11: 2205-2216.

28) Yang Z, Feng Z, Gu J, Li X, Dong Q, Liu K, Li Y, OuYang L. MicroRNA-488 inhibits chemoresistance of ovarian cancer cells by targeting Six1 and mitochondrial function. Oncotarget 2017; 8: 80981-80993.

29) Liang J, Bian ML, Chen QY, Liu X, Ou H, Li M, Liu J. Relationship between cyclin G1 and human papilloma virus infection in cervical intraepithelial neoplasia and cervical carcinoma. Chin Med Sci J 2006; 21: 81-85.

30) Zhao X, Liu M, Li D. Oleanolic acid suppresses the proliferation of lung carcinoma cells by miR-122/Cyclin G1/MEF2D axis. Mol Cell Biochem 2015; 400: 1-7.

31) Han H, Zhang Z, Yang X, Yang W, Xue C, Cao X. MiR-23b suppresses lung carcinoma cell proliferation through CCNG1. Oncol Lett 2018; 16: 4317-4324.

32) Zhao Y, Wang Y, Xing G. MiR-516b functions as a tumor suppressor by directly modulating CCNG1 expression in esophageal squamous cell carcinoma. Biomed Pharmacother 2018; 106: 1650-1660.

33) Smith ML, Kontny HU, Bortnick R, Fornace AJ Jr. The p53-regulated cyclin G gene promotes cell growth: p53 downstream effectors cyclin G and Gadd45 exert different effects on cisplatin chemosensitivity. Exp Cell Res 1997; 230: 61-68.

34) Dai L, Pan Q, Peng Y, Huang S, Liu J, Chen T, Wang X, Chen D, Wang J, Zhu Y, Wang H, Liu Y, Ou Y, Yu X, Cao K. p53 plays a key role in the apoptosis of human ovarian cancer cells induced by adenovirus-mediated CRM197. Hum Gene Ther 2018; 29: 916-926.

35) Yan J, Jiang JY, Meng XN, Xiu YL, Zong ZH. MiR-23b targets cyclin G1 and suppresses ovarian cancer tumorigenesis and progression. J Exp Clin Cancer Res 2016; 35: 31.