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RNA N6-methyladenosine modification participates in miR-660/E2F3 axis-mediated inhibition of cell proliferation in gastric cancer

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ABSTRACT

Increasing evidence has shown that dysregulation of mircoRNA (miRNA) is linked to the development and progression of human cancer, including gastric cancer (GC). In the current study, by analysing the GEO database (GSE78091), we found that miR-660 was significantly downregulated in GC. Consistently, quantitative real-time PCR (qRT-PCR) results showed that miR-660 was dramatically decreased in GC tissues and cell lines. Importantly, low miR-660 expression was closely related to larger tumor size ($P = 0.008$), lymph node metastasis ($P = 0.006$), advanced TNM stage ($P = 0.029$), and poor outcome ($P = 0.023$). Ectopic expression of miR-660 inhibited proliferation of MGC-803 and AGS cells and induced apoptosis. Further mechanism experiments suggested that the well-known oncogene E2F3 (E2F transcription factor 3) was a downstream target of miR-660. Overexpression of miR-660 reduced the activity of E2F3 by directly binding to the 3221˜3226 region of E2F3 3`-UTR, and there was a strong negative correlation between the expression of miR-660 and E2F3 in GC tissues ($r = -0.648$, $P < 0.001$). Furthermore, E2F3 overexpression abrogated the anti-proliferation effect of miR-660 in GC cell lines. Of note, we found an N6-methyladenosine (m^6A) motif at the 3063˜3067 region of E2F3 3`-UTR, and this $m^6A$-modified motif was required for the interaction between miR-660 and E2F3 3`-UTR. Collectively, our findings reveal the compelling role of m^6A in GC and highlight the regulatory function of the miR-660/E2F3 pathway in GC progression.

1. Introduction

Gastric cancer (GC) is one of the most common malignant tumors of the gastrointestinal tract with the third leading cause of cancer-related death worldwide [1]. Despite improvements in the diagnosis and treatment of gastric cancer in recent years, the survival rate of gastric cancer is still very poor, especially in patients with metastases (5-year survival rate of < 5%) [2]. Therefore, it is highly desirable to elucidate the underlying molecular mechanisms of GC, which will provide a new strategy for clinical treatment.

MircoRNA (miRNA) is a class of evolutionarily conserved small non-coding regulatory RNA consisting of 19–25 nucleotide [3]. Most miRNAs are present in the human genome in the form of single-copy, multi-copy or cluster [4]. It has been well proven that miRNA is involved in various developmental and pathophysiological processes. Mature miRNA exerts its biological function mainly by complementary pairing with the 3′ untranslated region (UTR) of its target gene, thereby leading to mRNA degradation or translational repression at the post-transcriptional level [5]. Multiple lines of evidence has shown that miRNA biogenesis is tightly controlled in a cell- or disease-context-dependent manner, and its dysregulation is linked to the development and progression of human cancer, including GC [6]. For example, miR-29c was reported to be significantly decreased in GC and retarded GC progression by directly targeting ITGB1 [7]; miR-423-3p was notably elevated in GC and promoted the malignant phenotype of GC through activating oncogenic autophagy [8]; miR-215 was shown to be a metastasis-promoting factor by regulating its target gene RB1 [9]. Although the roles of several miRNAs in GC have been well elucidated, there is still a large part of the miRNA's functions that are still unknown, and further research is needed to clarify this.

N6-methyladenosine (m^6A) modification is known to be the most prevalent internal modification of mRNAs in eukaryotes, which is determined by methyltransferases (writers-METTL3, METTL14, and WTAP), demethylases (erasers-FTO and ALKBH5) and binding proteins

Abbreviations: GC, gastric cancer; E2F3, E2F transcription factor 3; m^6A, N6-methyladenosine; 3′-UTR, 3′ untranslated region; EdU, 5-Ethynyl-2`-deoxyuridine; RIP, RNA immunoprecipitation; OS, overall survival

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(readers-YTHDF2 and YTHDF3) [10,11]. Accumulating evidence has shown that m^6A participates in almost every step in RNA metabolism including RNA stabilization, localization, transport, splicing and translation [12,13]. Recent studies suggest that m^6A is also involved in human cancer progression. Ma et al reported that m^6A modifications were reduced in liver cancer and were tightly controlled by METTL14, which markedly inhibited liver cancer metastasis [14]. Lin et al showed that METTL3 accelerated the translation process of multiple oncogenes to promote lung cancer progression [15]. Likewise, FTO was proposed to play a critical oncogenic effect in acute myeloid leukemia by decreasing m^6A levels of ASB2 and RARA genes [16]. However, the potential role of m^6A modification in GC remains unclear.

Herein, we identified that miR-660, as a tumor-suppressive gene, was significantly decreased in GC tissues and cell lines. Further mechanism study showed that miR-660 directly bound to oncogene E2F3 3`-UTR to inhibit E2F3 expression. More importantly, m^6A modification was involved in the inhibitory effect of miR-660 on E2F3.

2. Materials and methods

2.1. Patient samples

A total of 70 pairs of GC and adjacent normal tissues were retrospectively collected from patients during operation at Renmin Hospital of Wuhan University. All patients did not undergo radiotherapy or chemotherapy before surgery. The clinicopathological features of these patients are presented in Table 1. Before this study, we had obtained informed consent from patients. This study was approved by the ethics committee of Renmin Hospital of Wuhan University.

2.2. Cell culture and transfection

The GC cell lines including MGC-803, AGS, MKN28, SGC7901, MKN45 and human gastric epithelial GES-1 cells were obtained from ATCC (Manassas, USA) or Chinese Academy of Medical Sciences (Beijing, China). All cells were cultured in RPMI1640 or DMEM complete medium with 10% foetal bovine serum. Control mimics, miR-660 mimics (RiboBio, Guangzhou, China) and E2F3-overexpressing pcDNA3.1 vector were respectively transfected into MGC-803 and AGS cells by using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Table 1
Correlation between miR-660 expression and clinicopathological features in GC patients.

Parameters	All cases	miR-660 expression		*P* value
		Low	High	
Gender				
Male	51	25	26	0.788
Female	19	10	9	
Age (years)				
≤ 60	30	16	14	0.629
> 60	40	19	21	
Tumor size				
≤ 5	37	13	24	**0.008**
> 5	33	22	11	
Lymph node metastasis				
No	25	7	18	**0.006**
Yes	45	28	17	
TNM stage				
I-II	29	10	19	**0.029**
III-IV	41	25	16	
Differentiation grade				
Well-moderate	32	14	18	0.337
Poor-undifferentiation	38	21	17	

2.3. quantitative real-time PCR (qRT-PCR)

Total RNA in GC tissues and cells was extracted by TRIzol (Invitrogen),1 μg of RNA was reverse transcribed into cDNA. After that, quantitative PCR was conducted by using miSYBRGreenPCR kit (TransGen Biotech, China). $2^{-\Delta\Delta Ct}$ calculation method was used to evaluate RNA expression level. U6 and β-actin as an internal reference control for mir-660 and E2F3 expression, respectively.

2.4. Cell counting Kit-8 (CCK-8) assay

MGC-803 and AGS cells were seeded in 96-well plates (5000 cells in each well). After 0, 24, 48, 72 and 96 h, cells were treated with 10 μl CCK-8 solution (Beyotime, Shanghai, China) for 2 h at 37 °C. The absorbance at 450 nM of each well was recorded with a automatic microplate reader.

2.5. 5-Ethynyl-2`-deoxyuridine (EdU) incorporation assay

The DNA synthesis rate was tested by EdU kit (RiboBio, Guangzhou, China) according to the manufacturer's instructions. Briefly, MGC-803 and AGS cells were seeded in 96-well plates at a density of 1×10^4 cells. Then, cells were treated with 100 μM EdU reagent for 2 h, followed by fixation with 4% paraformaldehyde. Nucleus was visualized with DAPI (blue). Finally, EdU-positive cells (red) were counted by fluorescence microscopy in at least three randomly selected fields.

2.6. Cell cycle and apoptosis assays

For cell cycle analysis, MGC-803 and AGS cells were trypsinized and fixed with 70% ethanol overnight at 4 °C, followed by staining with PI reagent. The percentage of cells in the G0/G1, S, and G2/M phases was recorded using Flow cytometry (BD Biosciences, NY, USA). Cell apoptosis was carried out by using the Annexin V-FITC/ Propidium Iodide (PI) Apoptosis Detection Kit (BD Biosciences #556,547) based on manufacturer's manual. The percentage of early and late apoptosis in MGC-803 and AGS cells was determined by Flowjo V10 software.

2.7. Luciferase reporter assay

The wild type E2F3 3`-UTR full-length sequence and its corresponding three mutant sequences were cloned into the pmirGLO luciferase reporter vector (Promega), and then co-transfected into MGC-803 and AGS cells with miR-660 and control mimics, respectively. After 48 h of incubation, the luciferase activities in each group were tested by firefly luciferase reporter gene detection kit (#RG005, Beyotime, China).

2.8. Western blot

Total protein in MGC-803 and AGS cells transfected with miR-660 and control mimics was isolated by RIPA lysis buffer (Beyotime, China). Next, proteins were separated and transferred to the nitrocellulose membrane, followed by incubation with anti-E2F3 (#ab50917, Abcam) and IgG secondary antibody (#ab205718, Abcam). The proteins in each group were visualized by using chemiluminescence detection kit (Cyanagen, Italy). GAPDH was used as an internal reference control.

2.9. RNA immunoprecipitation (RIP)

The RIP protocol has been described in the previous studies [17,18]. Concretely, total RNA from GC tissues and cells was extracted and incubated with anti-m^6A antibody

(#ab151230, Abcam) for 2 h at 4 °C, followed by incubation with protein-A/G beads for another 2 h. Then, the m^6A antibody-bound RNAs were eluted and subjected to qRT-PCR using well-designed

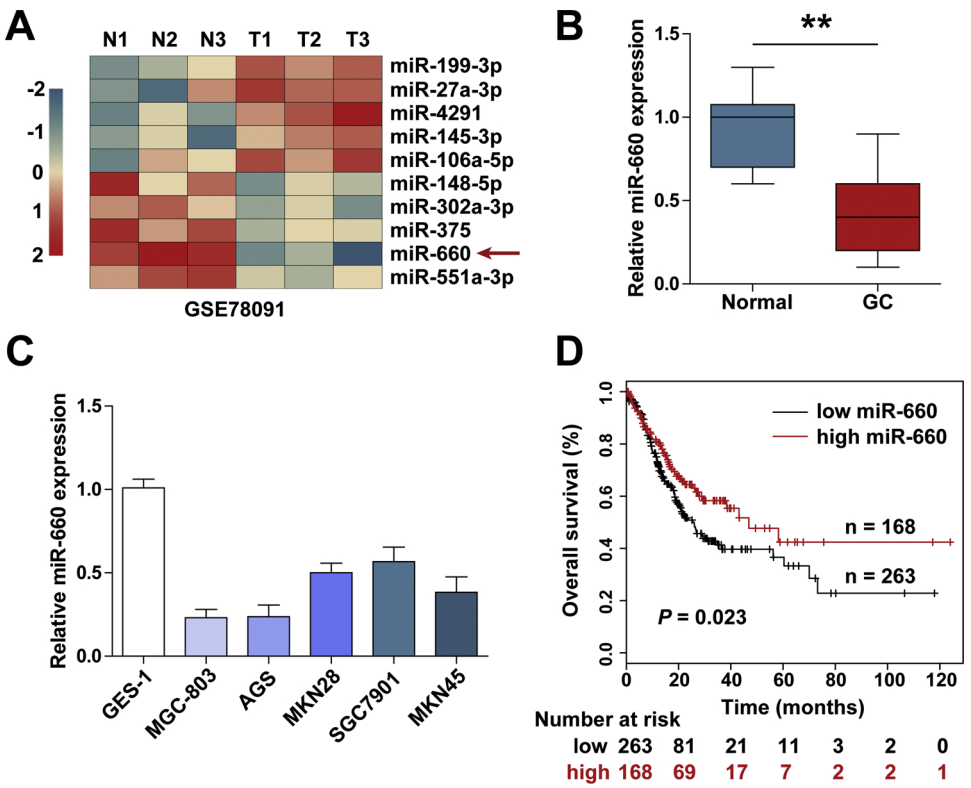

Fig. 1. miR-660 is identified to be downregulated in GC. (A) Hierarchical clustering analysis of the top five most increased and decreased miRNAs in GEO database (GSE78091) containing 3 pairs of GC and adjacent normal tissues. (B) qRT-PCR analysis for miR-660 expression in GC and normal tissues (n = 70). (C) qRT-PCR analysis for miR-660 expression in GC cell lines. (D) The overall survival curve of GC patients with low and high miR-660 expression (data from KM-plotter (http://kmplot.com/analysis/). $^{**}p < 0.01$.

primers for E2F3 3`-UTR.

2.10. Statistical analysis

Differences between groups were tested by Student's t-test. The association between miR-660 expression and clinicopathological features of GC patients was determined by Chi-square test. E2F3 and miR-660 expression correlation analysis was measured by Pearson's correlation coefficient. All statistical results are analyzed by SPSS Statistics 22.0 software. Data are shown as mean ± SD (standard deviation) of at least three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$.

3. Results

3.1. miR-660 is significantly downregulated in GC tissues and cell lines

By analysing the GEO database (GSE78091), we found that miR-660 expression was most decreased in GC tissues (Fig. 1A). To confirm this result, we collected 70 pairs of GC and adjacent normal tissues to conduct qRT-PCR analysis for miR-660 expression. As shown in Fig. 1B, miR-660 was dramatically decreased in GC tissues as compared with normal tissues. Also, low expression of miR-660 was observed in 5 GC cell lines (Fig. 1C). Importantly, loss of miR-660 was closely associated with malignant clinicopathological features, such as larger tumor size ($P = 0.008$), lymph node metastasis ($P = 0.006$), and advanced TNM stage ($P = 0.029$) (Table 1). Moreover, the survival data of GC patients in KM-plotter (http://kmplot.com/analysis/) database showed that GC patients with low miR-660 expression (n = 263) displayed shorter survival time than those with high miR-660 expression (n = 168). The above results indicate that dysregulation of miR-660 may play an important role in the development of GC.

3.2. Overexpression of miR-660 inhibits GC cell proliferation

Next, we transfected miR-660 and control mimics into MGC-803 and AGS cells. The results of qRT-PCR showed that miR-660 mimics could increased miR-660 expression almost 70 fold in these two cells (Fig. 2A). We then assessed the proliferative capacity of MGC-803 and AGS cells after transfection, the CCK-8 assay results indicated that miR-660 overexpression remarkably inhibited cell viability (Fig. 2B-C). As shown in Fig. 2D, more cells were exhibited in G0/G1 phase after ectopic expression of miR-660. Furthermore, EdU assay showed that overexpression of miR-660 reduced DNA synthesis levels in MGC-803 and AGS cells (Fig. 2E-H). In addition, more apoptotic cells were observed in miR-660-overexpressing cells (Fig. 2I). Altogether, there results suggest that miR-660 is an inhibitor of GC proliferation.

3.3. E2F3 is identified to be a direct target of miR-660

By searching Miranda (http://www.microrna.org/) and Targetscan (http://www.targetscan.org/) online database, we found that there are 3 potential miR-660 binding sites at the 3`-UTR of E2F3 gene (Fig. 3A). To test which binding site was functional, we performed the luciferase reporter assay. The results showed that miR-660 overexpression reduced the luciferase activities of wild-type E2F3 3`-UTR, mutant E2F3 3`-UTR#1, and mutant E2F3 3`-UTR#2 vectors in MGC-803 and AGS cells, but had no effect on that of mutant E2F3 3`-UTR#3 vector (Fig. 3B-C), suggesting that the 3221˜3226 region of E2F3 3`-UTR, but not the other two, was critical for the interaction between miR-660 and E2F3. Besides, we found that E2F3 was significantly elevated in GC tissues when compared with normal tissues (Fig. 3D) and increased E2F3 expression predicted poor outcome (data from KM-plotter) (Fig. 3E). Overexpression of miR-660 decreased both mRNA and protein expression of E2F3 in MGC-803 and AGS cells (Fig. 3F-G). Clinically, a strong negative correlation between miR-660 and E2F3 expression was observed in GC tissues ($r = -0.648$, $P < 0.001$). Moreover, ectopic expression of E2F3 abolished anti-proliferation effect caused by miR-660 overexpression (Fig. 3I-*J*). Collectively, these data indicate that miR-660 suppresses GC proliferation by directly targeting oncogene E2F3.

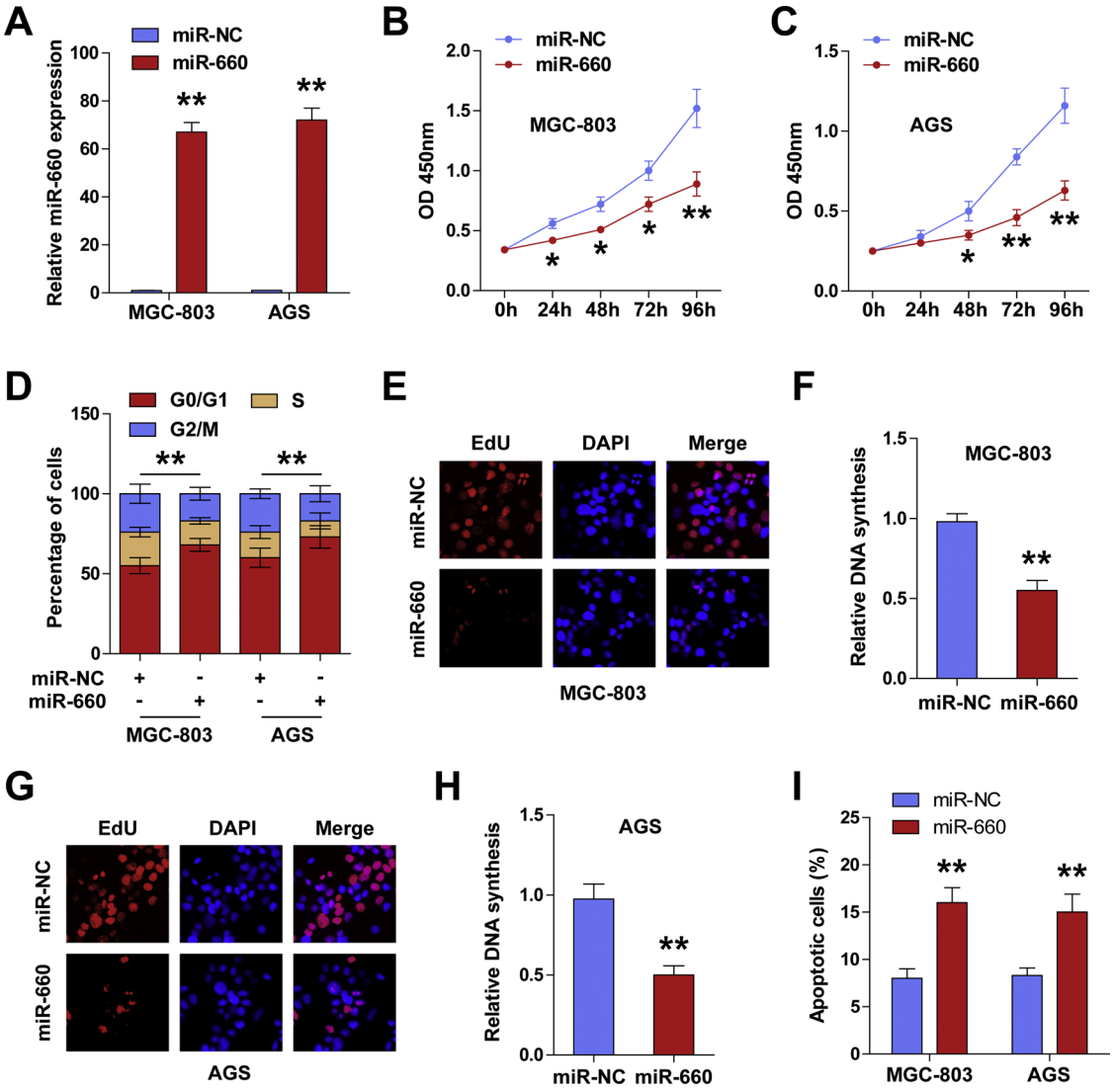

Fig. 2. Ectopic expression of miR-660 retards GC cell proliferation. (A) qRT-PCR analysis for overexpression efficiency of miR-660 in MGC-803 and AGS cells. (B–C) CCK-8 assay in miR-660-overexpressing MGC-803 and AGS cells. The absorbance at 450 nM was recorded at the indicated time. (D) Cell cycle assay with PI staining in miR-660-overexpressing MGC-803 and AGS cells. (E–H) EdU assay in miR-660-overexpressing MGC-803 and AGS cells. Nucleus was stained with DAPI (blue). EdU-positive cells (red) were counted in at least three randomly selected fields. (I) Cell apoptosis assay with FITC/PI staining in miR-660-overexpressing MGC-803 and AGS cells. $*p < 0.05$, $**p < 0.01$.

3.4. m^{6}A modification is indispensable for the interaction between miR-660 and E2F3

It has been reported that m^{6}A modification was involved in the interaction between miRNA and mRNA [18]. We then designed 3 pairs of specific primers that respectively contain the above 3 potential miR-660 binding sites at E2F3 3`-UTR. The RIP assay with anti-m$^6$A antibody was carried out to evaluate the m$^6$A modification level. The results showed that m$^6$A modification was only observed in the region that contains 3221˜3226 of E2F3 3`-UTR (Fig. 4A-B). Further, a RRACU (R denotes A or G) m^{6}A motif (AAACU) was found at the 3063˜3067 region of E2F3 3`-UTR (Fig. 4C). A series of vectors with this m^{6}A motif mutation were constructed to conduct m^{6}A-RIP assay (Fig. 4C). m^{6}A modification almost disappeared after the mutation of "AAACU" into "AACCU" or "AAAAU", but the m^{6}A level remained unchanged after the mutation into "AGACU" (Fig. 4D-E), indicating "RRACU" motif was critical for m^{6}A modification. To explore whether m^{6}A modification mediates the inhibitory effect of miR-660 on E2F3, luciferase reporter assay was performed. The results showed that overexpression of miR-660 significantly reduced luciferase activities of m^{6}A-motif-WT and m^{6}A-motif-mut#3 vectors in MGC-803 and AGS cells due to the presence of intact "RRACU" motif, but could not affect those of m^{6}A-motif-mut#1 and #2 vectors with destroyed m^{6}A motif (Fig. 4F-G). Clinically, we found that m^{6}A modification level was dramatically decreased in GC tissues compared with adjacent normal tissues (Fig. 4H). Taken together, these data reveal that m^{6}A modification is essential for the depression effect of miR-660 on E2F3 in GC.

4. Discussion

It is well accepted that miRNA exerts an important regulatory function in the process of cancer, including GC. In this study, we characterized the role of miR-660 that has not been previously reported in GC. We found that miR-660 was frequently downregulated in GC and its decrease was closely associated with aggressive clinicopathological parameters and unfavorable prognosis. Subsequent experiments showed that miR-660 retarded GC cell proliferation by altering oncogene E2F3 activity. Lastly, we found that m^{6}A modification acted as a regulator in miR-660-mediated inhibition of GC progression. Therefore, our findings provide a new insight into the molecular regulatory

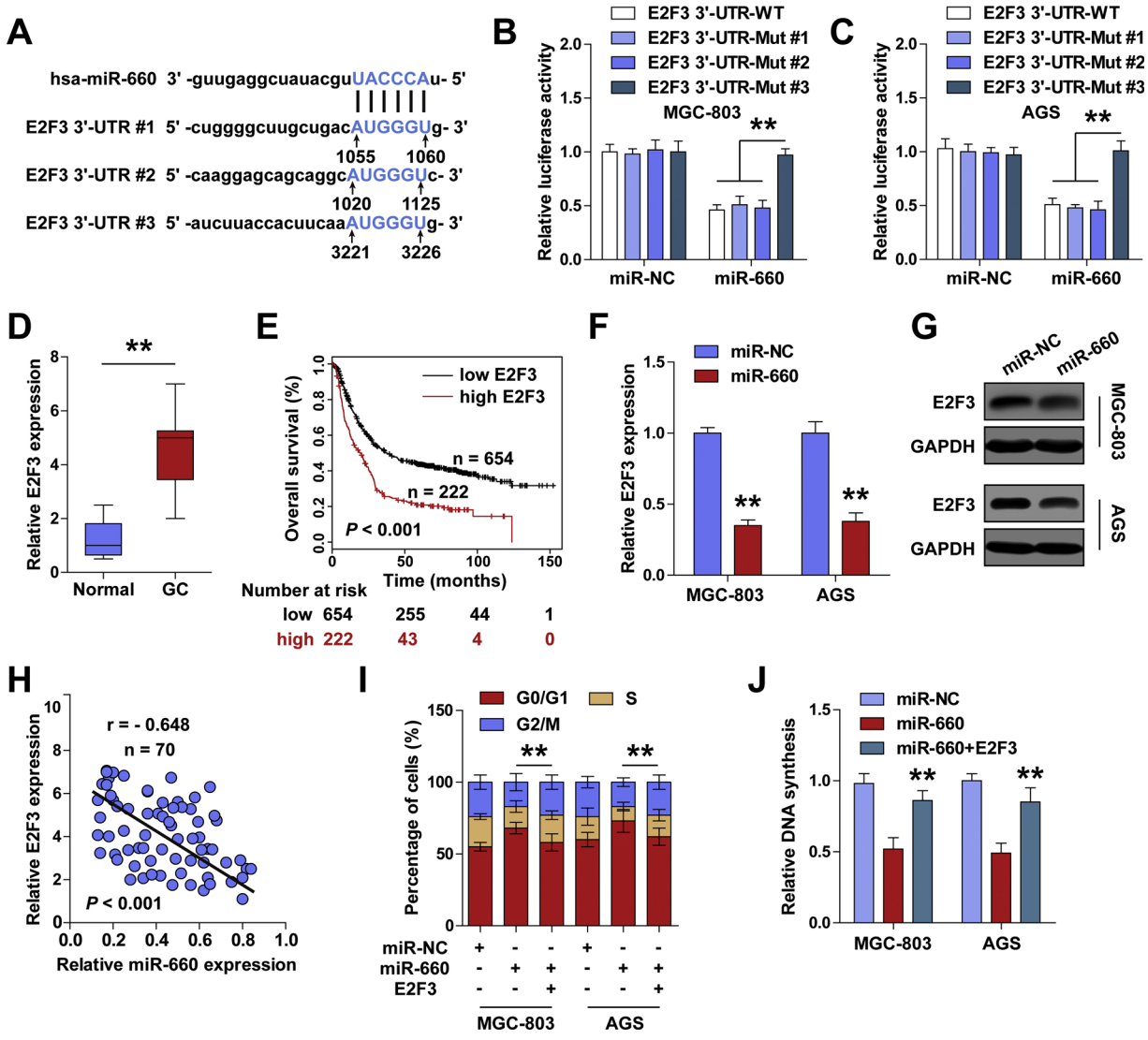

Fig. 3. E2F3 is a downstream target gene of miR-660 in GC. (A) Three potential miR-660 binding sites at E2F3 3`-UTR predicted by Miranda and Targetscan. (B–C) Luciferase reporter assay for wild-type and mutant E2F3 3`-UTR luciferase vectors in MGC-803 and AGS cells transfected with miR-660 and control mimics. (D) qRT-PCR analysis for E2F3 expression in GC and normal tissues (n = 70). (E) The overall survival curve of GC patients with low and high E2F3 expression (data from KM-plotter (http://kmplot.com/analysis/). (F–G) qRT-PCR and western blot assays for E2F3 mRNA (F) and protein (G) expression in miR-660-overexpressing MGC-803 and AGS cells. (H) Pearson's correlation analysis for miR-660 and E2F3 expression in GC tissues (n = 70). (I–J) Cell cycle (I) and apoptosis (*J*) assays in miR-660-overexpressing MGC-803 and AGS cells with or without co-transfection with E2F3-overexpressing vector. $^{**}p < 0.01$.

mechanism underlying GC.

Emerging evidence suggests that miRNA as an oncogene or tumor suppressor gene that controls the occurrence, development and progression of cancer [19]. Recently, miR-660 has been found to be dysregulated in a number of human cancers. Borzi et al reported that miR-660 was significantly decreased in lung cancer and inhibited lung tumorigenesis by modulating MDM2/p53 interaction [20]. Likewise, miR-660 was shown to be downregulated in Hodgkin lymphoma [21] and prostate cancer [22]. In contrast, high expression of miR-660 was identified in breast cancer and indicated a poor outcome [23,24]. One possible explanation for these discrepancies is that miRNA expression is tightly controlled in a cell- or disease-context-dependent manner [25]. In our study, miR-660 was found to be a tumor suppressor which was dramatically decreased in GC tissues and cell lines. In particular, the survival data from KM-plotter database showed that low miR-660 expression was correlated with shorter survival time in GC patients, implying that miR-660 is a promising prognosis biomarker for GC. Recent clinical studies have shown that miRNA-targeted therapy has achieved remarkable curative effect [26]. Thus, restore of miR-660 may be a new efficacious therapeutic approach for GC treatment.

Mature miRNA plays its biological role mainly at the post-transcriptional level by complementary pairing with the 3′-UTR of its target gene, thereby leading to mRNA degradation or translational repression [5]. Here, we found that miR-660 could bind to the 3221˜3226 region of E2F3 3`-UTR to suppress E2F3 expression. E2F3, belongs to E2F family, is a well-defined oncogene that accelerates the progression of G0/G1 to S phase [27]. E2F3 has been proposed to be notably overexpressed in multiple cancer, including endometrial cancer [28], hepatocellular carcinoma [29], lung cancer [30], prostate cancer [31] and GC [32]. In this study, we confirmed that E2F3 expression was significantly elevated in GC tissues and predicted unfavorable outcome. And ectopic expression of E2F3 rescued the decline in proliferative capacity of GC cells caused by miR-660 overexpression, indicating that miR-660 exerts the anti-proliferation effect, at least partly, by regulating oncogene E2F3 activity.

It has been proven that m^6A modification is widespread in the transcriptome with important regulatory functions. And the development of human diseases including cancer is strictly monitored by m^6A

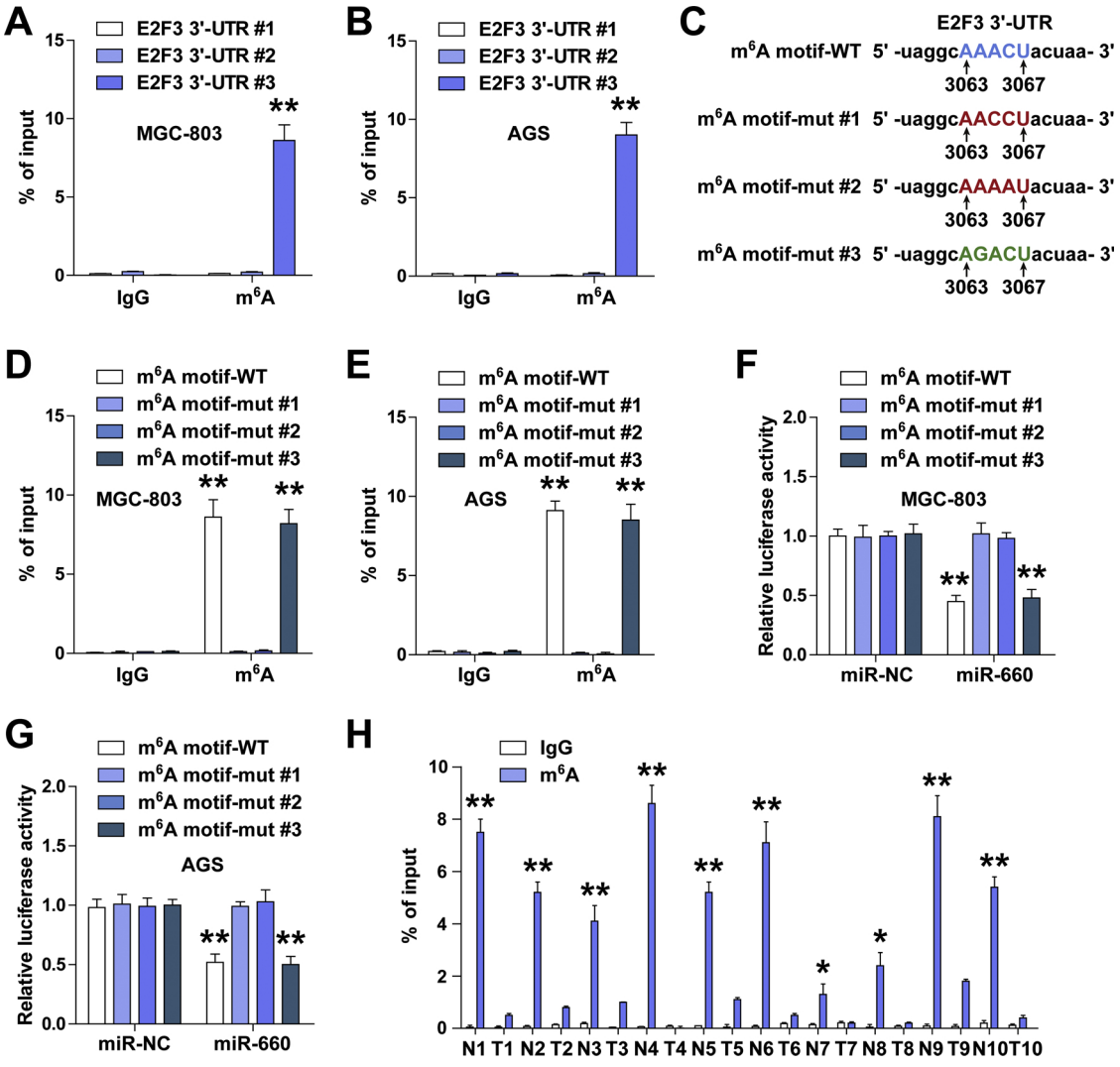

Fig. 4. m^{6}A modification is crucial for the interaction between miR-660 and E2F3 in GC. (A–B) m^{6}A RIP assay in MGC-803 and AGS cells with three pairs of specific primers that respectively comprise three potential miR-660 binding sites at E2F3 3`-UTR. (C) Wild-type and mutant sequences of m$^6$A motif at the 3063˜3067 region of E2F3 3`-UTR were shown. (D–E) m^{6}A RIP assay in MGC-803 and AGS cells transfected with wild-type and mutant m^{6}A motif vectors. (F–G) Luciferase reporter assay for wild-type and mutant m^{6}A motif luciferase vectors in MGC-803 and AGS cells transfected with miR-660 and control mimics. (H) m^{6}A RIP assay in 10 pairs of GC and adjacent normal tissues. *$p < 0.05$, **$p < 0.01$.

modification [33]. A very recent study revealed that m^{6}A modification was also involved in the interaction between miRNA and mRNA [18]. Herein, we found a m^{6}A motif at the 3063˜3067 region of E2F3 3`-UTR near the binding sites of miR-660 and E2F3, which is required for the interaction between miR-660 and E2F3 3`-UTR at 3221˜3226 region. This may be due to the fact that m^{6}A modification increased the accessibility of RNA binding motifs by changing the spatial structure of RNA transcripts to induce binding between RNA and RNA [34]. In addition, low m^{6}A modification level was identified in GC tissues compared with adjacent normal tissues, suggesting m^{6}A modification may play a key role in the malignant progression of GC.

In conclusion, to the best of our knowledge, this is the first study to explore the role of miR-660 in GC. Our data show that miR-660 is a new anti-proliferation gene by regulating oncogene E2F3 expression in GC. Of note, m^{6}A modification is essential for the inhibitory effect of miR-660 on E2F3. Therefore, our findings advance the understanding of molecular mechanism underlying GC.

Conflicts of interest

None.

Acknowledgments

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References

[1] E. Van Cutsem, X. Sagaert, B. Topal, K. Haustermans, H. Prenen, Gastric cancer, Lancet 388 (2016) 2654–2664.
[2] S.G. Thrumurthy, M.A. Chaudry, I. Chau, W. Allum, Does surgery have a role in managing incurable gastric cancer? Nat. Rev. Clin. Oncol. 12 (2015) 676–682.
[3] V. Ambros, The functions of animal microRNAs, Nature 431 (2004) 350–355.
[4] M. Mohajeri, M. Banach, S.L. Atkin, A.E. Butler, M. Ruscica, G.F. Watts, A. Sahebkar, MicroRNAs: novel molecular targets and response modulators of statin therapy, Trends Pharmacol. Sci. 39 (2018) 967–981.
[5] G. Michlewski, J.F. Caceres, Post-transcriptional control of miRNA biogenesis, Rna 25 (2019) 1–16.
[6] Y. Yan, R. Wang, W. Guan, M. Qiao, L. Wang, Roles of microRNAs in cancer associated fibroblasts of gastric cancer, Pathol. Res. Pract. 213 (2017) 730–736.
[7] T.S. Han, K. Hur, G. Xu, B. Choi, Y. Okugawa, Y. Toiyama, H. Oshima, M. Oshima, H.J. Lee, V.N. Kim, A.N. Chang, A. Goel, H.K. Yang, MicroRNA-29c mediates initiation of gastric carcinogenesis by directly targeting ITGB1, Gut 64 (2015) 203–214.
[8] P. Kong, X. Zhu, Q. Geng, L. Xia, X. Sun, Y. Chen, W. Li, Z. Zhou, Y. Zhan, D. Xu, The

microRNA-423-3p-Bim Axis Promotes Cancer progression and activates oncogenic autophagy in gastric Cancer, Mol. Ther. 25 (2017) 1027–1037.
[9] Z. Chen, K. Liu, L. Li, Y. Chen, Du S, miR-215 promotes cell migration and invasion of gastric cancer by targeting Retinoblastoma tumor suppressor gene 1, Pathol. Res. Pract. 213 (2017) 889–894.
[10] K.D. Meyer, Y. Saletore, P. Zumbo, O. Elemento, C.E. Mason, S.R. Jaffrey, Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons, Cell 149 (2012) 1635–1646.
[11] D. Dominissini, S. Moshitch-Moshkovitz, S. Schwartz, M. Salmon-Divon, L. Ungar, S. Osenberg, K. Cesarkas, J. Jacob-Hirsch, N. Amariglio, M. Kupiec, R. Sorek, G. Rechavi, Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq, Nature 485 (2012) 201–206.
[12] D. Dai, H. Wang, L. Zhu, H. Jin, X. Wang, N6-methyladenosine links RNA metabolism to cancer progression, Cell Death Dis. 9 (2018) 124.
[13] Y. Fu, D. Dominissini, G. Rechavi, C. He, Gene expression regulation mediated through reversible m(6)A RNA methylation, Nat. Rev. Genet. 15 (2014) 293–306.
[14] J.Z. Ma, F. Yang, C.C. Zhou, F. Liu, J.H. Yuan, F. Wang, T.T. Wang, Q.G. Xu, W.P. Zhou, S.H. Sun, METTL14 suppresses the metastatic potential of hepatocellular carcinoma by modulating N(6) -methyladenosine-dependent primary MicroRNA processing, Hepatology 65 (2017) 529–543.
[15] S. Lin, J. Choe, P. Du, R. Triboulet, R.I. Gregory, The m(6)A methyltransferase METTL3 promotes translation in human Cancer cells, Mol. Cell 62 (2016) 335–345.
[16] Z. Li, H. Weng, R. Su, X. Weng, Z. Zuo, C. Li, H. Huang, S. Nachtergaele, L. Dong, C. Hu, X. Qin, L. Tang, Y. Wang, G.M. Hong, H. Huang, X. Wang, P. Chen, S. Gurbuxani, S. Arnovitz, Y. Li, S. Li, J. Strong, M.B. Neilly, R.A. Larson, X. Jiang, P. Zhang, J. Jin, C. He, J. Chen, FTO Plays an Oncogenic Role in Acute Myeloid Leukemia as a N(6)-Methyladenosine RNA Demethylase, Cancer Cell 31 (2017) 127–141.
[17] K. Zeng, B. He, B.B. Yang, T. Xu, X. Chen, M. Xu, X. Liu, H. Sun, Y. Pan, S. Wang, The pro-metastasis effect of circANKS1B in breast cancer, Mol. Cancer 17 (2018) 160.
[18] X. Zhang, Y. Xu, Z. Qian, W. Zheng, Q. Wu, Y. Chen, G. Zhu, Y. Liu, Z. Bian, W. Xu, Y. Zhang, F. Sun, Q. Pan, J. Wang, L. Du, Y. Yu, circRNA_104075 stimulates YAP-dependent tumorigenesis through the regulation of HNF4a and may serve as a diagnostic marker in hepatocellular carcinoma, Cell Death Dis. 9 (2018) 1091.
[19] M.A. Iqbal, S. Arora, G. Prakasam, G.A. Calin, M.A. Syed, MicroRNA in lung cancer: role, mechanisms, pathways and therapeutic relevance, Mol. Aspects Med. (2018).
[20] O. Fortunato, M. Boeri, M. Moro, C. Verri, M. Mensah, D. Conte, L. Caleca, L. Roz, U. Pastorino, G. Sozzi, Mir-660 is downregulated in lung cancer patients and its replacement inhibits lung tumorigenesis by targeting MDM2-p53 interaction, Cell Death Dis. 5 (2014) e1564.
[21] S. Paydas, A. Acikalin, M. Ergin, H. Celik, B. Yavuz, K. Tanriverdi, Micro-RNA (miRNA) profile in Hodgkin lymphoma: association between clinical and pathological variables, Med. Oncol. 33 (2016) 34.
[22] E.A. Lekchnov, E.V. Amelina, O.E. Bryzgunova, I.A. Zaporozhchenko, M.Y. Konoshenko, S.V. Yarmoschuk, I.S. Murashov, O.A. Pashkovskaya, A.M. Gorizkii, A.A. Zheravin, P.P. Laktionov, Searching for the novel specific predictors of prostate Cancer in urine: the analysis of 84 miRNA expression, Int. J. Mol. Sci. 19 (2018).
[23] P. Krishnan, S. Ghosh, B. Wang, D. Li, A. Narasimhan, R. Berendt, K. Graham, J.R. Mackey, O. Kovalchuk, S. Damaraju, Next generation sequencing profiling identifies miR-574-3p and miR-660-5p as potential novel prognostic markers for breast cancer, BMC Genomics 16 (2015) 735.
[24] Y. Shen, Y.F. Ye, L.W. Ruan, L. Bao, M.W. Wu, Y. Zhou, Inhibition of miR-660-5p expression suppresses tumor development and metastasis in human breast cancer, Genet. Mol. Res. 16 (2017).
[25] Y. Wang, L. Wang, C. Chen, X. Chu, New insights into the regulatory role of microRNA in tumor angiogenesis and clinical implications, Mol. Cancer 17 (2018) 22.
[26] R. Rupaimoole, F.J. Slack, MicroRNA therapeutics: towards a new era for the management of cancer and other diseases, Nat. Rev. Drug Discov. 16 (2017) 203–222.
[27] E. De Keuckelaere, P. Hulpiau, Y. Saeys, G. Berx, F. van Roy, Nanos genes and their role in development and beyond, Cell. Mol. Life Sci. 75 (2018) 1929–1946.
[28] J. Wan, H. Liu, Q. Feng, J. Liu, L. Ming, HOXB9 promotes endometrial cancer progression by targeting E2F3, Cell Death Dis. 9 (2018) 509.
[29] L.N. Kent, S. Bae, S.Y. Tsai, X. Tang, A. Srivastava, C. Koivisto, C.K. Martin, E. Ridolfi, G.C. Miller, S.M. Zorko, E. Plevris, Y. Hadjiyannis, M. Perez, E. Nolan, R. Kladney, B. Westendorp, A. de Bruin, S. Fernandez, T.J. Rosol, K.S. Pohar, J.M. Pipas, G. Leone, Dosage-dependent copy number gains in E2f1 and E2f3 drive hepatocellular carcinoma, J. Clin. Invest. 127 (2017) 830–842.
[30] P. Trikha, N. Sharma, C. Pena, A. Reyes, T. Pecot, S. Khurshid, M. Rawahneh, J. Moffitt, J.A. Stephens, S.A. Fernandez, M.C. Ostrowski, G. Leone, E2f3 in tumor macrophages promotes lung metastasis, Oncogene 35 (2016) 3636–3646.
[31] S. Bilke, R. Schwentner, F. Yang, M. Kauer, G. Jug, R.L. Walker, S. Davis, Y.J. Zhu, M. Pineda, P.S. Meltzer, H. Kovar, Oncogenic ETS fusions deregulate E2F3 target genes in Ewing sarcoma and prostate cancer, Genome Res. 23 (2013) 1797–1809.
[32] T. Manicum, F. Ni, Y. Ye, X. Fan, B.C. Chen, Prognostic values of E2F mRNA expression in human gastric cancer, Biosci. Rep. (2018) 38.
[33] A.E. Erson-Bensan, O. Begik, m6A Modification and Implications for microRNAs, Microrna 6 (2017) 97–101.
[34] N. Liu, Q. Dai, G. Zheng, C. He, M. Parisien, T. Pan, N(6)-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions, Nature 518 (2015) 560–564.