ORIGINAL RESEARCH

The Clinical Significance and Expression Level of miR-411-5p in Colorectal Cancer

Tianliang Bai, MD; Mingxuan Zhang, MB; Yabin Liu, MD; Bindan Cai, MB; Shangkun Du, MB

ABSTRACT

Background • MicroRNAs (miRNAs) have been widely recognized as crucial regulators in the development and progression of various cancers, including colorectal cancer (CRC). Previous studies have highlighted the involvement of several miRNAs in CRC, such as miR-145, miR-29a-3p, and miR-196. However, the specific role and clinical significance of miR-411-5p in CRC have not been thoroughly investigated, representing a significant gap in the current understanding of CRC biology. While miR-411-5p has been implicated in the pathogenesis of other human malignancies, its precise mechanisms and impact on CRC development and prognosis remain largely unexplored. Understanding the functional relevance of miR-411-5p in CRC and elucidating its molecular interactions can provide valuable insights into the underlying mechanisms of CRC progression and potentially identify novel therapeutic targets. Therefore, this study aims to investigate the clinical value and level of miR-411-5p in colorectal cancer, shedding light on its potential as a diagnostic and prognostic biomarker. Additionally, we aim to explore the molecular mechanisms underlying the effects of miR-411-5p on CRC cells, particularly its interaction with the target gene NFE2L3. By filling this knowledge gap, our research contributes to a deeper understanding of the role of miR-411-5p in CRC and opens avenues for developing targeted therapies for this prevalent malignancy.

Methods • Colorectal cancer (CRC) tissue samples and corresponding normal paracancerous tissue samples were collected from 60 CRC patients treated at the Affiliated Hospital of Hebei University. Normal paracancerous tissue refers to the healthy tissue adjacent to the cancerous region. These tissue samples were obtained through biopsies, and the patients provided informed consent for their use in the study. To investigate the expression levels of miR-411-5p and NFE2L3, we employed quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis. This technique allowed us to measure the levels of miR-411-5p and NFE2L3 mRNA in both CRC and normal tissue samples. Additionally, we validated the protein levels of NFE2L3 using Western blot analysis. Furthermore, we assessed the functional impact of miR-411-5p on CRC cells through various assays. The MTT assay determined cell viability, the transwell migration assay evaluated cell migration and invasion abilities, and flow cytometry measured the rate of apoptosis in CRC cells. To confirm the molecular interaction between miR-411-5p and its target gene NFE2L3, we conducted dual-luciferase reporter assays. These assays enabled us to validate the binding between miR-411-5p and the 3' untranslated region (3'UTR) of the NFE2L3 mRNA. To investigate the potential therapeutic role of NFE2L3 in CRC, we transfected CRC cells with pcDNA3.0-NFE2L3, a plasmid overexpressing NFE2L3. This allowed us to assess the impact of NFE2L3 restoration on the behavior of CRC cells.

Results • Overexpression of miR-411-5p in CRC cells significantly reduced cell viability, inhibited migration and invasion, and increased the rate of apoptosis. Conversely, inhibition of miR-411-5p expression exerted the opposite effects on the biological behavior of CRC cells. Furthermore, our study revealed that NFE2L3 is a downstream target of miR-411-5p. Dual-luciferase reporter assays confirmed the binding between miR-411-5p and the 3'UTR of NFE2L3 mRNA, indicating a direct interaction between them. To investigate the therapeutic potential of targeting NFE2L3 in CRC, we transfected CRC cells with pcDNA3.0-NFE2L3, resulting in the restoration of NFE2L3 levels. This restoration effectively reversed the effects induced by miR-411-5p mimics on the behavior of CRC cells.

Conclusion • Our study provides compelling evidence for the tumor-suppressive role of miR-411-5p in CRC. The overexpression of miR-411-5p resulted in reduced cell viability, inhibited migration and invasion, and increased apoptosis in CRC cells. Importantly, we identified NFE2L3 as a downstream target of miR-411-5p and demonstrated its involvement in mediating the effects of miR-411-5p on CRC cell behavior. These findings not only confirm the tumor-suppressive role of miR-411-5p in CRC but also highlight NFE2L3 as a promising target for novel therapeutic strategies. Targeting NFE2L3 to modulate the biological function of CRC cells may hold therapeutic potential and serve as a basis for the development of targeted drugs. Further investigations are warranted to fully elucidate the underlying molecular mechanisms of miR-411-5p-NFE2L3 interaction and its impact on CRC progression. Additionally, future studies could explore the clinical implications of miR-411-5p as a diagnostic or prognostic biomarker in CRC patients. By advancing our understanding of the intricate regulatory networks involved in CRC, we can pave the way for personalized therapeutic approaches and improve patient outcomes. (*Altern Ther Health Med.* [E-pub ahead of print.])

Tianliang Bai, MD; **Shangkun Du**, MB, Department of Gastrointestinal Surgery; Affiliated Hospital of Hebei University; Baoding; Hebei; P.R. China. **Mingxuan Zhang**, MB, Shandong University School of Basic Medicine. **Yabin Liu**, MD, Department of General Surgery; Fourth Hospital of Hebei Medical University (Tumor Hospital of Hebei Province); Shijiiazhuang; Hebei; China. **Bindan Cai**, MB, Department of Neurology; Zhuozhou City Hospital; Zhuozhou; Hebei; P.R. China.

Corresponding author: Shangkun Du, XX
E-mail: 414559137@qq.com

INTRODUCTION

Colorectal cancer (CRC), encompassing cancers of the colon and rectum, is a leading cause of cancer-related deaths worldwide. According to the latest available data, CRC is responsible for approximately 900 000 deaths annually, making it the third most common cause of cancer mortality globally.[1,2] The incidence rates of CRC vary geographically, with higher rates observed in developed countries such as the United States, Canada, and parts of Europe, while lower rates are seen in certain Asian and African countries.[3]

The high incidence and mortality rates associated with CRC highlight the urgent need for improved early detection and effective therapeutic strategies. Unfortunately, early-

stage CRC often goes unnoticed, leading to delayed diagnosis and more advanced disease at presentation. This is partly due to the fact that the symptoms of early CRC can be nonspecific and easily attributed to less severe gastrointestinal conditions, such as irritable bowel syndrome or hemorrhoids.[4] Common symptoms include changes in bowel habits, rectal bleeding, abdominal discomfort, and unintended weight loss. As a result, individuals may delay seeking medical attention, leading to missed opportunities for early intervention and potentially curative treatments.[5]

MicroRNAs (miRNAs) have emerged as important regulators in cancer biology, including CRC. These small RNA molecules, typically consisting of 18-25 nucleotides, modulate gene expression by targeting messenger RNAs (mRNAs) and suppressing their translation or promoting their degradation.[6] Several miRNAs have been implicated in CRC pathogenesis, highlighting their potential as diagnostic markers and therapeutic targets. For instance, miR-145 has been found to be downregulated in CRC and is involved in inhibiting tumor growth and metastasis.[7] Similarly, miR-196 has been associated with CRC progression and invasion.[8]

In contrast, the specific interactions and functional implications of miR-411-5p in CRC remain poorly understood. Limited research has investigated the role of miR-411-5p in cancer, particularly in CRC. Therefore, our study aims to explore the regulatory effects of miR-411-5p on the target gene NFE2L3 in CRC. NFE2L3 is a transcription factor involved in important cellular signaling pathways, and its dysregulation has been linked to CRC development and progression (9). Investigating the interplay between miR-411-5p and NFE2L3 may provide novel insights into the molecular mechanisms underlying CRC and potentially identify new therapeutic targets.

In summary, this study aims to elucidate the role of miR-411-5p in CRC by investigating its interaction with NFE2L3 and exploring its impact on tumor growth, migration, and invasiveness. By uncovering the underlying molecular mechanisms, our findings may contribute to the development of innovative diagnostic approaches and targeted therapies for CRC.

METHODS

Clinical tissue samples

Biopsy samples of CRC and paracancerous tissue were obtained from 60 CRC patients treated at Affiliated Hospital of Hebei University. The patients signed an informed consent form before the samples were taken. The samples were then cleaned and placed in numbered centrifuge tubes. This study procedure was approved by the Ethics Committee of the Affiliated Hospital of Hebei University (Approval NO. HDFY-LL-2021-060).

Upon collection, the tissue samples were immediately processed to maintain RNA stability. The following methods were employed for sample preservation:

RNA later solution: Tissue samples were submerged in RNA later solution (e.g., Ambion RNAlater) immediately after collection. RNA later penetrates the tissue, preserving the RNA and preventing degradation by inactivating RNases. The samples were then stored at an appropriate temperature, typically 4°C, to maintain RNA integrity until further processing.

Flash-freezing in liquid nitrogen: Alternatively, tissue samples were rapidly frozen by immersing them in liquid nitrogen. This technique ensures the preservation of RNA integrity by preventing enzymatic degradation. After flash-freezing, the samples were transferred to a suitable storage vessel, such as cryovials, and stored at ultra-low temperatures, typically at -80°C, to maintain long-term RNA stability.

Cell culture transfection

Normal colorectal mucosal foetal human cells (FHC) and CRC cell lines (HT29 and HCT116) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's Modified Eagle Medium—purchased from Invitrogen Carlsbad, CA, USA—mixed with 10% foetal bovine serum (FBS) and 1% penicillin/streptomycin mixture at 37°C in a 5% CO_2 incubator. The miR-411-5p negative control (miR-411-5p NC) or mimic (miR-411-5p mimics) fragment synthesized by Sangon Biotech (Shanghai, China), or mixed with NFE2L3 overexpression plasmid (pcDNA3.0-NFE2L3) was used to co-transfect into HT29 and HCT116 cells. Fluorescence was observed 24 hours after transfection to confirm transfection efficiency. The transfection method was performed according to the instructions of Lipofectamine 2000 (Invitrogen).

qRT-PCR

Total RNA from CRC tissues and cells was extracted with TRIzol® (Invitrogen) and stored in liquid nitrogen. To quantify miR-411-5p expression, complementary DNA (cDNA) was synthesized using TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, Rockville, MD, USA), followed by qPCR with a TaqMan MicroRNA Assay Kit (Thermo Fisher Scientific). For NFE2L3 mRNA, cDNA was synthesized by reverse transcription using an Avian Myeloblastosis Virus Reverse Transcriptase (Solarbio, Beijing, China). Polymerase chain reaction (PCR) amplification was then performed by adding SYBR® *Premix Ex Taq II* (TaKaRa, Dalian, China). U6 and GAPDH were used as internal controls for miR-411-5p and NFE2L3 mRNA, respectively. The relative expression level was calculated using the $2^{-\Delta\Delta Ct}$ method. Primer information is as follows:

GAPDH: F: 5'-GGAGCGAGATCCCTCCAAAAT-3',
R: 5'-GGCTGTTGTCATACTTCTCATGG-3';
miR-411-5p; F: 5'-CGTACGCTTTATCTGTGACG-3',
R: 5'-GTCAAGTCGGTGGAACG-3'
NFE2L3: F: 5'-CTGACTGGGAAGGCAGAAAAG-3',
R: 5'-TCAGGCTGTGATGAAAGCAA-3';
U6: 5'-TGCGGGTGCTCGCTTCGGCAGC-3',
R: 5'-CCAGTGCAGGGTCCGAGGT-3'

Reverse Transcription: The reverse transcription step was performed using a commercially available reverse transcription kit (e.g., SuperScript III First-Strand Synthesis System, Thermo Fisher Scientific). The reaction mixture contained 1 μg of RNA template, 0.5 μM reverse transcription primer, and the reaction was carried out at 50°C for 60 minutes. The reaction was then terminated by heating the sample at 70°C for 15 minutes.

PCR Amplification: For PCR amplification, 2 μL of cDNA template was added to a PCR reaction mixture containing 10 μL of SYBR Green PCR Master Mix, 0.2 μM forward primer, 0.2 μM reverse primer, and nuclease-free water to make a final volume of 20 μL. The thermal cycling conditions consisted of an initial denaturation step at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension step at 72°C for 5 minutes.

Internal Control (U6 and GAPDH): U6 small nuclear RNA (snRNA) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were chosen as internal controls in this study. U6 is commonly used as a reference gene in many studies due to its stable expression across various tissues and experimental conditions. Similarly, GAPDH is a widely used reference gene known for its consistent expression levels in different cell types. The inclusion of these internal controls allows for normalization of the qRT-PCR data, providing a reliable comparative analysis of the target gene expression levels.

Western blot analysis

RIPA lysate (Beyotime, Shanghai, China) was used to obtain protein from the cells in each group. Once denaturation had occurred, the supernatant was taken for protein sampling. The standard steps of western blot analysis were then followed: sample loading; electrophoresis; membrane transfer; closure; incubation of primary antibody NFE2L3 (Invitrogen, diluted at 1:1000, PA5-102015) and GAPDH (Abcam, Cambridge, MA, USA, diluted at 1:1000, ab8245); incubation of secondary antibody Goat Anti-Rabbit IgG (H&L) HRP (Abcam, diluted at 1:10,000; ab7090); and development exposure. After scanning polyvinylidene difluoride membrane, the strip's grey scale was analysed using ImageJ.

MTT assay

Cells (1×10^4 cells/well) were inoculated on 96-well plates and incubated at 37°C for 24 hours. After incubation for two hours with 10 microlitres of MTT reagent (Solarbio), the cell viability was expressed as a D value by measuring the optical density at 490 nanometres with an enzyme marker. In the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, the cell viability was determined and expressed as a D value. The D value represents the absorbance or optical density (OD) of the formazan dye formed by viable cells.

Transwell assay

After transfection, the cell density was adjusted and 200 microlitres of cell suspension was added to the upper layer of the transwell chambers (Corning, Tewksbury, MA, USA); 500 microlitres of complete medium containing 10% FBS was added to the lower layer of the chambers, which were then placed in an incubator for 48 hours. Cells remaining in the upper chamber after washing in phosphate-buffered saline were removed and treated with 4% paraformaldehyde and crystal violet, respectively, and five random fields of view were taken for staining cell counting. For invasion assays, the transwell of the upper chamber was pre-layered with Matrigel matrix gel.

In the Transwell assay, cell migration or invasion capabilities were measured using Transwell inserts with porous membranes. The pore size of the Transwell membrane was 8 μm.

Flow cytometry assay

After transfection, cells were transferred to 1.5 milliliter Eppendorf tubes and centrifuged (4°C, 1000 revolutions per minute, for 5 minutes) to collect cells for flow cytometry analysis according to Annexin V FITC/PI apoptosis kit (Solarbio) instructions.

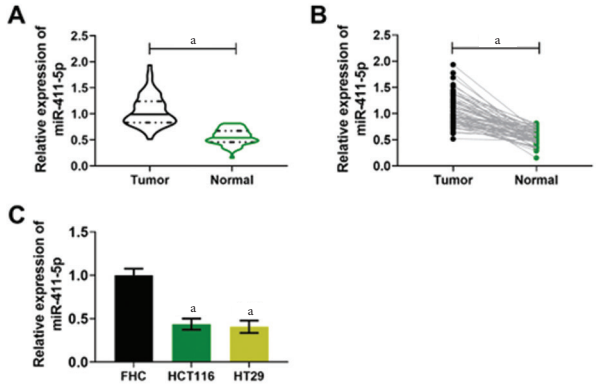
For flow cytometry analysis of apoptosis, the specific fluorophores used were FITC (fluorescein isothiocyanate) and PI (propidium iodide). FITC is commonly used as a fluorophore to label Annexin V, which binds to phosphatidylserine exposed on the outer leaflet of apoptotic cells. PI is used as a DNA-binding dye to distinguish between viable and non-viable cells based on their DNA content.

The choice of FITC and PI fluorophores for apoptosis detection in flow cytometry is based on their specific binding properties and compatibility with the flow cytometry instrument's excitation and emission wavelengths. FITC-conjugated Annexin V allows for sensitive identification of apoptotic cells, while PI staining helps distinguish between early apoptotic (Annexin V-positive, PI-negative) and late apoptotic or necrotic (Annexin V-positive, PI-positive) cell populations.

Dual-luciferase reporter assay

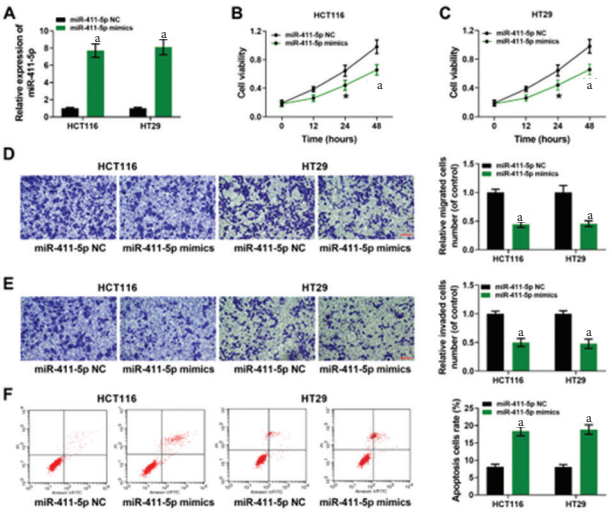
Four bioinformatic sites—TargetScan7.1, READ, COAD and the miRNA Pathway Dictionary Database—were used to predict the targeting relationship between miR-411-5p and NFE2L3.Wild-type (WT) NFE2L3 and mutant (MUT) NFE2L3 were PCR amplified and inserted into pmirGLO reporter vectors as NFE2L3-3'UTR-WT and NFE2L3-3'UTR-MUT. Subsequently, NFE2L3-3'UTR-WT and miR-411-5p NC, NFE2L3-3'UTR-WT and miR-411-5p mimics, NFE2L3-3'UTR-MUT and miR-411-5p NC, NFE2L3-3'UTR-MUT and miR-411-5p mimics were co-transfected into HCT116 and HT29 cells using Lipofectamine 2000, respectively. Cells were collected after 48 hours of incubation, and luciferase activity was detected using Dual-Lucy Assay Kit (Solarbio) and Varioskan™ LUX multifunctional enzyme marker (Thermo Fisher Scientific).

Figure 1. The expression of miR-411-5p in colorectal cancer. (A, B) QRT-PCR detected the expression of miR-411-5p in CRC tumour tissues (n = 60) and normal tissues (n = 60). (C) The expression of miR-411-5p in FHC, HCT116 and HT29 cells were examined using qRT-PCR.



[a]$P < .05$

Figure 2. The impact of overexpressed miR-411-5p in colorectal cancer cells. HCT116 and HT29 cells transfected with miR-411-5p NC and miR-411-5p mimics. (A) QRT-PCR examined the transfection efficiency. (B, C) MTT assay detected the cell viability. (D, E) Transwell assay examined the cell migration and invasion ability. Scale bar, 20 μm. (F) Flow cytometry assay tested the cell apoptosis rate. $P < .05$.

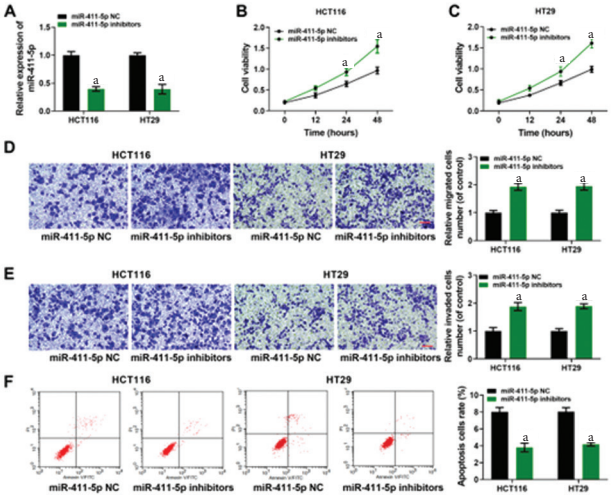


[a]$P < .05$

Statistical analysis

The data were displayed as mean ± standard deviation. Statistical analysis was carried out using the SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). All investigations were repeated more than three times. Cancer and adjacent normal tissue samples were compared using paired *t* tests. Differences between two groups were compared using unpaired *t* tests, and differences between multiple groups were determined using one-way ANOVA followed by Tukey's post hoc test. A result of $P < .05$ was deemed statistically significant.

Figure 3. The impact of knockdown of miR-411-5p in colorectal cancer cells. HCT116 and HT29 cells transfected with miR-411-5p NC and miR-411-5p inhibitors. (A) QRT-PCR examined the transfection efficiency. (B, C) MTT assay detected the cell viability. (D, E) Transwell assay examined the cell migration and invasion ability. Scale bar, 20 μm. (F) Flow cytometry assay tested the cell apoptosis rate.



[a]$P < .05$

RESULTS

miR -411-5p was lowly expressed in CRC tissues.

We found that miR-411-5p was clearly lower in CRC tumor tissues (n = 60) than in normal tissues (n = 60) (all $P < .001$, Figure 1A and 1B). Likewise, the content of miR-411-5p was significantly reduced in HCT116 and HT29 cells compared to FHC (all $P < .001$, Figure 1C).

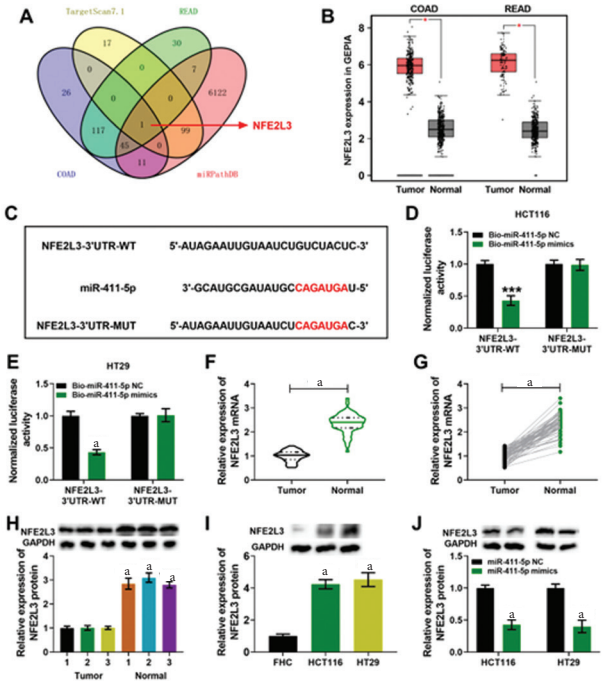
Effect of overexpressed miR-411-5p in CRC cells

HCT116 and HT29 cells were treated with miR-411-5p mimics and their negative control segments, and their overexpression efficiency was examined using qRT-PCR (all $P < 0.001$, Figure 2A). Overexpressed miR-411-5p inhibited the growth of CRC cells (all $P < 0.001$, Figure 2B and 2C). Treating CRC cells with miR-411-5p mimics considerably restricts the cells' migration and invasion abilities ($P < .001$, $P < .001$, Figure 2D and 2E). In addition, the ratio of apoptotic cells was heightened among cells transfected with miR-411-5p mimics (all $P < .001$, Figure 2F).

Effect of knockdown of miR-411-5p in CRC cells

Assay results showed that transfection of miR-411-5p inhibitors in CRC cell lines suppressed the expression of miR-411-5p (all $P < .001$, Figure 3A). A series of cell biological characterization experiments were further performed, and it was found that inhibiting miR-411-5p expression induced a statistically considerable rise in the activity and the migration and invasion abilities of HCT116 and HT29 cells, while the apoptosis rate of CRC cells decreased (all $P < .001$, Figure 3B–3F).

Figure 4. NFE2L3 is a target of miR-411-5p. (A) Four bioinformatic sites predicted the downstream mRNA of miR-411-5p. (B) Analysis of NFE2L3 expression in the GEPIA database. (C) Binding sites of miR-411-5p to NFE2L3 and their mutant forms. (D, E) Binding of miR-411-5p to NFE2L3 was detected by dual-luciferase reporter assay. (F, G) QRT-PCR used to detect the expression of NFE2L3 mRNA in CRC tissues and normal tissues. (H) Western blot detection of NFE2L3 protein expression in CRC tissues and normal tissues. (I) Western blot detection of NFE2L3 protein expression in FHC, HCT116 and HT29 cells. (J) Western blot detection of NFE2L3 protein expression in CRC cells transfected with miR-411-5p mimics.



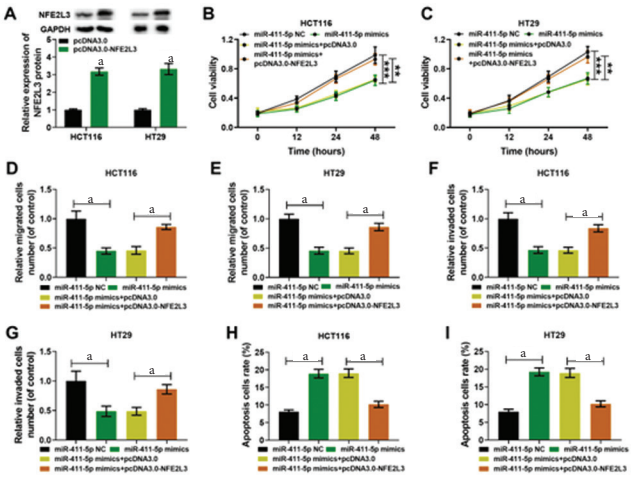
[a]$P < .05$

NFE2L3 is a target gene of miR-411-5p

NFE2L3 was projected to be a downstream target gene of miR-411-5p by four bioinformatics sites (Figure 4A). By analyzing the expression of NFE2L3 in the Gene Expression Profiling Interactive Analysis (GEPIA) database, NFE2L3 was found to be elevated in colon adenocarcinoma (COAD) and rectal adenocarcinoma (READ) tumor tissues ($P < .001$, Figure 4B). Figure 4C shows the binding site of miR-411-5p in the 3'UTR of NFE2L3 and its mutated form.

Further, we constructed a luciferase reporter gene plasmid containing the NFE2L3 3'UTR targeting sequence by synthesizing the mimic fragment of miR-411-5p. Dual-luciferase report assay revealed that overexpression of the mimic fragment of miR-411-5p significantly inhibited the wild-type (NFE2L3-3'UTR-WT) signal of NFE2L3 (all $P < .001$) but was ineffective for its mutant plasmid (NFE2L3-3'UTR-MUT) (all $P > .001$, Figure 4D and 4E). QRT-PCR testing showed higher levels of NFE2L3 in CRC tissues than in normal tissues (all $P < .001$, Figure 4F and 4G). This result was also reflected in the western blot analysis of CRC tissues (all $P < .001$, Figure 4H). Likewise, NFE2L3 protein expression was elevated in HCT116 and HT29 cells (all $P < .001$, Figure 4I). However, in HCT116 and HT29 cells transfected with miR-411-5p mimics, NFE2L3 expression was supressed. (all $P < .001$, Figure 4J). The above data suggest that miR-411-5p inversely modulates NFE2L3 in CRC.

Figure 5. Effect of co-transfection of NFE2L3 and miR-411-5p on colorectal cancer cells. (A) Western blot detection of NFE2L3 expression in HCT116 and HT29 cells transfected with pcDNA3.0-NFE2L3. HCT116 and HT29 cells after co-transfection with miR-411-5p mimics and pcDNA3.0-NFE2L3. (B, C) MTT assay for cell viability. (D–G) Transwell assay for cell migration and invasion. (H, I) Flow cytometry for apoptosis.



[a]$P < .05$

miR -411-5p/NFE2L3 axis regulated the biological behavior of CRC cells.

Western blot analysis showed that transfection of pcDNA3.0-NFE2L3 in CRC cells significantly increased the amount of NFE2L3 (all $P < .001$, Figure 5A). MTT assay revealed that miR-411-5p mimics constrained the growth of CRC cells; conversely, overexpression of NFE2L3 neutralized the effect of miR-411-5p and restored cell growth to normal levels (all $P < .001$, Figure 5B and 5C). It was also found that miR-411-5p inhibited the migratory ability of HCT116 and HT29 cells but that NFE2L3 significantly reduced this inhibitory impact and restored the migratory ability of cancer cells (all $P < .001$, Figure 5D and 5E). Similarly, miR-411-5p and NFE2L3 displayed the same regulatory trends on the invasive ability of CRC cells (all $P < .001$, Figure 5F and 5G). In addition, overexpression of NFE2L3 restored the influence of miR-411-5p mimics on CRC cell apoptosis (all $P < .001$, Figure 5H and 5I).

DISCUSSION

MiRNAs have a wide range of gene-regulating functions and are essential to multiple aspects of tumor development.[11,12] Studies have shown that an imbalance in the regulation of miRNA expression is intimately related to colorectal carcinogenesis and metastasis and malignant phenotype.[13,14]

In the context of our findings, miR-411-5p was underexpressed in CRC, and aberrant expression of miR-411-5p regulated growth, apoptosis, migration, and invasion in CRC cell lines. This analysis may be because aberrant manifestation of miR-411-5p was present in tumor tissues and peripheral blood. In contrast, several types of solid tumor tissues displayed low levels of miR-411-5p expression. MiR-411-5p has already been used to target VASP and GRB2 to restrain the development of breast cancer cells.[15,16] In addition, some studies reported that miR-411-5p was involved in regulating the progression of hepatocellular carcinoma (HCC) through AKT or STAT3 pathways as a downstream of lncRNA KDM4A-AS1, lncRNA MIAT or circ_001569.[17-19] Furthermore, C. Zhang *et al.* found that increased levels of miR-411-5p in head and neck squamous cell carcinomas (HNSCC) positively correlated with lymph node metastasis and metastatic potential in HNSCC patients; this is possibly due to the role of RYBP in regulating lymph node metastasis in HNSCC.[20] B. Zhang *et al.* reported that miR-411 had a low expression in CRC samples and that miR-411 takes part in the development of CRC through the regulation of PRRG4.[21] Our findings and previous findings demonstrate that the role of miR-411-5p in malignancy is heterogeneous and plays different roles in different cancers.

Our experimental results show that NFE2L3 is upregulated in CRC, where it promotes cancer cell growth and is an oncogene, and that NFE2L3 is regulated by miR-411-5p. NFE2L3 is highly expressed in a diversity of malignancy samples, including lymphomas, breast, colorectal and pancreatic cancers, and has a critical role in the biosynthesis of tumor development. NFE2L3 expression was high in HCC, and suppressing NFE2L3 inhibits the proliferation of HCC cells and induces apoptosis; this mechanism may act through the Wnt/β-catenin pathway.[22] Likewise, elevated levels of NFE2L3 in pancreatic cancer patients have a positive correlation with TNM, and the prognosis is poorer in patients with high expression, suggesting that NFE2L3 is a pro-oncogene in pancreatic cancer.[23] In relation to CRC, Bury *et al.* found that NFE2L3 was significantly upregulated in samples from patients with CRC and that inhibition of NFE2L3 by small molecule interference suppressed the growth and migration of CRC cells, possibly by acting through the DUX4/CDK1 complex.[24] Chowdhury *et al.* found that NFE2L3 has multiple regulatory mechanisms in CRC. Under a normal physiological state, NFE2L3 can be degraded by ubiquitination through labeling by HRD1/VCP complex; additionally, NFE2L3 promotes the expression of UHMK1 by binding to sMaf, thus promoting the growth of cancer cells.[25] L. Zhang *et al.* report that overexpression of NFE2L3 leads to a poor prognosis and low survival rate in CRC patients, and inhibition of NFE2L3 by downregulation of CCND1 and p Rb1-ser807/811 can induce cell cycle arrest.[26] Therefore, NFE2L3 is equally tumor-heterogeneous; this may be related to its different upstream and downstream mechanisms, which should be explored in depth.

The intricate interplay between miR-411-5p, NFE2L3, and molecular pathways like AKT and STAT3 provides mechanistic insights into how these molecules influence CRC pathology.

miR-411-5p and AKT pathway: miR-411-5p has been shown to interact with the AKT signaling pathway, which plays a crucial role in cell survival, proliferation, and apoptosis. Activation of AKT pathway promotes cell growth and inhibits apoptosis, contributing to cancer progression. miR-411-5p can target and downregulate key components or regulators of the AKT pathway, such as AKT1 or AKT2, thereby suppressing the pathway's activity. This inhibition of the AKT pathway by miR-411-5p may result in decreased cell survival, proliferation, and enhanced apoptosis, ultimately impeding CRC progression.

miR-411-5p and STAT3 pathway: The STAT3 pathway is another important signaling pathway implicated in cancer development and progression. miR-411-5p has been found to interact with the STAT3 pathway in CRC. STAT3 is a transcription factor that, when activated, promotes cell survival, proliferation, and immune evasion. miR-411-5p can directly target and downregulate STAT3 expression, leading to the inhibition of its pathway. By modulating the STAT3 pathway, miR-411-5p can suppress the growth-promoting and anti-apoptotic effects mediated by STAT3, thereby impeding CRC progression.

NFE2L3 and CRC progression: NFE2L3, also known as NRF3, is a transcription factor involved in cellular stress response and regulation of antioxidant genes. In the context of CRC, NFE2L3 has been implicated in promoting tumor growth and metastasis. It can regulate genes involved in cell cycle progression, apoptosis, and epithelial-mesenchymal transition (EMT). By modulating these pathways, NFE2L3 can contribute to CRC progression by promoting cell proliferation, inhibiting apoptosis, and enhancing invasive and metastatic potential.

The above evidence suggests that the functions of miR-411-5p and NFE2L3 are favorable for clinical use as potential biomarkers or therapeutic targets for CRC. One study found miR-411 to be a risk factor and predictor of poor prognosis in glioblastoma patients.[27] In addition, miR-411 is highly expressed in lung cancer patients, which is clinically associated with patients' lymph node metastasis and TNM staging, and high expression of miR-411 predicts low patient survival.[28] Studies have shown that high expression of NFE2L3 can predict poor prognosis in pancreatic cancer and bladder cancer.[23,29] The above studies suggest that high expression of miR-411-5p and NFE2L3 in cancer can be used as an indicator of poor prognosis for patients, and increased detection of miR-411-5p and NFE2L3 can be considered in clinical care to predict patient prognosis. In our current study, 60 CRC patients were selected, and no clinical data were analyzed due to the small sample size. Follow-up studies were considered to increase the sample size to evaluate the clinical prognosis of CRC patients with miR-411-5p and NFE2L3.

The findings regarding miR-411-5p and NFE2L3 in colorectal cancer (CRC) have promising implications for their potential clinical applications. These molecules could be

developed as biomarkers or therapeutic targets in CRC management. Here are some specific strategies and technologies that could be used to leverage these findings in clinical settings:

Biomarkers for CRC diagnosis and prognosis: miR-411-5p and NFE2L3 have shown differential expression patterns in CRC tissues compared to normal tissues. Therefore, they hold potential as biomarkers for CRC diagnosis and prognosis. Further studies could focus on developing diagnostic assays, such as PCR-based or next-generation sequencing approaches, to detect and quantify the levels of miR-411-5p and NFE2L3 in patient samples, including blood or tissue biopsies. Validation studies across larger patient cohorts are necessary to establish their clinical utility as biomarkers.

Therapeutic targeting of miR-411-5p and NFE2L3: The dysregulation of miR-411-5p and NFE2L3 in CRC suggests their potential as therapeutic targets. Strategies such as antisense oligonucleotides, small interfering RNAs (siRNAs), or miRNA mimics could be explored to modulate the expression and activity of miR-411-5p and NFE2L3. Additionally, the development of small molecule inhibitors or monoclonal antibodies targeting NFE2L3 could be investigated. Preclinical studies using CRC cell lines and animal models are necessary to assess the efficacy and safety of these targeted therapies.

Based on these findings, several key areas for future research can be outlined:

Elucidation of molecular mechanisms: Further studies are needed to unravel the precise molecular mechanisms by which miR-411-5p and NFE2L3 exert their effects on CRC progression. These investigations could involve transcriptomic profiling, protein-protein interaction analyses, and pathway enrichment studies to identify downstream targets and signaling pathways influenced by miR-411-5p and NFE2L3.

Clinical trials: Conducting clinical trials to evaluate the efficacy and safety of targeting miR-411-5p and NFE2L3 in CRC treatment is crucial. These trials can assess the therapeutic potential of miRNA-based therapies or NFE2L3 inhibitors in improving patient outcomes, such as overall survival, disease-free survival, and response rates.

Epidemiological studies: Exploring the roles of miR-411-5p and NFE2L3 across different populations and stages of CRC can provide valuable insights into their clinical relevance and potential as personalized therapeutic targets. Epidemiological studies involving diverse patient cohorts and long-term follow-up data are warranted.

It is important to acknowledge and address any limitations in the study that could affect the interpretation of the results. Some potential limitations may include a small sample size, a lack of long-term follow-up data, or potential confounding factors. Future research efforts should aim to address these limitations by conducting larger studies with diverse patient populations, incorporating long-term follow-up data, and implementing rigorous statistical analyses to ensure robustness and reliability of the findings.

CONCLUSION

In conclusion, the findings regarding miR-411-5p and NFE2L3 in CRC provide valuable insights into their potential clinical applications as biomarkers and therapeutic targets. Leveraging these findings through the development of diagnostic assays, targeted therapies, and further research efforts can significantly impact CRC management and advance personalized treatment strategies. Continued research into the molecular mechanisms, clinical trials, and epidemiological studies will further enhance our understanding of miR-411-5p and NFE2L3 and their roles in CRC progression, ultimately benefiting patients in the future.

DISCLOSURE OF INTEREST

The authors declare that they have no financial conflicts of interest.

FUNDING

President's Fund of Hebei University (School 20220036) Hospital Foundation of the Affiliated Hospital of Hebei University (2022QC32) Baoding Science and Technology Plan (2241ZF297)

ACKNOWLEDGMENTS

None

REFERENCES

1. Baidoun F, Elshiwy K, Elkeraie Y, et al. Colorectal Cancer Epidemiology: Recent Trends and Impact on Outcomes. *Curr Drug Targets*. 2021;22(9):998-1009. doi:10.2174/18735592MTEx9NTk2y
2. Brady LA, Tumiel-Berhalter LM, Schad LA, et al. Increasing Breast, Cervical, and Colorectal Cancer Screenings: A Qualitative Assessment of Barriers and Promoters in Safety-Net Practices. *J Patient Cent Res Rev*. 2021;8(4):323-330. doi:10.17294/2330-0698.1857
3. Patel SG, Karlitz JJ, Yen T, Lieu CH, Boland CR. The rising tide of early-onset colorectal cancer: a comprehensive review of epidemiology, clinical features, biology, risk factors, prevention, and early detection. *Lancet Gastroenterol Hepatol*. 2022;7(3):262-274. doi:10.1016/S2468-1253(21)00426-X
4. Bocchetti M, Ferraro MG, Ricciardiello F, et al. The Role of microRNAs in Development of Colitis-Associated Colorectal Cancer. *Int J Mol Sci*. 2021;22(8):3967. doi:10.3390/ijms22083967
5. Hill M, Tran N. miRNA interplay: mechanisms and consequences in cancer. *Dis Model Mech*. 2021;14(4):dmm047662. doi:10.1242/dmm.047662
6. Zhu Y, Wang C, Becker SA, et al. miR-145 Antagonizes SNAI1-Mediated Stemness and Radiation Resistance in Colorectal Cancer. *Mol Ther*. 2018;26(3):744-754. doi:10.1016/j.ymthe.2017.12.023
7. Mo WY, Cao SQ. MiR-29a-3p: a potential biomarker and therapeutic target in colorectal cancer. *Clin Transl Oncol*. 2023;25(3):563-577. doi:10.1007/s12094-022-02978-6
8. Pourdavoud P, Pakzad B, Mosallaei M, et al. MiR-196: emerging of a new potential therapeutic target and biomarker in colorectal cancer. *Mol Biol Rep*. 2020;47(12):9913-9920. doi:10.1007/s11033-020-05949-8
9. Dai YC, Pan Y, Quan MM, et al. MicroRNA-1246 Mediates Drug Resistance and Metastasis in Breast Cancer by Targeting NFE2L3. *Front Oncol*. 2021;11:677168. doi:10.3389/fonc.2021.677168
10. Saliba J, Coutaud B, Makhani K, et al. Loss of NFE2L3 protects against inflammation-induced colorectal cancer through modulation of the tumor microenvironment. *Oncogene*. 2022;41(11):1563-1575. doi:10.1038/s41388-022-02192-2
11. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov*. 2017;16(3):203-222. doi:10.1038/nrd.2016.246
12. Van Roosbroeck K, Calin GA. Cancer Hallmarks and MicroRNAs: The Therapeutic Connection. *Adv Cancer Res*. 2017;135:119-149. doi:10.1016/bs.acr.2017.06.002
13. He J, Wu F, Han Z, et al. Biomarkers (mRNAs and Non-Coding RNAs) for the Diagnosis and Prognosis of Colorectal Cancer - From the Body Fluid to Tissue Level. *Front Oncol*. 2021;11:632834. doi:10.3389/fonc.2021.632834
14. Tang Y, Zong S, Zeng H, et al. MicroRNAs and angiogenesis: a new era for the management of colorectal cancer. *Cancer Cell Int*. 2021;21(1):221. doi:10.1186/s12935-021-01920-0
15. Liu LB, Jiang ZJ, Jiang XL, Wang S. Up-regulation of SNHG15 facilitates cell proliferation, migration, invasion and suppresses cell apoptosis in breast cancer by regulating miR-411-5p/VASP axis. *Eur Rev Med Pharmacol Sci*. 2020;24(4):1899-1912.
16. Zhang Y, Xu G, Liu G, et al. miR-411-5p inhibits proliferation and metastasis of breast cancer cell via targeting GRB2. *Biochem Biophys Res Commun*. 2016;476(4):607-613. doi:10.1016/j.bbrc.2016.06.006
17. Chen T, Liu R, Niu Y, et al. HIF-1α-activated long non-coding RNA KDM4A-AS1 promotes hepatocellular carcinoma progression via the miR-411-5p/KPNA2/AKT pathway. *Cell Death Dis*. 2021;12(12):1152. doi:10.1038/s41419-021-04449-2
18. Zhang X, Pan B, Qiu J, et al. lncRNA MIAT targets miR-411-5p/STAT3/PD-L1 axis mediating hepatocellular carcinoma immune response. *Int J Exp Pathol*. 2022;103(3):102-111. doi:10.1111/iep.12440
19. Liu H, Xue L, Song C, Liu F, Jiang T, Yang X. Overexpression of circular RNA circ_001569 indicates poor prognosis in hepatocellular carcinoma and promotes cell growth and metastasis by sponging miR-411-5p and miR-432-5p. *Biochem Biophys Res Commun*. 2018;503(4):2659-2665. doi:10.1016/j.bbrc.2018.08.020
20. Zhang C, Wang H, Deng M, et al. Upregulated miR4115p levels promote lymph node metastasis by targeting RYBP in head and neck squamous cell carcinoma. *Int J Mol Med*. 2021;47(4):36. doi:10.3892/ijmm.2021.4869
21. Zhang B, Cao W, Liu Y, Zhao Y, Liu C, Sun B. Circ_0056618 enhances PRRG4 expression by competitively binding to miR-411-5p to promote the malignant progression of colorectal cancer. *Mol Cell Biochem*. 2023;478(3):503-516. doi:10.1007/s11010-022-04525-x
22. Ren Y, Wang Y, Hao S, et al. NFE2L3 promotes malignant behavior and EMT of human hepatocellular carcinoma (HepG2) cells via Wnt/βcatenin pathway. *J Cancer*. 2020;11(23):6939-6949. doi:10.7150/jca.48100

23. Wang H, Zhan M, Yang R, Shi Y, Liu Q, Wang J. Elevated expression of NFE2L3 predicts the poor prognosis of pancreatic cancer patients. *Cell Cycle*. 2018;17(17):2164-2174. doi:10.1080/15384101.2018.1520558
24. Bury M, Le Calvé B, Lessard F, et al. NFE2L3 Controls Colon Cancer Cell Growth through Regulation of DUX4, a CDK1 Inhibitor. *Cell Rep*. 2019;29(6):1469-1481.e9. doi:10.1016/j.celrep.2019.09.087
25. Chowdhury AMMA, Katoh H, Hatanaka A, et al. Multiple regulatory mechanisms of the biological function of NRF3 (NFE2L3) control cancer cell proliferation. *Sci Rep*. 2017;7(1):12494. doi:10.1038/s41598-017-12675-y
26. Zhang L, Hu DL, Tang B, et al. NFE2L3 Inhibition Induces Cell Cycle Arrest at the G0/G1 Phase in Colorectal Cancer Cells through Downregulating CCND1 and pRb1-ser807/811. *Dis Markers*. 2019;2019:2829798. doi:10.1155/2019/2829798
27. Fan W, Yu X, Li K, Zhu M. The alerting expression of microRNA-411 predicts clinical prognosis and regulates tumor progression of glioblastoma. *Bioengineered*. 2021;12(1):8650-8657. doi:10.1080/21655979.2021.1988365
28. Wang SY, Li Y, Jiang YS, Li RZ. Investigation of serum miR-411 as a diagnosis and prognosis biomarker for non-small cell lung cancer. *Eur Rev Med Pharmacol Sci*. 2017;21(18):4092-4097.
29. Qian J, Huang C, Zhu Z, et al. NFE2L3 promotes tumor progression and predicts a poor prognosis of bladder cancer. *Carcinogenesis*. 2022;43(5):457-468. doi:10.1093/carcin/bgac006