Original Article

Clinical Value and Regulatory Mechanism of miR-767-5p in Colorectal Cancer

(colorectal cancer / miR-767-5p / NFIA / interaction)

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Abstract. The poor prognosis of colorectal cancer (CRC) contributes to a yearly increase in CRC mortality, while microRNAs (miRNAs) were found to play a regulatory function in diversiform cancers, including CRC. The objective of this research was to evaluate the clinical value and possible regulatory mechanisms of miR-767-5p in CRC. The expression level of miR-767-5p in CRC tissues and cells was examined. The Kaplan-Meier curve was utilized to analyse the function of miR-767-5p in CRC prognosis. The independent prognostic factors in CRC were assessed by a multivariate COX regression analysis. Additionally, the regulatory mechanism of miR-767-5p in CRC was determined through an *in vitro* cell experiment. The miR-767-5p expression was downregulated in CRC tumour tissues and CRC cells. Indicators such as tumour differentiation, TNM, LNM and miR-767-5p were identified as independent prognostic factors for a poor CRC prognosis. The regulatory relationship between miR-767-5p and nuclear factor I A (*NFIA*) was verified by the dual-luciferase reporter assay, and the *NFIA* expression level was significantly suppressed by over-expressed miR-767-5p. The proliferation, migration and invasion of CRC cells were inhibited by over-expressing miR-767-5p, while the inhibition effect could be reversed by over-expressing *NFIA*. The over-expressed miR-767-5p could serve as a tumour suppressor to inhibit the progression of CRC by suppressing the expression level of *NFIA*.

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Abbreviations: CRC – colorectal cancer, miRNAs – microRNAs, MUT – mutant reporter, NFI – nuclear factor I, *NFIA* – nuclear factor I A gene, WT – wild-type.

Introduction

Colorectal cancer (CRC), along with lung, prostate and breast cancers, is considered to be the greatest killer threatening human life (Brouwer et al., 2020). The mortality rate of CRC is rising worldwide, and the diagnosed new cases are approximately 1.2 million annually, with a five-year survival rate of only 40–60 % (Labianca et al., 2010; Brouwer et al., 2020). Currently, surgery, radiation and chemotherapy are the main treatments for CRC in the clinic. Despite the benefits of surgical resection combined with preoperative chemoradiotherapy, postoperative chemotherapy, targeted immunization and multidisciplinary therapy for most CRC patients, some of the aforementioned treatment methods can result in irreversible bodily harm for patients with CRC, and tumour recurrence (both local and distant) post-primary tumour resection still results in high cancer mortality rates (Longo and Johnson, 2002). Therefore, the identification of novel therapeutic targets for CRC has the potential to generate innovative insights for the clinical management of CRC. Additionally, in the process of

postoperative nursing, the effective monitoring and intervention of poor prognostic factors can effectively prevent the recurrence of CRC and then reduce the mortality of postoperative recurrence.

MicroRNAs (miRNAs), a class of non-coding RNAs, have been demonstrated to exert diverse functions in various aspects of cell biology, including cell proliferation, apoptosis, migration and invasion (Saliminejad et al., 2019). Given the multifarious functions of miRNAs, the involvement of miRNAs in the progression of CRC has attracted considerable attention. It has been established that dysregulation of miRNA exerts an effect on the growth, infiltration or metastasis of the tumours through regulating its downstream genes, thereby influencing the progression of CRC, which will open up a new road for the treatment of CRC and provide novel biomarkers for CRC prognosis (Hill and Tran, 2021; Huang et al., 2021). For instance, the circPVT1/miR-45 axis can be regarded as a therapeutic target of CRC, and the proliferation of CRC cells could be promoted by miR-130b-3p (Wang et al., 2019; Song et al., 2022). Additionally, miR-29a-3p, miR-196 and miR-485-5p have also been identified as potential biomarkers and therapeutic targets in CRC (Pan et al., 2020; Pourdavoud et al., 2020; Mo and Cao, 2023). miR-767-5p, dysregulated in the progression of multiple cancers such as thyroid cancer, glioma and hepatocellular carcinoma, was identified as one of the key regulators of a variety of genes implicated in CRC following the findings of the Long study, which screened a network of miRNAs for CRC and identified 26 miRNAs targeting the relevant genes or transcription factors of CRC (Zhang et al., 2019; Jia et al., 2020; Zhang et al., 2020; Long et al., 2022). Nevertheless, the dysregulated expression of miR-767-5p in CRC remains to be substantiated by clinical or *in vitro* research.

According to the above information, we suppose that miR-767-5p may show clinical significance in CRC progression. The miR-767-5p expression level in CRC tumour tissues and the independent prognostic factors that may predict a poor prognosis for CRC were investigated in this research. Additionally, the possible regulatory mechanism of miR-767-5p in the CRC was explored to provide a novel therapeutic target for the treatment of CRC.

Material and Methods

Subjects and Materials

Clinical subjects

All procedures used in this study adhere to the tenets of the Declaration of Helsinki. The study was permitted by the Ethics Review Committee of Hunan Cancer Hospital (No. 20190031) and the informed consent was signed by all participants after understanding the purpose of this research.

This study enrolled 133 patients diagnosed with CRC who underwent surgical treatment at Hunan Cancer Hospital between 2019 and 2021. Inclusion criteria: the patients were diagnosed with CRC by pathological diagnosis. No anti-cancer treatment was received by the patients prior to surgery.

Exclusion criteria: 1) Patients who underwent neoadjuvant chemoradiotherapy or Chinese medicine treatment before the operation. 2) Patients who underwent metastatic and recurrent CRC. 3) Patients suffered from two or more types of tumours. 4) Patients with serious diseases, such as cardiovascular disease.

Tissues

The CRC tumour tissues and the normal paracancer tissues were collected during the surgery and then stored at –80 °C for later research. The tissues were observed and confirmed by at least two clinical pathologists.

Cells

NCM460 cells (human colonic epithelial cells) and four CRC cell lines (SW837, LoVo, SW480 and SNU796) were sourced from the Shanghai Cell Bank (Shanghai, China).

Methods

Clinical general data collection

Key indicators, including age, gender and tumour status, were recorded and analysed. The subjects were followed up for 5 years (3 to 60 months), and a 5-year survival rate study was performed to collect related indicators (differentiation, location, TNM, LNM, tumour size, survival time and subject state) for survival data evaluation.

Cell culture and transfection

DMEM complete medium with 10 % foetal bovine serum (FBS, Gibco, NY) was used for the cell culture. The cell culture conditions were 37 °C and 5 % CO_2.

Before transfection, the cells were counted, and then the cells were seeded in 96-well plates and cultured in DMEM complete medium at 37 °C with 5 % CO_2. miR-767-5p mimic and inhibitor, pcDNA 3.1 *NFIA* (oe *NFIA*) and the negative control (mimic NC and oe NC) were ordered from Merck KGaA (Darmstadt, Germany) and Thermo Fisher Scientific (Waltham, MA). The miR-767-5p mimic and oe *NFIA* were used for the over-expression of miR-767-5p and *NFIA*, respectively. The suppression of miR-767-5p was performed by a miR-767-5p inhibitor. miR-767-5p mimic, miR-767-5p inhibitor, oe *NFIA* and negative control were transfected several times into LoVo and SW837 cells with the help of Lipofectamine 2000 (Thermo Fisher Scientific) following different experimental operations. Post-transfection, the cells were cultured for 24–72 hours in an incubator (Thermo Fisher Scientific).

Analysis of the miR-767-5p and NFIA expression levels

The collected tissues obtained from the subjects were disintegrated, and the cells were also collected after

transfection for 24–72 h. Trizol (Sigma-Aldrich, Saint Louis, MO) was added to the tissues and cells followed by centrifugation at 3,000 *g* to collect the supernatant. The supernatant was mixed with chloroform for 15 s, and after centrifugation at 12,000 *g*, the supernatant was collected. Isopropanol was used to precipitate the RNA in the supernatant and the precipitate was washed with 75 % ethanol and then collected after centrifugation at 8,000 *g*. The purity and concentration of the extracted RNA were analysed by NanoDrop-2000 (Thermo Fisher Scientific). Then, the Invitrogen SuperScript reverse transcription kit (Thermo Fisher Scientific) was used to reverse RNA to cDNA. The quantification of miR-767-5p and NFIA was performed in the 7500 qRT-PCR system (Applied Biosystems, Carlsbad, CA) using the SYBR kit (Invitrogen, Carlsbad, CA). Finally, the equation $2^{-\Delta\Delta Ct}$ was used to calculate the miR-767-5p and *NFIA* expression levels. *U6* and *GAPDH* were used as internal references. The sequences of the primers and gene vectors were listed in Supplementary Table (ST) 1.

Analysis of cell proliferation

The CCK-8 assay was utilized for the proliferation analysis. After different timings (0, 24, 48, and 72 h) of cell transfection, CCK-8 (Dojindo Molecular Technologies, Kumamoto Prefecture, Japan) was added into each well of the plates and incubated for an hour. After the incubation, the OD450 value was analysed.

Analysis of cell migration and invasion

The migration and invasion of CRC cells were analysed by a Transwell assay. The transfected cells were counted and seeded in the upper chambers of Transwell plates (Corning, NY) following a 24-hour starvation period. Matrigel was pre-coated in the upper chamber for the invasion assay. FBS-free DMEM medium was added into the upper chamber, while DMEM complete medium serving as the chemoattractant was added into the bottom chamber. The Transwell plates were incubated for 48 hours at 37 °C and 5 % CO_2. After removal of the upper chamber, the cells in the bottom chamber were immobilized using methol (Merck), and then 0.1 % crystal violet (Solarbio, Beijing, China) was used for the cell staining. Thirty minutes later, the cells in the bottom chamber were washed with PBS softly, and then the cells were observed and counted using a microscope (Nippon Kogaku Kogyo Kabushiki Kaisha, Tokyo, Japan).

Prediction of the miR-767-5p downstream genes and their regulatory relationship validation

The downstream genes of miR-767-5p were searched using TargetScanHuman (https://www.targetscan.org/vert_72/), and the retrieval results were collected and analysed. The final downstream target gene of miR-767-5p was determined by the Venn diagram. As CRC progression could be inhibited by suppressing the expression of *NFIA* (Hu et al., 2020). *NFIA* was selected as the miR-767-5p downstream gene for further analysis.

In the dual-luciferase reporter assay, the binding site of *NFIA* on miR-767-5p was predicted by TargetScanHuman (https://www.targetscan.org/vert_72/). The sequences of *NFIA* 3'UTR with miR-767-5p binding sites sub-cloning into pGL-3 luciferase plasmids (Promega Corporation, Madison, WI) were taken as the *NFIA* wild-type reporter (*NFIA*-WT), and the *NFIA* mutant reporter (*NFIA*-MUT) was established by point mutation performed at the binding site. Mimic and inhibitor of miR-767-5p with a negative control were co-transfected with *NFIA*-WT/*NFIA*-MUT into CRC cells by using Lipofectamine 2000. After 48 hours of co-transfection, the relative luciferase activity of *NFIA*-WT and *NFIA*-MUT was evaluated by the Dual-Glo® Luciferase Assay System (Promega), normalized to renilla.

Statistical analysis

Data analysis and diagram creation were performed using SPSS and GraphPad Prism, with all data presented as mean values ± SD. Each experiment was performed at least three times. The χ^2 test and *t*-test were utilized for the difference assessment. The Kaplan-Meier curve was used for the 5-year survival rate evaluation, and multivariate COX regression analysis was utilized for investigation of independent prognostic factors. Results were considered significant differences if $P < 0.05$.

Results

The miR-767-5p expression level in CRC tumour tissues and cells

Compared to normal paracancerous tissues, miR-767-5p expression levels in CRC tumour tissues were obviously decreased (Fig. 1A). The decreasing miR-767-5p expression levels were also observed in CRC cells (SW480,

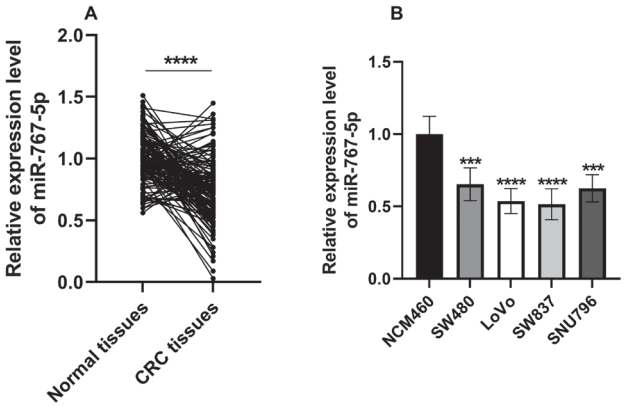

Fig. 1. Comparison of miR-767-5p expression levels. (**A**) The miR-767-5p expression levels in tumour and normal tissues. (**B**) The miR-767-5p expression level in NCM460 and CRC cell lines (SW837, LoVo, SW480, and SNU796). The experiment was performed six times. $^{***}P < 0.001$, $^{****}P < 0.0001$.

LoVo, SW837 and SNU796) compared to NCM460 (Fig. 1B), and the difference was significant. The LoVo and SW837 cell lines were selected for further research.

The association between miR-767-5p expression level and clinical indicators

Based on the median miR-767-5p expression level in the CRC tumour tissues, all subjects were divided into a high miR-767-5p group (N = 60) and a low miR-767-5p group (N = 73). The association between the miR-767-5p expression level and clinical indicators (age, gender, tumour differentiation, tumour location, TNM, LNM and tumour sizes) was assessed by the χ^2 test, and the result is shown in Table 1. There was a significant association between miR-767-5p expression and tumour differentiation (P = 0.035), TNM (P = 0.014) and LNM (P = 0.046). The number of subjects with poorly differentiated tumour tissues, TNM stage III and positive LNM at a low miR-767-5p level was higher than that at a high miR-767-5p level. No significant difference was found between the miR-767-5p expression level and the other indicators (age, gender, location and tumour size).

The role of miR-767-5p expression level in the prognosis of CRC

The Kaplan-Meier survival curve was utilized to evaluate the 5-year survival rate. The group with high miR-767-5p levels demonstrated a higher cumulative survival rate compared to the group with low miR-767-5p levels (Fig. 2). A further multivariate COX regression analysis was used to assess the independent prognostic factors for poor CRC prognosis (Table 2). The miR-767-5p expression level was confirmed to serve as an independent prognostic indicator of CRC with an HR value of 0.333 (95% CI = 1.073–8.427, P = 0.036), together with differentiation (HR 0.423, 95% CI = 1.032–5.422, P = 0.042), TNM (HR 2.450, 95% CI = 1.033-5.812, P = 0.042) and LNM (HR 2.499, 95% CI = 1.055–5.916, P = 0.037).

The effect of up-regulated miR-767-5p on the proliferation, migration and invasion of LoVo and SW837 cells

The effect of miR-767-5p over-expression on the cell proliferation, migration and invasion of LoVo and SW837 cells was evaluated by transfection of the miR-767-5p mimic. The miR-767-5p expression level in LoVo and SW837 cells after transfection with the miR-767-5p mimic was analysed by qRT-PCR and is shown in Figure 3A. No significant difference was noticed in the mimic NC group compared to the control group, indicating that there was no influence of the mimic NC on the expression of miR-767-5p in both LoVo and SW837 cells. However, the miR-767-5p expression level was increased after transfection with the miR-767-5p mimic, implying that the miR-767-5p mimic was successfully transfected into both LoVo and SW837 cells.

Table 1. Association of miR-767-5p expression level and clinical indicators of the subjects

Indicators		**Number of cases (N = 133)**	**Expression level of miR-767-5p**		**P value**
			Low (N = 73)	**High (N = 60)**	
Age (yrs)					0.645
	< 60	65	37	28	
	≥ 60	68	36	32	
Gender					0.795
	Male	56	30	26	
	Female	77	43	34	
Differentiation					0.035*
	Poor	46	31	15	
	High-moderate	87	42	45	
Location					0.669
	Rectum	67	38	29	
	Colon	66	35	31	
TNM					0.014*
	I-II	92	44	48	
	III	41	29	12	
LNM					0.046*
	Negative	83	40	43	
	Positive	50	33	17	
Tumour size (cm)					0.291
	< 5	89	46	43	
	≥ 5	44	27	17	

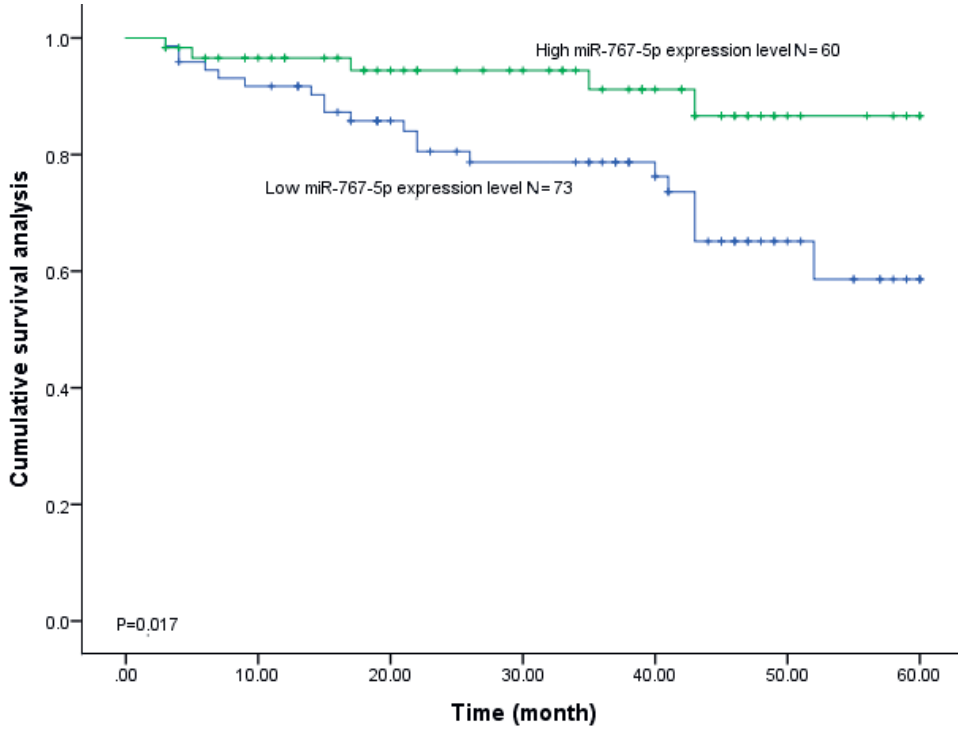

Fig. 2. The Kaplan-Meier curve of the 5-year survival analysis

Table 2. Evaluation of independent prognostic factors for CRC poor prognosis by multivariate COX regression analysis

Indicators	P value	HR	95% CI
miR-767-5p	**0.036***	3.007	1.073–8.427
Age	0.313	1.545	0.663–3.602
Gender	0.732	1.175	0.466–2.960
Differentiation	**0.042***	2.365	1.032–5.422
Location	0.710	1.175	0.502–2.745
TNM	**0.042***	2.450	1.033–5.812
LNM	**0.037***	2.499	1.055–5.916
Tumour size	0.070	2.289	0.935–5.602

Note: *P < 0.05.

After 48 hours of miR-767-5p mimic transfection, the proliferation of LoVo and SW837 cells was evaluated by the CCK-8 assay, and the over-expressed miR-767-5p could significantly decrease the proliferation of LoVo (Fig. 3B) and SW837 (Fig. 3C) cells compared to the control group. The migration and invasion of the LoVo and SW837 cells were assessed by the Transwell assay. According to the results of migration (Fig. 3D and E), over-expression of miR-767-5p showed a suppressive effect on the migration of both LoVo and SW837 cells. Additionally, the over-expression of miR-767-5p also inhibited the invasion (Fig. 3F and G) of LoVo and SW837 cells, as invasive cells were decreased by the up-regulated miR-767-5p.

Validation of the interaction between miR-767-5p and NFIA

The binding site between miR-767-5p and *NFIA* (Fig. 4A), which was predicted by TargetScanHuman, indicated that there was a regulatory relationship between miR-767-5p and *NFIA*. The interaction between miR-767-5p and *NFIA* was validated by the dual-luciferase reporter assay. For *NFIA*-WT in both LoVo (Fig. 4B) and SW837 (Fig. 4C) cells, the relative luciferase activity was inhibited by over-expressed miR-767-5p compared to the control group, while suppressing the expression level of miR-767-5p could increase the relative luciferase activity of *NFIA*-WT, suggesting that the expression of *NFIA* was regulated by miR-767-5p. Furthermore, no significant difference was observed among the groups for *NFIA*-MUT, demonstrating that the mutated *NFIA* lost its capacity to interact with miR-767-5p. The regulatory relationship between miR-767-5p and *NFIA* was further evaluated by qRT-PCR. After miR-767-5p was over-expressed in the LoVo (Fig. 4D) and SW837 (Fig. 4E) cells, the *NFIA* expression level was notably suppressed, while over-expression of *NFIA* could enhance the *NFIA* expression level that was suppressed by miR-767-5p. The dual-luciferase reporter assay and qRT-PCR results indicated a regulatory relationship between miR-767-5p and *NFIA*; the expression level of *NFIA* could be inhibited by over-expressed miR-767-5p.

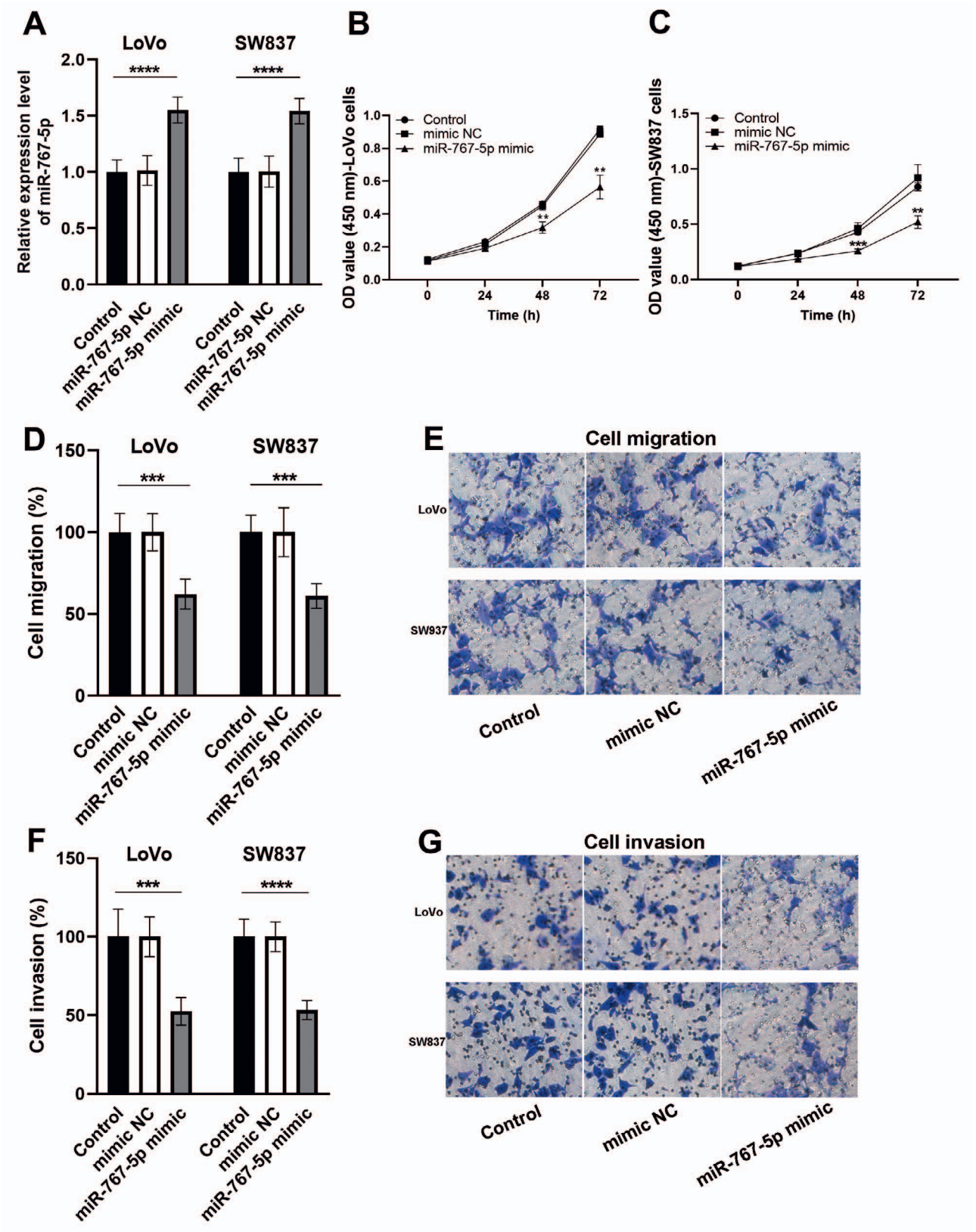

Fig. 3. The impact of over-expressed miR-767-5p on the LoVo and SW837 cells. (**A**) The expression level of miR-767-5p in LoVo and SW837 cells post-transfection. (**B**) and (**C**), the proliferation of LoVo and SW837 cells. (**D**) and (**E**), the migration of LoVo and SW837 cells. (**F**) and (**G**), the invasion of LoVo and SW837 cells. Each experiment was performed at least three times. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

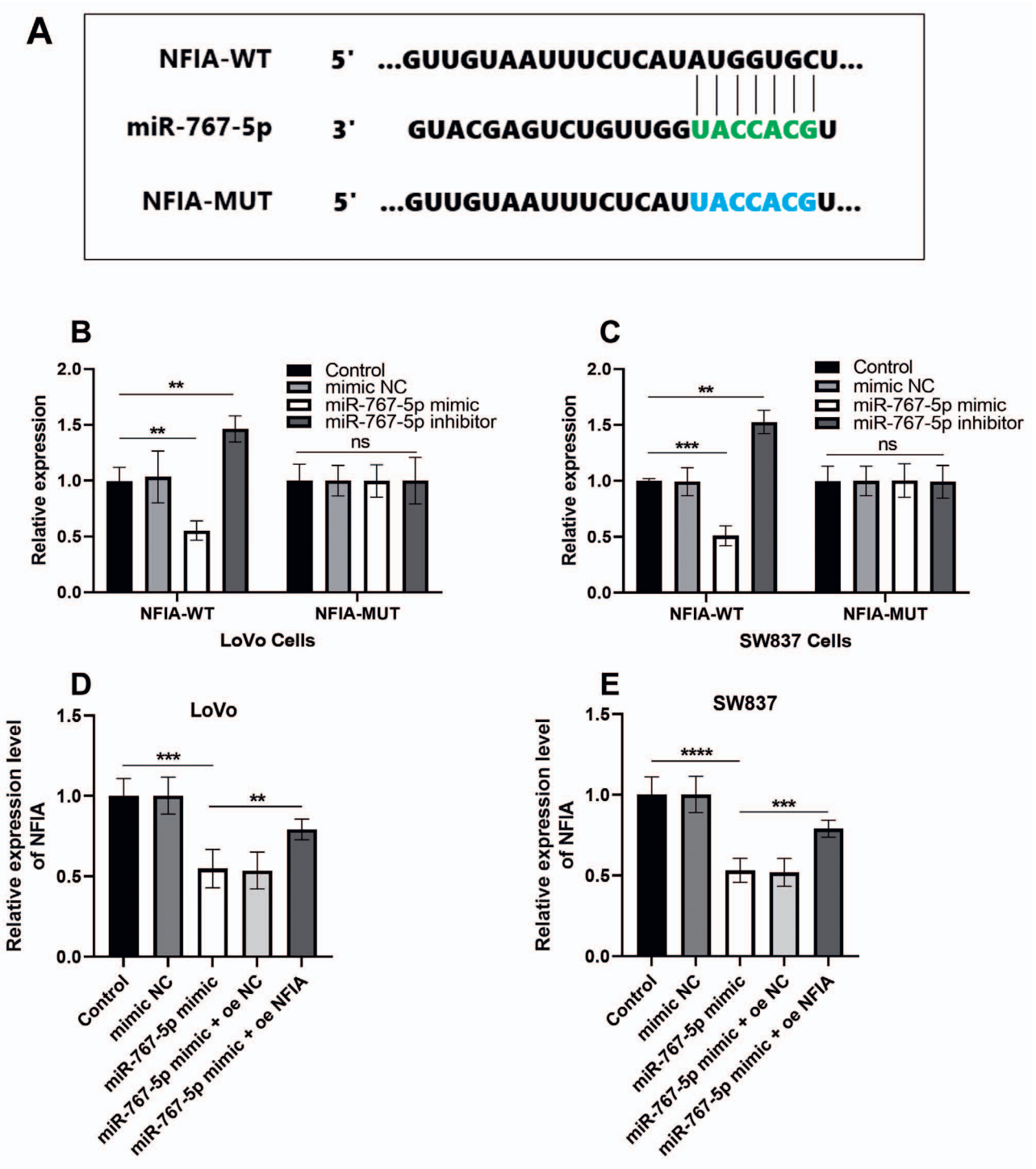

Fig. 4. The validation of the interaction between miR-767-5p and *NFIA*. (**A**) Sequences of the binding site of miR-767-5p and *NFIA*. (**B**) and (**C**), the relative luciferase activity of *NFIA*-WT in LoVo and SW837 cells was significantly suppressed by over-expressed miR-767-5p and enhanced by miR-767-5p silencing. (**D**) and (**E**), the expression of *NFIA* was visibly down-regulated by over-expressed miR-767-5p and could be enhanced by the oe *NFIA* vector. Each experiment was performed at least three times. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, ns: no significant difference.

The effect of the miR-767-5p/NFIA axis on the proliferation, migration and invasion of CRC cells

The effect of the miR-767-5p/*NFIA* axis on cell proliferation was evaluated by the CCK-8 assay. Over-expression of *NFIA* could counteract the inhibition of proliferation caused by over-expressed miR-767-5p in LoVo and SW837 cells and showed a promotive effect on the proliferation of LoVo (Fig. 5A) and SW837 (Fig. 5B) cells. In addition, the migration and invasion of

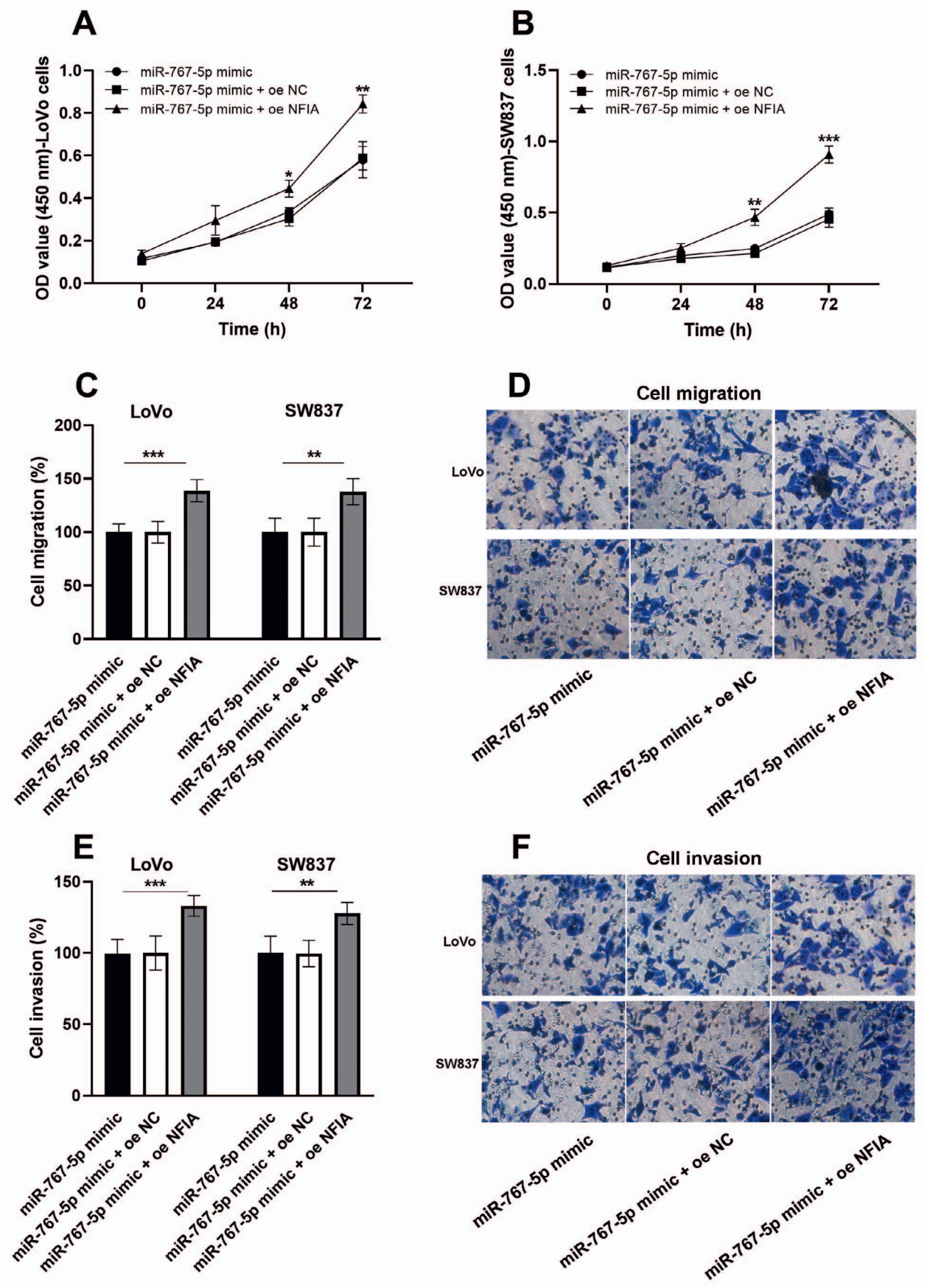

Fig. 5. The impact of the interaction between miR-767-5p and *NFIA* on the proliferation, migration, and invasion of LoVo and SW837 cells. (**A**) and (**B**), the proliferation of LoVo and SW837 cells. (**C**) and (**D**), the migration of LoVo and SW837 cells. (**E**) and (**F**), the invasion of LoVo and SW837 cells. Each experiment was performed at least three times. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

both LoVo and SW837 cells were assessed by the Transwell assay. In comparison with the miR-767-5p mimic group, the over-expressed *NFIA* could enhance the migration (Fig. 5C and D) and invasion (Fig. 5E and F) that were inhibited by over-expressed miR-767-5p in the LoVo and SW837 cells, demonstrating an enhancing effect of *NFIA* on the migration and invasion of CRC cells.

Discussion

CRC is the most common gastrointestinal malignancy worldwide and is primarily treated by drug therapy, surgical resection and endoscopic resection. However, many patients develop drug resistance after treatment, leading to a poor prognosis (Labianca et al., 2013; Tomasello et al., 2017). An effective CRC monitoring indicator and postoperative nursing indicator are needed to strengthen the monitoring and nursing for CRC post operation and to avoid the occurrence of a poor CRC prognosis. miRNAs have proved to be highly sensitive biomarkers for a variety of diseases and could play a diagnostic role in the early asymptomatic stages of different kinds of diseases. miRNAs could also predict the prognosis of diseases and cancers (He et al., 2020; Hill and Tran, 2021). miR-767-5p was shown to be expressed differentially in various tumours. A previous study reported that miR-767-5p was down-regulated in human glioblastoma multiforme, and the proliferation, migration and invasion of glioma cells could be suppressed by the over-expressed miR-767-5p (Zhang et al., 2019). miR-767-5p was also up-regulated in thyroid cancer and could be inhibited by LINC-PINT to induce TET2 expression and then suppress the development of thyroid cancer (Jia et al., 2020). The dysregulated expression level of miR-767-5p was also found in multiple myeloma and breast cancer (Feng et al., 2019; Wang et al., 2021).

In this research, down-regulation of miR-767-5p expression was observed in CRC tumour tissues and CRC cells, suggesting that over-expressed miR-767-5p might have a protective value in CRC. The Kaplan-Meier analysis showed that subjects with a high miR-767-5p expression level showed a higher survival rate than those with a low miR-767-5p expression level, further confirming the protective value of the up-regulated miR-767-5p expression in CRC. Tumour differentiation, TNM and LNM were three indicators used for cancer severity assessment (Manikantan et al., 2009; Li et al., 2021; Yuan et al., 2023). The risk is higher in poorly differentiated tumours with advanced TNM stages compared to highly differentiated tumours with low TNM stages. LNM positivity indicates lymph node metastasis, posing a serious threat to the patient's life (Yu et al., 2013; Zhang et al., 2022). This study revealed a significant association between tumour differentiation, TNM, LNM and miR-767-5p expression levels, and the correlation between miR-767-5p and CRC development was then verified. Moreover, the up-regulation of miR-767-5p expression served as an independent prognostic factor for CRC poor prognosis and could act as a nursing indicator for postoperative monitoring. NFIA belongs to the nuclear factor I (NFI) family and was demonstrated to have a carcinogenic effect in different kinds of cancer. According to Yang's study, *NFIA* could serve as an independent prognostic factor for oesophageal squamous carcinoma (Yang et al., 2018). The expression level of *NFIA* could be inhibited by miR-212-3p over-expression to suppress the development of bladder cancer (Wu et al., 2019). In this study, the over-expressed *NFIA* reversed the inhibitory effects of over-expressed miR-767-5p on CRC cell proliferation, migration and invasion, confirming the carcinogenic effect of *NFIA*. Uncontrolled cell proliferation, invasion and migration caused by dysregulation of adequate protein-coding gene expression and abnormal activation of signalling pathways were the greatest characteristics of CRC cells and other malignant tumour cells (Agarwal et al., 2017). Inhibition of these processes could suppress the progression of tumours. A previous study reported that proliferation, migration, invasion and many other cellular processes could also be regulated by miRNAs (Garofalo and Croce, 2011), and the regulatory mechanism should be due to the fact that specific bases of target mRNA could be complemented and paired by miRNAs after transcription, causing degradation of target mRNA or inhibiting its translation, thus achieving negative regulation of the target gene (Lee et al., 1993). For instance, the dysregulated miR-767-5p expression could suppress the proliferation, migration, and promote the apoptosis of glioma cells via regulating its downstream gene, SUZ_{12} (Zhang et al., 2019). miR-29c-3p showed a regulatory relationship with *NFIA* and was involved in the proliferation, migration and invasion in CRC cell lines, thereby influencing the progression of CRC (Hu et al., 2020). In our study, the effects of miR-767-5p and *NFIA* on the proliferation, migration and invasion of CRC cell lines were also evaluated. As miR-767-5p over-expression could inhibit CRC cell proliferation, migration and invasion, which could be attenuated by over-expression of *NFIA*, we confirmed their involvement in CRC progression and highlighted the regulatory function of miRNAs in tumour development.

The investigation of the functions of miRNA in human diseases is still at the initial stage, and most functions of the genes have not been explored. The role of miRNAs in human diseases is broad and diverse, and any research that may bring breakthroughs in the diagnosis and treatment of human diseases is meaningful. miR-767-5p was down-regulated in CRC, and the possible regulatory mechanism of the miR-767-5p/*NFIA* axis in CRC progression was first demonstrated by this study, which provided a theoretical therapeutic target for clinical treatment of CRC. However, some limitations still exist in this study that warrant further evaluation. Firstly, it is not clear whether CRC patients in other regions have similar clinical tumour indicators or whether regional differences exist due to the small sam-

ple size. Secondly, the down-regulated miR-767-5p expression promoted the proliferation of CRC cells, while the tumour size showed no significant relationship with the miR-767-5p expression level, and this result may be affected by the small sample size. Lastly, in the regulatory effect of miR-767-5p on CRC, only one of the downstream genes of miR-767-5p, *NFIA*, was evaluated. Whether any other downstream or upstream genes could be regulated by miR-767-5p in CRC progression remains to be further assessed. In further studies, the sample size will be increased, and a more complete network of the miR-767-5p regulatory mechanisms in CRC progression will be investigated. Furthermore, we will delve into the full regulatory mechanism of CRC progression.

Conclusion

In summary, miR-767-5p was down-regulated in CRC tumour tissues and cell lines and could serve as an independent prognostic factor for evaluating the poor prognosis of CRC. miR-767-5p was regarded as a tumour suppressor to inhibit the progression of CRC by suppressing *NFIA* expression.

Conflict of interest

Authors declare no conflict of interests for this article.

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