Tohoku J. Exp. Med., 2023, **259**, 77-84

LncRNA MHENCR Predicts Poor Outcomes in Patients with Colorectal Carcinoma and Modulates Tumorigenesis by Impairing MiR-532-3p

Dong Zhou,[1,*] Zhiwei Liao,[1,*] Xiaojun Chen,[1] Yijuan Fan[2] and Hua Zuo[1]

[1]Department of General Surgery, Renhe Hospital, Shanghai, China
[2]Department of Pathology, Renhe Hospital, Shanghai, China

The long noncoding RNAs (lncRNAs) are widely involved in the progression of various malignant tumors. The current study investigated the role and mechanism of lncRNA melanoma highly expressed noncoding RNA (MHENCR) in colorectal carcinoma. The expression of MHENCR was measured by real-time quantitative reverse transcription PCR (RT-qPCR). The chi-square analysis was used to analyze the correlation between MHECNR and miR-532-3p. Kaplan-Meier curve and multivariate Cox regression analysis were conducted to assess the significance of MHENCR in clinic. The interaction of MHENCR and miR-532-3p was probed using Pearson analysis and dual-luciferase reporter assay. Cellular experiments were implemented to explore the effects of MHENCR/miR-532-3p on colorectal carcinoma cells. Compared with para-cancerous tissues, MHENCR expression was increased and miR-532-3p expression was decreased in tumor tissues. High expression of MHENCR exhibited shorter overall survival. Interfering of MHENCR suppressed cellular activities while the silence of miR-532-3p diminished the decreased cellular behaviors in colorectal carcinoma cells. Interfering with MHENCR expression represses colorectal carcinoma cell proliferation, migration, and invasion by regulating miR-532-3p. MHENCR may act as a novel prognostic marker in colorectal carcinoma and MHENCR/miR-532-3p may serve as a potential target for treating colorectal carcinoma.

Keywords: colorectal carcinoma; lncRNA MHENCR; miR-532-3p; prognosis; progression
Tohoku J. Exp. Med., 2023 January, **259** (1), 77-84.
doi: 10.1620/tjem.2022.J089

Introduction

Colorectal carcinoma (CRC), a high-risk gastrointestinal tumor, is the third most common malignant tumor in humans (Siegel et al. 2022). In China, the prevalence of CRC is the fifth-highest cancer with the increase in obesity rate and westernized lifestyle (Cao et al. 2021). With the development of society, the increase of CRC diagnostic approach and the improvement of strategies have made the early detection of CRC easier, however, due to factors such as untimely early screening and inconspicuous symptoms, leading to delayed diagnosis and poor survival (Roncucci and Mariani 2015). With the development of biology and bioinformatics, it is easier to find drug targeting molecules and molecular markers (Walcher et al. 2020; Zhong et al. 2021). Therefore, it is extremely important to investigate the pathogenesis of CRC, and explore predictive markers, and novel treatment strategies for treating CRC.

Recently, long non-coding RNAs (lncRNAs) containing no less than 200 nucleotides have emerged as important participants in cancer (Bhan et al. 2017; Taniue and Akimitsu 2021). Increasing studies indicated that lncRNAs affect tumorigenesis and development by participating in the regulation of cell apoptosis, tumor infiltration, and metastasis, as well as associated with the prognosis of tumor patients (Galamb et al. 2019). However, the functions of most lncRNAs remain unclear. LncRNAs can act as competing endogenous RNAs (ceRNAs) or natural miRNA sponges and are often involved in post-transcriptional regulation by interacting with miRNAs or mRNAs

Received September 15, 2022; revised and accepted October 17, 2022; J-STAGE Advance online publication October 27, 2022
*These two authors contributed equally to this work.
Correspondence: Hua Zuo, Department of General Surgery, Renhe Hospital, No.1999, Changjiang West Road, Baoshan District, Shanghai 200431, China.
e-mail: zuohua_renhe@163.com

(Grelet et al. 2017; Ghasemi et al. 2020). Exploring the function of lncRNA/miRNA regulation and discovering the molecular mechanism of CRC is crucial. The melanoma highly expressed noncoding RNA (MHENCR, also named CTD-3184A7.4) is upregulated in melanoma and facilitates melanoma progression (Chen et al. 2017). Recent studies also indicated that MHENCR was abnormally expressed and linked with the prognosis of patients with lung adenocarcinoma or clear cell renal cell (Zhong et al. 2021; Cao et al. 2022). These studies revealed that MHENCR expression may participate and play important role in tumorigenesis.

In a previous study identifying differently expressed lncRNAs in CRC tissues, we noted that MHENCR was upregulated in CRC (Xu et al. 2019). We speculate that MHENCR may participate in the progression of CRC. In our own clinical CRC tissues, we confirmed the increased expression of MHENCR. Herein, the association between MHENCR expression and clinical parameters and CRC patients' overall survival was analyzed. Moreover, the underlying molecular mechanisms devoted to the functions of MHENCR in CRC were also explored.

Materials and Methods

Clinical specimens

One hundred forty-three paired CRC tissues and adjacent para-cancerous tissue specimens were obtained from patients who received surgical resection treatment at the Renhe Hospital from February 2016 to January 2019. Tissue specimens were stored in liquid nitrogen. Inclusion criteria for CRC patients include: 1) All specimens were confirmed by histological diagnosis. 2) The patients with CRC had not received anti-tumor treatments before specimen collection. 3) Patients have complete clinical parameters and postoperation overall survival information (up to five years, median 42 months). The clinical parameters were listed in Table 1. All patients and their families signed written informed consent, and the study protocol was approved by the Ethics Committee of Renhe Hospital.

Cell source

Origin of cell lines: Normal colon epithelial cells (NCM460) and CRC cell lines (HT29, HCT116, SW480, SW620, and LoVo) were obtained at the Institute of Biochemistry and Cell Science, Chinese Academy of Sciences (Shanghai, China). NCM460 and CRC cell line HCT116 were incubated in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS). The other CRC cells were incubated in Roswell Park Memorial Institute (RPMI)-1640 medium with 10% FBS and maintained in a humidified atmosphere at 37°C with 5% CO_2.

Cell plasmids and miRNAs transfection

Two cell lines with high expression of MHENCR (HCT116 and SW480) were selected for further culture, and further cell silencing experiments and subsequent functional experiments were performed. The MHENCR shRNA (sh-MHENCR), shRNA NC, miRNA mimics (miR-532-3p mimic), mimic NC, inhibitors of miR-532-3p (anti-miR-532-3p), and inhibitor NC (anti-miR NC) were synthesized from GenePharma (Shanghai, China) and plasmids were transfected into CRC cell lines using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

RNA extraction and real-time quantitative PCR (RT-qPCR)

Trizol was used to extract total RNA in tissue specimens and cells, and the concentration and purity of the extracted RNA were detected by A260/A280 value. The extracted RNA was reverse transcribed using Quantitect Reverse Transcription Kit (QIAGEN, Hilden, Germany) with a 20 μl reverse transcription system. Subsequently, qPCR was carried out using SYBR® Premix Ex Taq™ II (Takara, Dalian, China) and PrimeScript™ miRNA qPCR Starter Kit Ver.2.0 (Takara). The reference genes for lncRNA and miRNA normalization were β-actin and U6, respectively. The sequences were listed in Table 1. The Comparative cycle threshold (Ct) methods were used to evaluate the relative expression levels of lncRNA and miRNA.

Nucleoplasmic separation assay

The nuclear/cytoplasmic RNA of HCT116 and SW480 cells were separated and isolated using a Minute™ Cytoplasmic and Nuclear Extraction Kit (Invent Biotechnologies, Plymouth, MN, USA). The cells were

Table 1. The primer sequences for RT-qPCR.

Genes		Sequences (5' to 3')
MHENCR	Forward	5'-ATGGTCAGTGGACGGACG-3'
	Reverse	5'-ACAGCAAGCACAAGGGTG-3'
miR-532-3p	Forward	5'-ACACTCCCCTCCCACACCCAAGG-3'
	Reverse	5'-GTGCAGGGTCCGAGGT-3'
β-actin	Forward	5'-GGGAAATCGTGCGTGACATTAAG-3'
	Reverse	5'-TGTGTTGGCGTACAGGTCTTTG-3'
U6	Forward	5'-CTCGCTTCGGCAGCACA-3'
	Reverse	5'-AACGCTTCACGAATTTGCGT-3'

washed with phosphate buffered saline (PBS), resuspended in ice-cold cell fractionation buffer, and then centrifuged (500 × *g*, 4°C) for 4 min to separate cytoplasmic fraction away from the nuclear pellet. The cytoplasm supernatant was transferred to a new tube for RNA extraction. The pellet containing the nucleus was mixed with ice-cold disruption buffer and cell fractionation buffer. After washing, centrifugation, and filtration, the isolated nuclear/cytoplasmic RNA was obtained and stored at −80°C until use.

Dual-luciferase reporter assay

The miRNAs that sponged to MHENCR were predicted by LncBase Predicted v.2. The 3'UTR fragments of MHENCR with binding sites of miR-532-3p were amplified and cloned into pmirGLO vectors (Promega, Madison, WI, USA) to construct MHENCR-wt. Meanwhile, the 3'UTR mutant fragments of MHENCR were cloned into pmirGLO vectors to build MHENCR-mut. Then, the wt or mut vectors were cotransfected with miR-532-3p mimic or mimic NC using transfection reagent. The luciferase activities were detected utilizing the Dual-luciferase Assay (Promega).

MTT assay

Cell proliferation abilities were monitored using an MTT kit (Sigma-Aldrich, Shanghai, China). The transfected cells (3,000 cells/well) were incubated in 96-well plates. The MTT kit was added to each well and further incubated for 4 h. Then 100 μl dimethyl sulfoxide (DMSO) was used to dissolve the precipitate. The absorbance was measured under a microplate spectrophotometer at 490 nm.

Transwell assays

Cell migratory capacities were measured using Transwell migration assay without coated matrigel. Cell invasive potential was assessed using Transwell invasion assay in which the upper surface of the well precoated with matrigel (BD Bioscience, San Jose, CA, USA) at 4°C. Briefly, 1×10^5 resuspend cells in medium without serum were put into the top chamber and spread evenly. Medium with 10% FBS was added to lower chambers. After 24 h of incubation, the migratory or invasive cells on the lower surface were fixed and dyed with 0.1% crystal violet.

Statistical analysis

SPSS 20.0 and GraphPad 7.0 software were used for the statistical analysis of data. Student's t-test or one-way ANOVA was used for comparison of measurement data groups. A Chi-square test was used to compare the association of clinical characteristics and MHENCR expression. A *P* value under 0.05 indicates that the difference was significant statistically.

Results

MHENCR was in high-expression level in CRC

The levels of MHENCR were estimated in CRC tissues and cell lines. The RT-qPCR results revealed that MHENCR expression was increased in CRC tissues versus paracancerous normal tissues ($P < 0.001$, Fig. 1A). The CRC cell lines (HT29, HCT116, SW480, SW620, and LoVo) have higher MHENCR expression levels than the normal cells (NCM460), especially in HCT116 and SW480 cells ($P < 0.05$, Fig. 1B). Therefore, the CRC cell lines, HCT116 and SW480, were selected as follow-up experiments.

MHENCR expression was associated with the development of CRC and patients' overall survival

In the patients with CRC, patients with MHENCR expression levels above the mean level of all selected 143 CRC patients were assigned to a high-level group, while patients with MHENCR expression levels below the mean level of all selected 143 patients were assigned to the low-level group. Through the correlation analysis of clinicopathological parameters, we found that MHENCR expression was not related to the patients' sex, age, location,

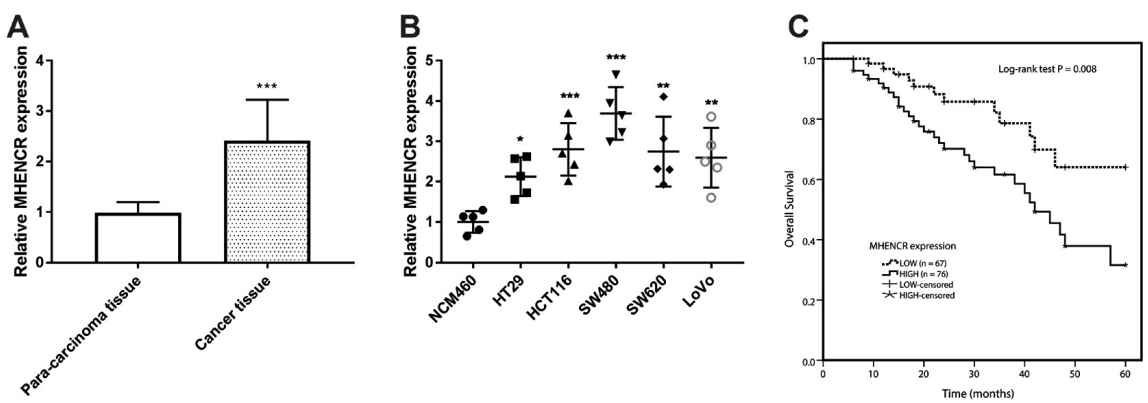

Fig. 1. MHENCR expression was increased in colorectal carcinoma (CRC) and associated with overall survival.
A. The MHENCR expression was elevated in CRC tissues (measured by RT-qPCR). ***$P < 0.001$. B. The levels of MHENCR were increased in CRC cells compared with normal cell NCM460. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. C. Kaplan-Meier analysis showed that high expression of MHENCR obtained an adverse overall survival. Log-rank test $P = 0.008$.

differentiation, invasion, but was related to tumor growth size, TNM stage, and lymph node metastasis (Table 2).

The Kaplan-Meier analysis results revealed that the overall survival rate of CRC patients with higher MHENCR expression levels was shorter than that of patients with lower MHENCR levels ($P = 0.008$, Fig. 1C). Additionally, multivariate Cox analysis showed that in addition to lymph node metastasis and TNM stage, high expression of MHENCR ($P = 0.029$) was also associated with poor prognosis in patients with CRC (Table 3).

Table 2. Correlations between MHENCR expression and clinical characteristics in colorectal carcinoma (CRC) patients (n = 143).

Parameters	Total (n = 143)	MHENCR expression Low (n = 67)	High (n = 76)	*P* value
Sex				0.187
Male	83	35	48	
Female	60	32	28	
Age (years)				0.264
< 60	74	38	36	
≥ 60	69	29	40	
Location				0.14
Colon	76	40	36	
Rectum	67	27	40	
Tumor size (cm)				0.041
< 5	66	37	29	
≥ 5	77	30	47	
Differentiation				0.348
Well/Moderate	73	37	36	
Poor	70	30	40	
Lymph node metastasis				0.024
Negative	84	46	38	
Positive	59	21	38	
Invasion				0.074
No	51	29	22	
Yes	92	38	54	
TNM stage				0.004
I-II	82	47	35	
III-IV	61	20	41	

Table 3. Multivariate Cox analysis of risk factors-related prognosis in colorectal carcinoma (CRC) patients based on overall survival.

Parameters	Multivariate analysis		*P* values
	HR	95%CI	
MHENCR	0.402	0.178-0.911	0.029
Sex	0.442	0.193-1.011	0.053
Age	1.610	0.774-3.353	0.203
Location	0.535	0.278-1.031	0.062
Tumor size	0.497	0.224-1.101	0.085
Differentiation	0.567	0.296-1.087	0.088
Lymph node metastasis	0.347	0.123-0.981	0.046
Invasion	1.774	0.863-3.646	0.119
TNM stage	0.321	0.112-0.921	0.035

HR, hazard ratio; CI, confidence interval.

Knockdown of MHENCR can inhibit cellular activities of CRC cells

Two cell lines with high MHENCR expression, HCT116, and SW480, were selected to transfect MHENCR shRNA, and the interference efficiency was detected after 48 h of transfection. MHENCR shRNA could downregulate the expression of MHENCR in HCT116 and SW480, with an interference efficiency of more than 60% ($P < 0.001$, Fig. 2A). MTT assays were performed to investigate the influence of MHENCR knockdown on cell proliferative capacities. The results indicated that the proliferative potential was significantly decreased at 48 and 72 h by the silence of MHENCR ($P < 0.01$, Fig. 2B, C). The Transwell results revealed that the migration ($P < 0.001$, Fig. 2D) and invasion abilities ($P < 0.001$, Fig. 2E) of HCT116 and SW480 cells were weakened after MHENCR knockdown.

The targeted relationship between MHENCR and miR-532-3p in cell lines

The nuclear and cytoplasmic separation assay illustrated that most of the MHENCR can be found in the cytoplasm (Fig. 3A). A further mechanism of knockdown of MHENCR depressing the progression of CRC cells was studied and miR-532-3p was identified as a factor that was negatively regulated by MHENCR. The binding prediction using LncBase Predicted v.2 databases showed that there were continuous MHENCR wild-type binding sites with miR-532-3p sequences (Fig. 3B). The expression levels of miR-532-3p were decreased in CRC tissues ($P < 0.001$, Fig. 3C). MHENCR expression was negatively correlated with miR-532-3p expression (Pearson r = −0.2923, $P = 0.004$, Fig. 3D). Whereafter, the expression of miR-532-3p was measured in CRC cells transfected with sh-MHENCR. We observed that miR-532-3p levels were elevated in MHENCR-silence CRC cells ($P < 0.001$, Fig. 3E). The dual-luciferase reporter assay results confirmed the relationship between MHENCR and miR-532-3p. Increased expression of miR-532-3p reduced the luciferase activities of MHENCR-wt, but not MHENCR-mut ($P < 0.001$, Fig. 3F, G).

MHENCR/miR-532-3p axis regulates the CRC cellular behaviors

To assess the potential function of MHENCR/miR-532-3p in CRC progression, CRC cells were grouped into control group, sh-MHENCR group, sh-MHENCR + anti-miR NC group, sh-MHENCR + anti-miR-532-3p group. Transfection results showed that the expression of MHENCR was decreased by sh-MHENCR ($P < 0.001$), while miR-532-3p expression was increased in MHENCR knockdown CRC cells ($P < 0.001$, Fig. 4A). Cotransfected with sh-MHENCR and anti-miR-532-3p did not affect the decreased expression of MHENCR by sh-MHENCR, whereas the increased expression of miR-532-3p was reversed by anti-miR-532-3p ($P < 0.001$, Fig. 4A). As displayed in Fig. 4B, knockdown of miR-532-3p reversed the decreased cell proliferation abilities induced by sh-MHENCR ($P < 0.01$). A similar phenomenon was observed: Inhibition of miR-532-3p reversed the effects caused by sh-MHENCR on cell migration capacities ($P < 0.001$, Fig. 4C) and invasion abilities ($P < 0.01$, Fig. 4D).

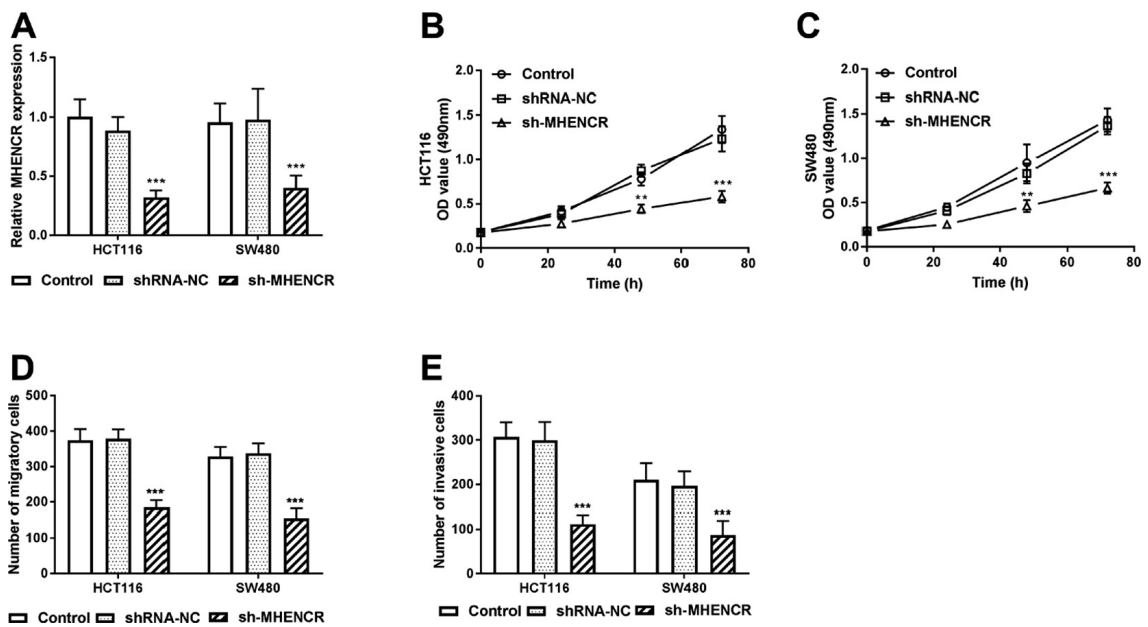

Fig. 2. Silence of MHENCR affected cellular behaviors in colorectal carcinoma (CRC) cells.
A. Transfection efficiency of sh-MHENCR was confirmed by RT-qPCR. B. and C. Decreased MHENCR weakened cell proliferation in HCR116 (B) and SW480(C) cells. D. The cell migratory capacities were decreased by sh-MHENCR. E. Cell invasive abilities were decreased by sh-MHENCR. $^{**}P < 0.01$, $^{***}P < 0.001$.

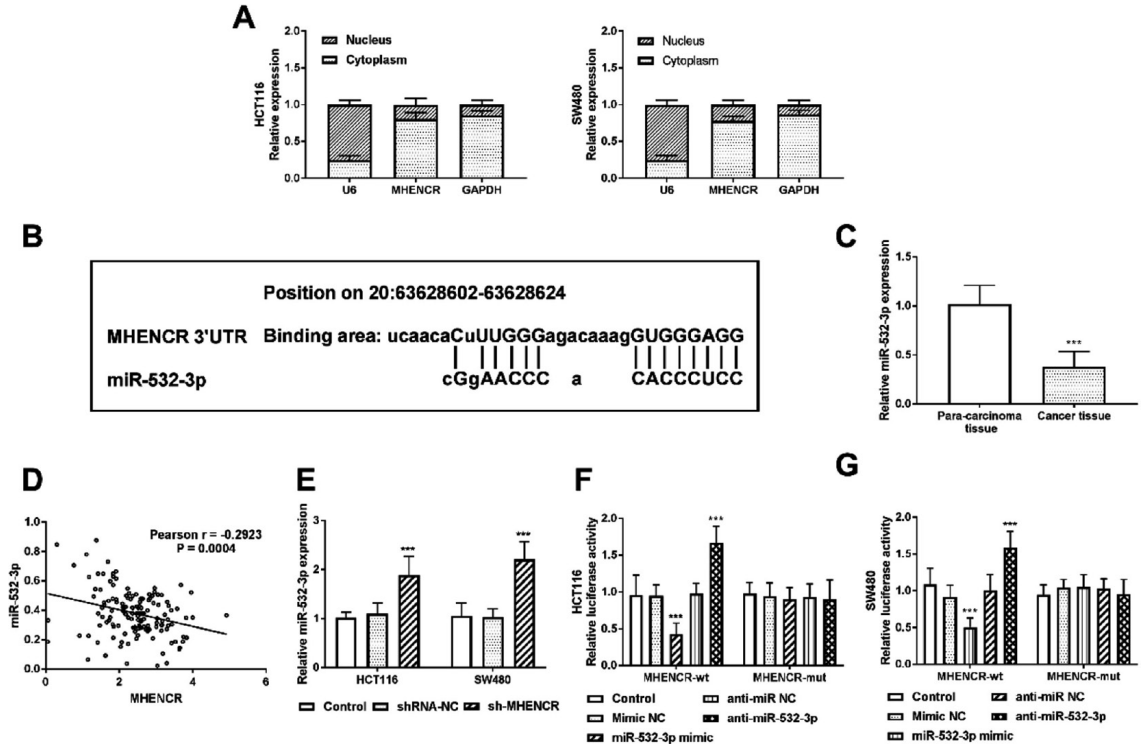

Fig. 3. miR-532-3p, a downstream miRNA of MHENCR.

A. Detection of lncRNA MHENCR location in HCT116 and SW480 cells. B. The binding sites between MHENCR and miR-532-3p. C. miR-532-3p expression levels were lower in tumor tissues. ***$P < 0.001$. D. Pearson r correlation analysis between MHENCR expression and miR-532-3p expression. r = −0.2923, $P = 0.0004$. E. miR-532-3p expression was increased in MHENCR-silence cells. ***$P < 0.001$. F. Overexpression of miR-532-3p decreased the luciferase activities of HCT116 cells that co-transfected with MHENCR-wt and miR-532-3p mimic while not affecting the luciferase activities of MHENCR-mut. ***$P < 0.001$. G. The dual-luciferase reporter assay verified the target relationship between MHENCR and miR-532-3p. ***$P < 0.001$.

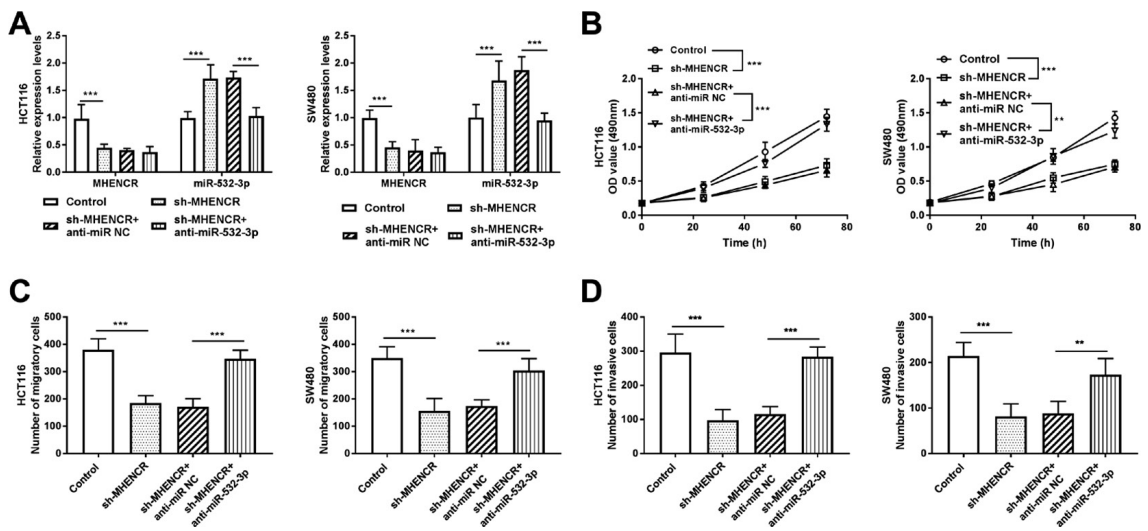

Fig. 4. MHENCR/miR-532-3p axis influences cellular activities.

A. Transfection efficiency was confirmed by RT-qPCR. B. miR-532-3p downregulation reversed the decreased cell proliferation induced by the silence of MHENCR. C. miR-532-3p silence eliminated the sh-MHENCR-induced decreased cell migration abilities. D. Decreased expression miR-532-3p increased the sh-MHENCR-induced downregulation of cell invasion capacities. **$P < 0.01$, ***$P < 0.001$.

Discussion

In the current study, we observed that MHENCR was upregulated in CRC tissues and associated with poor overall survival. The depletion of MHENCR repressed CRC cell proliferation, invasion, and migration by regulating miR-532-3p. The findings provide a theoretical basis for further developing targeted therapy for treating CRC.

LncRNAs do not encode proteins but can regulate cellular behaviors by interacting with miRNAs or proteins (Ferrè et al. 2016). Studies have shown that lncRNAs are involved in the occurrence and progression of CRC by regulating tumor cell growth, apoptosis, and metastasis (Wei and Wang 2017; Zhao et al. 2018). At present, the incidence and mortality of CRC are still increasing year by year. Although the gene therapy of CRC has achieved some effect, the survival time of patients is still unsatisfactory (Aguiar Junior et al. 2020). Therefore, this study actively explored novel lncRNA and provided potential therapeutic strategies for CRC treatment by analyzing the molecular mechanisms in the occurrence and development of CRC.

Previous studies reported that MHENCR was upregulated and associated with prognosis in clear cell renal cell carcinoma (Zhong et al. 2021) and melanoma (Chen et al. 2017). Herein, MHENCR expression was also increased in CRC tissues. High MHENCR expression in tumor tissues is related to clinical stage, tumor size, and lymph node metastasis. The data suggest that MHENCR may be an oncogene and involved in the progression of CRC. In melanoma, MHENCR expression was associated with TNM stage and lymph node metastasis (Chen et al. 2017). In addition to MHENCR, other lncRNAs are also indicated to have a function in CRC. For instance, lncRNA MIR4435-2HG exhibits high expression in CRC and contributes to cancer development, as well as predicts a worse prognosis (Shen et al. 2020). CRC cell proliferation was promoted by lncRNA-cCSC1 through targeting miR-124-3p and upregulating CD44 (Zhang et al. 2021). LncRNA small nucleolus RNA host gene 14 (SNHG14) with miR-3940-5p and NAP1L2 have performance characteristics in CRC and may be potential value for CRC prognosis (Matboli et al. 2021). This evidence revealed the clinical significance of lncRNAs in CRC. In the current study, the clinical analysis indicated that high MHENCR expression displayed shorter overall survival, suggesting that MHENCR may be a potential prognostic biomarker for CRC. The findings were consistent with a previous study, in which high MHENCR expression was related to shorter overall survival in colon adenocarcinoma (Tan et al. 2021).

LncRNAs function as oncogenes or tumor-suppressers in tumor progression through modulating cellular activities. Herein, functional experiments identified the critical role of MHENCR in CRC cells. The depletion of MHENCR repressed CRC cell proliferation, invasion, and migration *in vitro*. Our results revealed that MHENCR has functions in CRC, suggesting MHENCR may be a therapeutic target for CRC. Consistent with MHENCR, many lncRNAs also have functions in CRC. For instance, lncRNA small nucleolar RNA host gene 15 (SNHG15) could enhance tumor growth and inhibit cell apoptosis by inhibiting miR-338-3p in CRC (Li et al. 2019). These studies combined with current data demonstrated the crucial role of lncRNAs in CRC, and indicate that targeting lncRNAs may act as a potential strategy for the treatment of CRC patients.

To explore the mechanism of MHENCR in CRC, its location and the downstream miRNAs of MHENCR were explored. MHENCR accounted for a higher proportion in the cytoplasm, which suggest that MHENCR may participate in the progression of CRC through posttranscriptional regulation. Using online bioinformatic prediction and verification, MHENCR was specifically related to miR-532-3p. miR-532-3p plays a tumor inhibitory role in several cancers (Jiang et al. 2019; Gao et al. 2021; Huang et al. 2021). For instance, miR-532-3p repressed proliferation and promoting apoptosis of lymphoma cells by targeting β-catenin in lymphoma (Liu et al. 2020). miR-532-3p could weaken cell proliferation viability, adhesion, and migration of tongue squamous cell carcinoma by modulating podoplanin (Liu and Zhao 2021). In the current study, knockdown of miR-532-3p reversed the decreased proliferation abilities, migratory capacities, and invasive power caused by the downregulation of MHENCR. A previous study also revealed that miR-532-3p acted as a tumor inhibitor by suppressing CRC progression through Wnt/β-catenin signaling (Gu et al. 2019). These results suggest that MHENCR may promote CRC tumorigenesis through regulating miR-532-3p.

Taken together, lncRNA MHENCR expression was an oncogene in CRC tissues and cells, as well as associated with overall survival. LncRNA MHENCR could devote to CRC progression and tumorigenesis by sponging miR-532-3p. The finding of the MHENCR/miR-532-3p axis might supply a potential prognosis marker and a more effective therapeutic strategy for the treatment of CRC patients.

Conflict of Interest

The authors declare no conflict of interest.

References

Aguiar Junior, S., Oliveira, M.M., Silva, D., Mello, C.A.L., Calsavara, V.F. & Curado, M.P. (2020) Survival of patients with colorectal cancer in a cancer center. *Arq. Gastroenterol.*, **57**, 172-177.

Bhan, A., Soleimani, M. & Mandal, S.S. (2017) Long noncoding RNA and cancer: a new paradigm. *Cancer Res.*, **77**, 3965-3981.

Cao, P., Li, F., Xiao, Y., Hu, S., Kong, K., Han, P., Yue, J., Deng, Y., Zhao, Z., Wu, D., Zhang, L. & Zhao, B. (2022) Identification and validation of 7-lncRNA signature of epigenetic disorders by comprehensive epigenetic analysis. *Dis. Markers*, **2022**, 5118444.

Cao, W., Chen, H.D., Yu, Y.W., Li, N. & Chen, W.Q. (2021) Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. *Chin.*

Med. J. (*Engl.*), **134**, 783-791.
Chen, X., Dong, H., Liu, S., Yu, L., Yan, D., Yao, X., Sun, W., Han, D. & Gao, G. (2017) Long noncoding RNA MHENCR promotes melanoma progression via regulating miR-425/489-mediated PI3K-Akt pathway. *Am. J. Transl. Res.*, **9**, 90-102.
Ferrè, F., Colantoni, A. & Helmer-Citterich, M. (2016) Revealing protein-lncRNA interaction. *Brief. Bioinform.*, **17**, 106-116.
Galamb, O., Bartak, B.K., Kalmar, A., Nagy, Z.B., Szigeti, K.A., Tulassay, Z., Igaz, P. & Molnar, B. (2019) Diagnostic and prognostic potential of tissue and circulating long non-coding RNAs in colorectal tumors. *World J. Gastroenterol.*, **25**, 5026-5048.
Gao, B., Wang, L., Zhang, Y., Zhang, N., Han, M., Liu, H., Sun, D. & Liu, Y. (2021) MiR-532-3p suppresses cell viability, migration and invasion of clear cell renal cell carcinoma through targeting TROAP. *Cell Cycle*, **20**, 1578-1588.
Ghasemi, T., Khalaj-Kondori, M., Hosseinpour Feizi, M.A. & Asadi, P. (2020) lncRNA-miRNA-mRNA interaction network for colorectal cancer; an in silico analysis. *Comput. Biol. Chem.*, **89**, 107370.
Grelet, S., Link, L.A., Howley, B., Obellianne, C., Palanisamy, V., Gangaraju, V.K., Diehl, J.A. & Howe, P.H. (2017) A regulated PNUTS mRNA to lncRNA splice switch mediates EMT and tumour progression. *Nat. Cell Biol.*, **19**, 1105-1115.
Gu, C., Cai, J., Xu, Z., Zhou, S., Ye, L., Yan, Q., Zhang, Y., Fang, Y., Liu, Y., Tu, C., Wang, X., He, J., Li, Q., Han, L., Lin, X., et al. (2019) MiR-532-3p suppresses colorectal cancer progression by disrupting the ETS1/TGM2 axis-mediated Wnt/beta-catenin signaling. *Cell Death Dis.*, **10**, 739.
Huang, J., Liu, Y., Wang, M., Wang, R., Ling, H. & Yang, Y. (2021) FoxO4 negatively modulates USP10 transcription to aggravate the apoptosis and oxidative stress of hypoxia/reoxygenation-induced cardiomyocytes by regulating the Hippo/YAP pathway. *J. Bioenerg. Biomembr.*, **53**, 541-551.
Jiang, W., Zheng, L., Yan, Q., Chen, L. & Wang, X. (2019) MiR-532-3p inhibits metastasis and proliferation of non-small cell lung cancer by targeting FOXP3. *J. BUON*, **24**, 2287-2293.
Li, M., Bian, Z., Jin, G., Zhang, J., Yao, S., Feng, Y., Wang, X., Yin, Y., Fei, B., You, Q. & Huang, Z. (2019) LncRNA-SNHG15 enhances cell proliferation in colorectal cancer by inhibiting miR-338-3p. *Cancer Med.*, **8**, 2404-2413.
Liu, Y., Li, Q., Dai, Y., Jiang, T. & Zhou, Y. (2020) miR-532-3p inhibits proliferation and promotes apoptosis of lymphoma cells by targeting beta-catenin. *J. Cancer*, **11**, 4762-4770.
Liu, Z.Y. & Zhao, C.G. (2021) miR-532-3p inhibits the progression of tongue squamous cell carcinoma by targeting podoplanin. *Chin. Med. J.* (*Engl.*), **134**, 2999-3008.
Matboli, M., Shafei, A.E., Ali, M.A., El-Din Ahmed, T.S., Naser, M., Abdel-Rahman, T., Anber, N. & Ali, M. (2021) Role of extracellular LncRNA-SNHG14/miRNA-3940-5p/NAP12 mRNA in colorectal cancer. *Arch. Physiol. Biochem.*, **127**, 479-485.
Roncucci, L. & Mariani, F. (2015) Prevention of colorectal cancer: how many tools do we have in our basket? *Eur. J. Intern. Med.*, **26**, 752-756.
Shen, M.Y., Zhou, G.R. & Zhang, Y.Z. (2020) LncRNA MIR4435-2HG contributes into colorectal cancer development and predicts poor prognosis. *Eur. Rev. Med. Pharmacol. Sci.*, **24**, 1771-1777.
Siegel, R.L., Miller, K.D., Fuchs, H.E. & Jemal, A. (2022) Cancer statistics, 2022. *CA Cancer J. Clin.*, **72**, 7-33.
Tan, X., Li, Q., Zhang, Q., Fan, G., Liu, Z. & Zhou, K. (2021) Integrative analysis reveals potentially functional N6-methyladenosine-related long noncoding RNAs in colon adenocarcinoma. *Front. Genet.*, **12**, 739344.
Taniue, K. & Akimitsu, N. (2021) The functions and unique features of lncRNAs in cancer development and tumorigenesis. *Int. J. Mol. Sci.*, **22**, 632.
Walcher, L., Kistenmacher, A.K., Suo, H., Kitte, R., Dluczek, S., Strauss, A., Blaudszun, A.R., Yevsa, T., Fricke, S. & Kossatz-Boehlert, U. (2020) Cancer stem cells-origins and biomarkers: perspectives for targeted personalized therapies. *Front. Immunol.*, **11**, 1280.
Wei, G.H. & Wang, X. (2017) lncRNA MEG3 inhibit proliferation and metastasis of gastric cancer via p53 signaling pathway. *Eur. Rev. Med. Pharmacol. Sci.*, **21**, 3850-3856.
Xu, M., Chen, X., Lin, K., Zeng, K., Liu, X., Xu, X., Pan, B., Xu, T., Sun, L., He, B., Pan, Y., Sun, H. & Wang, S. (2019) lncRNA SNHG6 regulates EZH2 expression by sponging miR-26a/b and miR-214 in colorectal cancer. *J. Hematol. Oncol.*, **12**, 3.
Zhang, H.R., Wu, S.Y. & Fu, Z.X. (2021) LncRNA-cCSC1 promotes cell proliferation of colorectal cancer through sponging miR-124-3p and upregulating CD44. *Biochem. Biophys. Res. Commun.*, **557**, 228-235.
Zhao, W., Geng, D., Li, S., Chen, Z. & Sun, M. (2018) LncRNA HOTAIR influences cell growth, migration, invasion, and apoptosis via the miR-20a-5p/HMGA2 axis in breast cancer. *Cancer Med.*, **7**, 842-855.
Zhong, L., Li, Y., Xiong, L., Wang, W., Wu, M., Yuan, T., Yang, W., Tian, C., Miao, Z., Wang, T. & Yang, S. (2021) Small molecules in targeted cancer therapy: advances, challenges, and future perspectives. *Signal Transduct. Target. Ther.*, **6**, 201.