

• 专题研究:肿瘤 •

METTL3 介导的 KIF11 mRNA m6A 修饰通过 PI3K/AKT 信号通路促进结直肠癌进展

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[摘要] 目的: 探讨驱动蛋白家族成员 11(kinesin family member 11, KIF11)在结直肠癌(colorectal cancer, CRC)中的生物学功能及其分子调控机制。方法: 采用定量实时 PCR 技术结合公共数据库分析 KIF11 在 CRC 中的表达情况。通过 CCK-8 细胞增殖实验、集落形成实验、EdU 染色实验和 Transwell 迁移实验评估 KIF11 对 CRC 细胞增殖和迁移能力的影响。运用 Western blot、RNA 免疫共沉淀定量 PCR(RIP-qPCR)、甲基化 RNA 免疫共沉淀定量 PCR(MeRIP-qPCR)和 RNA 稳定性实验解析 KIF11 的 N6-甲基腺苷(N6-methyladenosine, m6A)修饰机制。采用 RNA 测序技术和相关性分析方法探究 KIF11 调控的下游机制。结果: KIF11 在 CRC 组织中高表达, 其表达水平与 CRC 细胞的增殖和迁移能力呈正相关。甲基转移酶样蛋白 3(methyltransferase-like 3, METTL3)和胰岛素样生长因子 2 mRNA 结合蛋白 2(insulin like growth factor 2 mRNA binding protein 2, IGF2BP2)通过 m6A 修饰调控 KIF11 mRNA 的稳定性, 从而正向调控 KIF11 的表达。进一步研究发现, KIF11 通过激活 PROM1/PI3K/AKT 通路促进 CRC 的恶性进展。结论: METTL3/IGF2BP2 介导的 KIF11 mRNA m6A 修饰通过 PI3K/AKT 信号通路促进 CRC 的发生发展, 提示 KIF11 可能作为 CRC 潜在的预后标志物和治疗靶点。

[关键词] 结直肠癌; KIF11; m6A; METTL3; PI3K/AKT 信号通路**[中图分类号]** R735.34**[文献标志码]** A**[文章编号]** 1007-4368(2025)11-1546-17**doi:** 10.7655/NYDXBNSN250235

METTL3 - mediated m6A modification of KIF11 mRNA promotes colorectal cancer progression through the PI3K/AKT signaling pathway

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[Abstract] **Objective:** To investigate the biological functions and molecular regulatory mechanisms of kinesin family member 11 (KIF11) in colorectal cancer (CRC). **Methods:** The expression of KIF11 in CRC was examined by qRT-PCR and public databases. Functional assays (CCK-8, colony formation, EdU, and Transwell) were employed to evaluate KIF11's roles in CRC progression. Western blot, RIP-qPCR, MeRIP-qPCR, and RNA stability assays were performed to elucidate the molecular mechanism of N6-methyladenosine (m6A) modification for KIF11. RNA sequencing (RNA-seq) and correlation analysis were used to examine the downstream mechanism of KIF11 regulation. **Results:** KIF11 was highly expressed in CRC and promoted CRC proliferation and migration. Mechanistically, methyltransferase-like 3 (METTL3)/insulin like growth factor 2 mRNA binding protein 2 (IGF2BP2) enhanced KIF11 mRNA stability and expression in an m6A-dependent way. Furthermore, by means of the PROM1/PI3K/AKT pathway, KIF11 facilitated the progression of CRC. **Conclusion:** The m6A modification of KIF11 by METTL3/IGF2BP2 contributes to CRC progression via the PI3K/AKT signaling pathway, highlighting its potential as a prognostic biomarker and therapeutic target.

[Key words] colorectal cancer; KIF11; m6A; METTL3; PI3K/AKT pathway

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Colorectal cancer (CRC) is the third most common cancer globally and the second leading cause of death from cancer^[1]. According to GLOBOCAN 2020 estimates, 3 154 674 new CRC cases are projected worldwide by 2040^[2], highlighting its significance as a public health crisis. Despite advances in diagnostic and therapeutic strategies, patient prognosis remains poor^[3]. Thus, exploring the molecular mechanisms of CRC and promising therapeutic targets are essential.

Kinesin family member 11 (KIF11), a plus-end-directed kinesin motor protein of the kinesin family, plays a critical role in spindle pole separation and chromosome alignment during mitosis^[4]. Accumulating evidence demonstrates that KIF11 upregulation is associated with poor prognosis in many cancers. For example, KIF11 is highly expressed and promotes cell proliferation in gallbladder cancer^[5]; in hepatocellular carcinoma, KIF11 is negatively correlated with senescence biomarkers; its knockdown induces cellular senescence and suppresses hepatocellular carcinoma (HCC) progression^[6]. Epigenetic modifications, particularly N6-methyladenosine (m6A), are pivotal in disease pathogenesis. As the most prevalent internal mRNA modification in humans (affecting almost 90% of transcripts)^[7], m6A regulates post-transcriptional processes. However, whether m6A modifications and their regulatory enzymes control KIF11 expression in CRC remains unexplored.

The dynamic regulation of m6A methylation modification is performed by methyltransferases (“writers”), demethylases (“erasers”), and binding proteins (“readers”)^[8]. METTL3 is one of the major methyltransferases proven to be highly expressed in various cancers and to accelerate tumor malignant development, including colorectal and lung cancers^[9-12]. Similarly, IGF2BP2 is a key m6A modification “reader” that encourages the growth of malignancies by affecting the stability and translation of downstream target mRNAs^[13-15].

PROM1, also known as CD133, is a cancer stem cell (CSC) marker involved in tumorigenesis, drug resistance, and apoptosis in CSCs. For example, CD133 can promote angiogenesis through VEGF-A^[16]; it can also promote gastric cancer (GC) progression through the PI3K/AKT signaling pathway^[17].

In this study, we demonstrated that KIF11 was highly expressed in CRC, and its knockdown inhibited the proliferation and migration of CRC cells. We also found that the METTL3 - IGF2BP2 axis regulated KIF11 in an m6A-dependent way. In addition, KIF11 promoted CRC progression by regulating PROM1 and PI3K/AKT signaling pathway. Collectively, these findings position KIF11 as a potential therapeutic target and prognostic biomarker for CRC.

1 Materials and Methods

1.1 Materials

1.1.1 Clinical samples

This study collected 30 pairs of CRC tissues and adjacent normal tissues from patients undergoing surgery at the Second Affiliated Hospital of Nanjing Medical University. Before surgery, none of the patients underwent radiotherapy or chemotherapy. All patients who participated in this study signed informed consent forms. The whole process was in strict accordance with the Declaration of Helsinki. The ethical approval of this study was provided by the Institutional Review Board of Nanjing Medical University (Ethics No. 2024-KY-274-01).

1.1.2 Animals

Four-week-old, 16–18 g specific pathogen free (SPF) male BALB/c nude mice were purchased from GemPharmatech Co., Ltd. The nude mice were housed in an environment of 20–27 °C with relative humidity of 40%–60%. The Nanjing Medical University Animal Ethics Committee granted its approval for animal studies (IACUC-2410071).

1.1.3 Reagents and instruments

TRIzol (Invitrogen, USA); anti-KIF11, anti-IGF2BP2, anti-ACTIN, anti-GAPDH (Proteintech, USA); anti-METTL3 (Youmengbiology, China); anti-PI3K, anti-p-PI3K, anti-AKT (CST, USA); p-AKT (Affinity, USA); anti-IgG (Millipore, USA); SYBR Green premix (CWBIO, Taizhou, China); BeyoClick™ EdU cell proliferation kit with AF555 (Beyotime, Shanghai, China); Annexin V-FITC/PI Apoptosis Detection Kit (Vazyme, Nanjing, China); Magna RIP kit, anti-m6A, IgG antibody (Millipore, USA). Light Cycler480 II real-time fluorescence PCR Instrument (Roche, USA); Cen-

trifuges (Eppendorf, Germany); Cell incubator (ThermoFisher Scientific, USA); Chemiluminescence imager (Tanon, Guangdong, China); Electrophoresis apparatus, Thermal cycler (BIORAD, USA); Flow cytometer (Beckman Coulter, USA); Fluorescence microscope (Olympus, Japan); Microplate reader(Biotek, USA).

1.2 Methods

1.2.1 Lentiviral and plasmid transfections

shRNA and overexpression plasmids were designed for genes including KIF11. They were transfected into cells according to the instructions^[18].

1.2.2 RNA Extraction and qRT-PCR

Total cellular RNA was extracted. The cells were fully lyzed using TRIzol reagent and allowed to stand for 5 min. Then 0.2 mL of chloroform was added and the

mixture was shaken vigorously for 15 s, followed by a 3-minute rest, The sample was centrifuged at 12 000 g at 4 °C for 15 min. We transfered the upper layer of liquid to a new centrifuge tube, added 0.5 mL of isopropanol to precipitate the RNA follaued by a 10-minute rest, and then centrifuged the sample at 12 000 g at 4 °C for 10 min. Subsequently, we discarded the supernatant and then washed the RNA precipitate with 75% ethanol twice, and finally dissolved the RNA precipitate with 20–50 μL of RNase-free water. SYBR Green premix was used for qRT-PCR. The comparative threshold cycle $2^{-\Delta\Delta CT}$ method was used to evaluate the relative gene expression and normalize it to GAPDH^[19]. The primer sequences used for qRT-PCR are shown in Table 1.

表1 引物序列表
Table 1 Primer sequences

Gene	Forward(5'→3')	Rerverse(5'→3')
KIF11	TTAATTTGGCAGAGCGGAAA	CCATACGCAAAGATAGTGCAA
METTL3	GCTGACCATTCCAAGCTCTC	ATTTCTTGGCTGGCTCCTTT
IGF2BP2	TTGCAGGAATTGACGCTGTA	ACCCAAGGCGTTCAGATTTA
PROM1	TTTGCTGCTTGTGGAATAGAC	ATAGGAAGGACTCGTTGCTGG
GAPDH	GAAGGTGAAGGTCGGAGT	GAAGATGGTGATGGGATTTTC

1.2.3 Western blot

Total protein of cells was extracted and then separated by 10% SDS-PAGE. The concentrated gel was 80 V and the separated gel was 120 V for constant voltage electrophoresis and then transferred the protein to a PVDF membrane. After sealing the membrane with 5% BSA for 2 h, the primary antibody was incubated overnight at 4 °C. The membrane was rinsed in TBST for 10 min on day 2 and repeated three times, followed by incubation with the corresponding secondary antibody for 2 h. Finally, an ECL chemiluminescent solution was used to visualize the protein bands.

1.2.4 CCK-8 assay, colony formation, and EdU experiments

To determine cell proliferation, a CCK-8 test was performed. According to the experimental design, HCT116, DLD1, or SW480 cells were spread in 96-well plates at a density of 2 000 cells per well. After 24–36 h, CCK-8 solution was added for another 24, 36, 48, 72, and 96 h. The absorbance at 450 nm was

detected after 2 h of incubation. Cells were spread at a density of 1 000–2 000 cells per well for colony formation experiments in 6-well plates, and the cell colonies were fixed with paraformaldehyde after 10–14 days and then stained with crystal violet. For EdU experiments, HCT116, DLD1, and SW480 cells were spread evenly in 96-well plates at a density of 5 000 cells per well. After 24 h, the 96-well plates were incubated with 20 μmol/L EdU working solution for 2 h. Subsequently, the medium was discarded, and the cells were washed three times with PBS. Then 4% paraformaldehyde was added and fixed at room temperature for 15 min, followed by permeabilization with 0.5% Triton X-100 for 10 min to enhance cell membrane permeability. Next, the cells were incubated with click reaction buffer for 30 min away from light. and the cell proliferation ratio was detected under a fluorescence microscope.

1.2.5 Transwell experiments

After adding 60 000–80 000 HCT116, DLD1, or SW480 cells to the upper chamber and medium con-

taining 20% FBS to the lower chamber, the cells that had passed through the membrane were fixed with para-formaldehyde and stained with crystal violet 36–48 h later. Then, the cells were photographed and observed to assess cell migration capacity according to previous studies^[20].

1.2.6 Cell apoptosis assay

HCT116, DLD1, or SW480 cells were spread evenly in 6-well plates, after 48 h, the cells were washed twice with pre-cooled PBS, followed by resuspension of the cells with $1\times$ binding buffer to a concentration of 1×10^6 cells/mL; 100 μ L of cell suspension was incubated with 5 μ L of Annexin V-FITC and 5 μ L of PI staining solution for 15 min in the dark. Flow cytometry analysis was performed within 1 h.

1.2.7 Subcutaneous xenograft experiments

BALB/c nude mice were administered subcutaneous injections of HCT116 cells transfected with shNC/shKIF11-1, containing approximately 3.5×10^6 cells per 150 μ L PBS, and the volume and weight of the tumors were measured every four days (volume=length $\times$ width $^2/2$).

1.2.8 RNA immunoprecipitation(RIP) assay

Following the instructions, adequate amounts of cells were lysed with lysates using the Magna RIP kit. The cells were incubated overnight with anti-IGF2BP2 or IgG antibody and magnetic beads at 4 $^{\circ}$ C. The magnetic beads were then washed and treated with proteinase K to remove proteins. Lastly, the purified RNA was reverse-transcribed to cDNA and analyzed using qRT-PCR^[21].

1.2.9 Methylated RNA immunoprecipitation(MeRIP)

The Manga MeRIP m6A kit was used. According to the instructions, total cell RNA was extracted and reduced into 100 or fewer nucleotide fragments. Then the magnetic bead-coupled anti-m6A antibody was incubated in immunoprecipitation buffer with the fragmented RNA for at 4 $^{\circ}$ C 2 h, while an IgG control was set up to exclude nonspecific binding. Next, the magnetic bead complexes were washed with pre-cooled washing buffer to remove nonspecific RNA, and finally the enriched m6A-modified RNA was recovered by proteinase K digestion and phenol-chloroform extraction, and the resulting products were analyzed by qRT-PCR to determine the methylation level of the target RNA. The

whole experiment was operated on ice to maintain RNA stability.

1.2.10 RNA stability assays

According to the experimental design, HCT116 and DLD1 cells were spread evenly in a 6-well plate. After 18 h, the 5 μ g/mL of actinomycin D was added to each well, and the cells were collected after 0, 3, and 6 h, respectively. RNA was extracted and subjected to qRT-PCR^[22], and half-life was calculated using the comparative threshold cycle $2^{-\Delta\Delta C_t}$ method.

1.3 Statistical analysis

GraphPad Prism version 9.5 and Image J version 5.4 were utilized to analyze the data. Every experiment was independently performed three times. When the data conformed to normal distribution, the data differences between the two groups were examined using the Student's *t*-test, while ANOVA was used to compare more than two groups. The Fisher's exact test was used to evaluate the clinicopathological data. $P < 0.05$ were considered statistically significant.

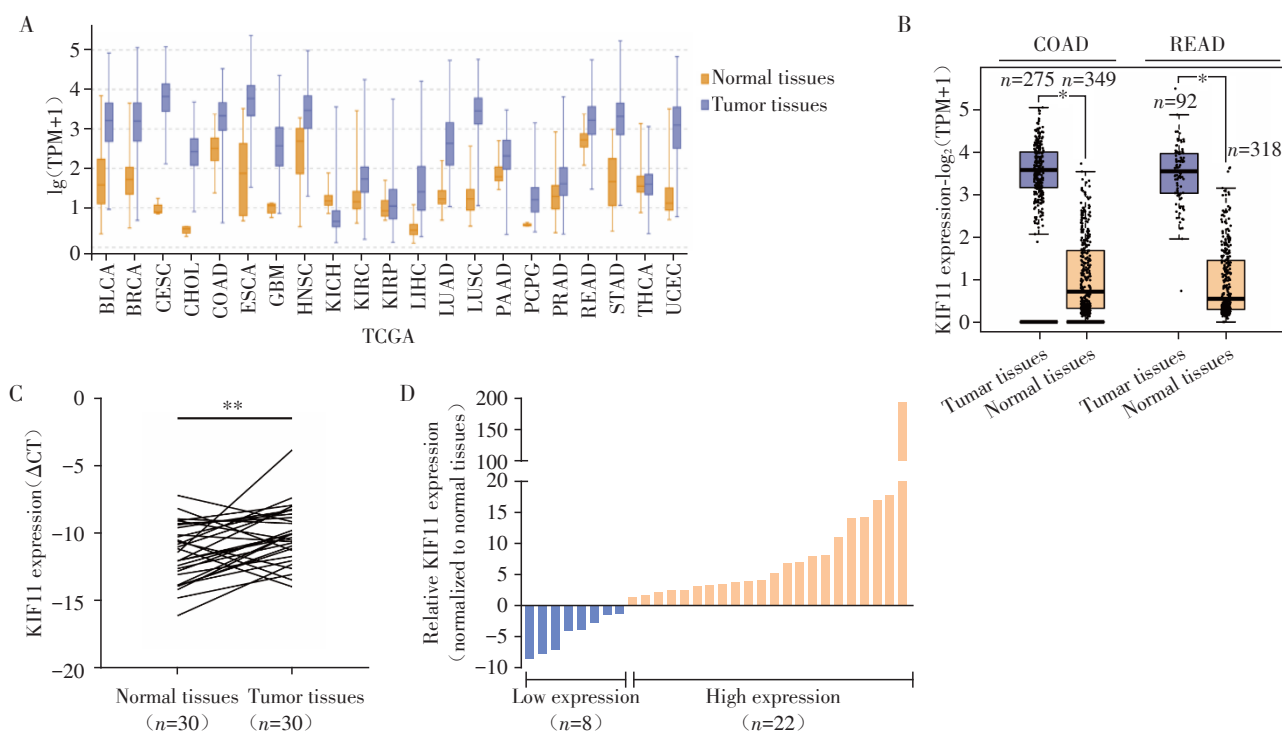
2 Results

2.1 KIF11 expression was upregulated in CRC tissues

According to OmicShare tools (<https://www.omicshare.com/tools/>)^[23] and the GEPIA database (<http://gepia2.cancer-pku.cn/>), the expression of KIF11 was highly expressed in various types of cancers, including colon and rectal cancers (Figure 1A, B). We examined the KIF11 expression levels in tumor tissues and adjacent paired normal tissues from 30 pairs of CRC patients by qRT-PCR. The findings demonstrated that KIF11 was highly expressed in CRC tissues compared with normal tissues (Figure 1C). Normalized to normal tissues, KIF11 expression levels in 30 pairs of the CRC tissues were divided into the high and low expression groups by qRT-PCR (Figure 1D). Subsequently, we analyzed the relationship between KIF11 expression and clinicopathological factors in the samples, and the results showed that a high level of KIF11 was associated with lymph node metastasis and distant metastasis (Table 2).

2.2 KIF11 promoted CRC cell proliferation and migration *in vitro*

CCK-8 and colony formation assays revealed that



A: Expression of KIF11 in TCGA in various types of cancers. B: GEPIA database showed that KIF11 was highly expressed in CRC tissues (COAD: colon adenocarcinoma; READ: rectum adenocarcinoma). C, D: KIF11 expression levels in 30 pairs of clinical tissues were examined (C) and divided into high and low expression groups by qRT-PCR (D). * $P < 0.05$ and ** $P < 0.01$.

图1 KIF11在结直肠癌组织中高表达
Figure 1 KIF11 was highly expressed in CRC tissues

表2 CRC患者KIF11的表达水平与临床病理参数的相关性
Table 2 Correlations between KIF11 expression and clinicopathological characteristics in CRC patients [n(%)]

Variable	Expression of KIF11		P
	Low (n=8)	High (n=22)	
Age (years)			>0.990
≤60	3 (37.5)	7 (31.8)	
>60	5 (62.5)	15 (68.2)	
Sex			>0.990
Male	5 (62.5)	12 (54.5)	
Female	3 (37.5)	10 (45.5)	
TNM stage			0.078
I / II	5 (62.5)	5 (22.7)	
III / IV	3 (37.5)	17 (77.3)	
Lymph node metastasis			0.034
Negative	6 (75.0)	6 (27.3)	
Positive	2 (25.0)	16 (72.7)	
Distant metastasis			0.049
Negative	6 (75.0)	7 (31.8)	
Positive	2 (25.0)	15 (68.2)	

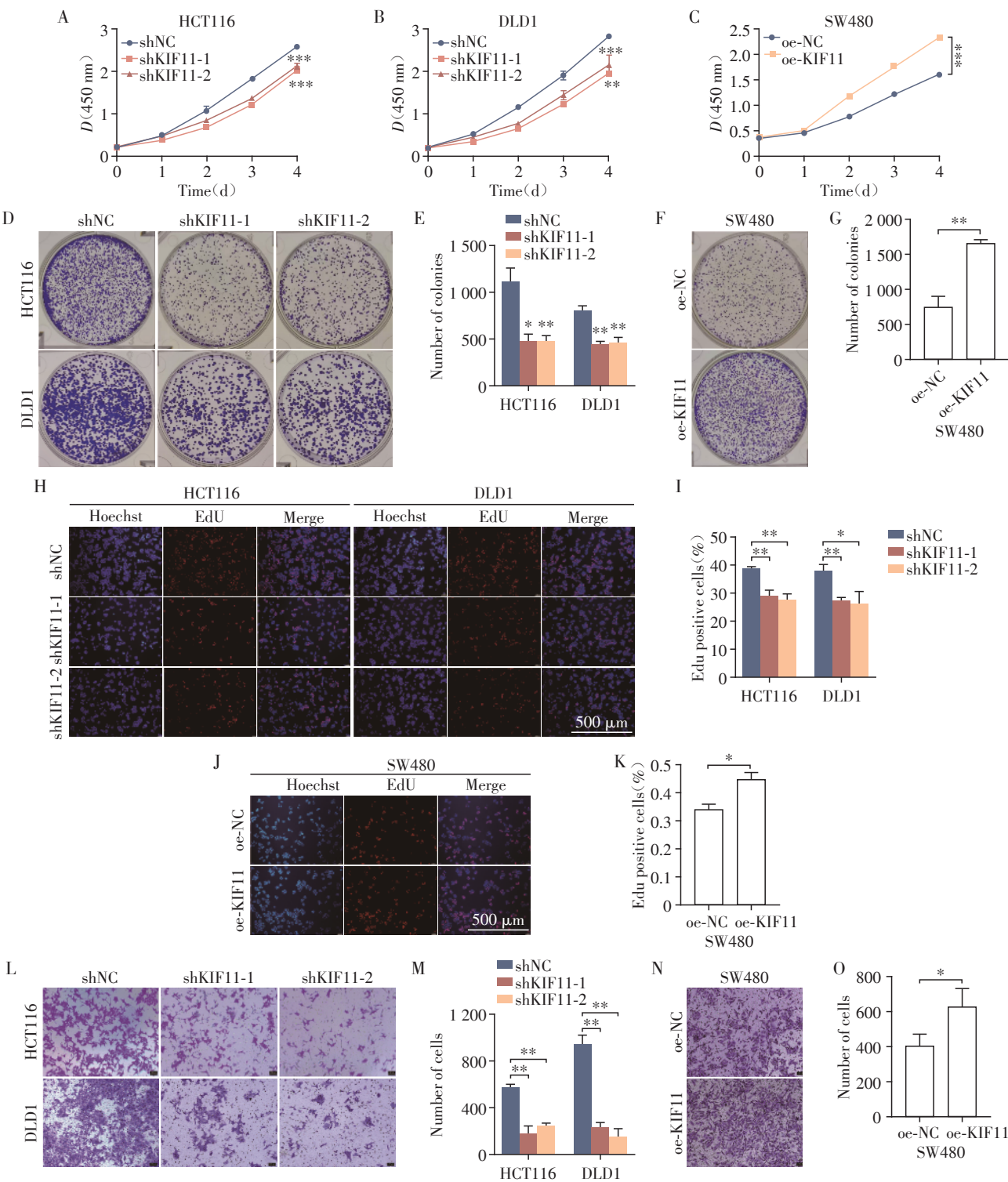
the knockdown of KIF11 inhibited the proliferative ability of cells (Figure 2A, B, D, E), while overex-

pression of KIF11 enhanced cell viability (Figure 2C, F, G). In addition, the EdU assay revealed the same results (Figure 2H–K). According to transwell assay, KIF11 knockdown weakened the migration ability of cells compared with the control, while overexpression of KIF11 significantly enhanced the cell migration capacity (Figure 2L–O). Flow cytometry analysis revealed that KIF11 knockdown significantly increased apoptosis rate in CRC cells, whereas KIF11 overexpression suppressed cell apoptosis (Figure 3).

2.3 KIF11 promoted CRC progression *in vivo*

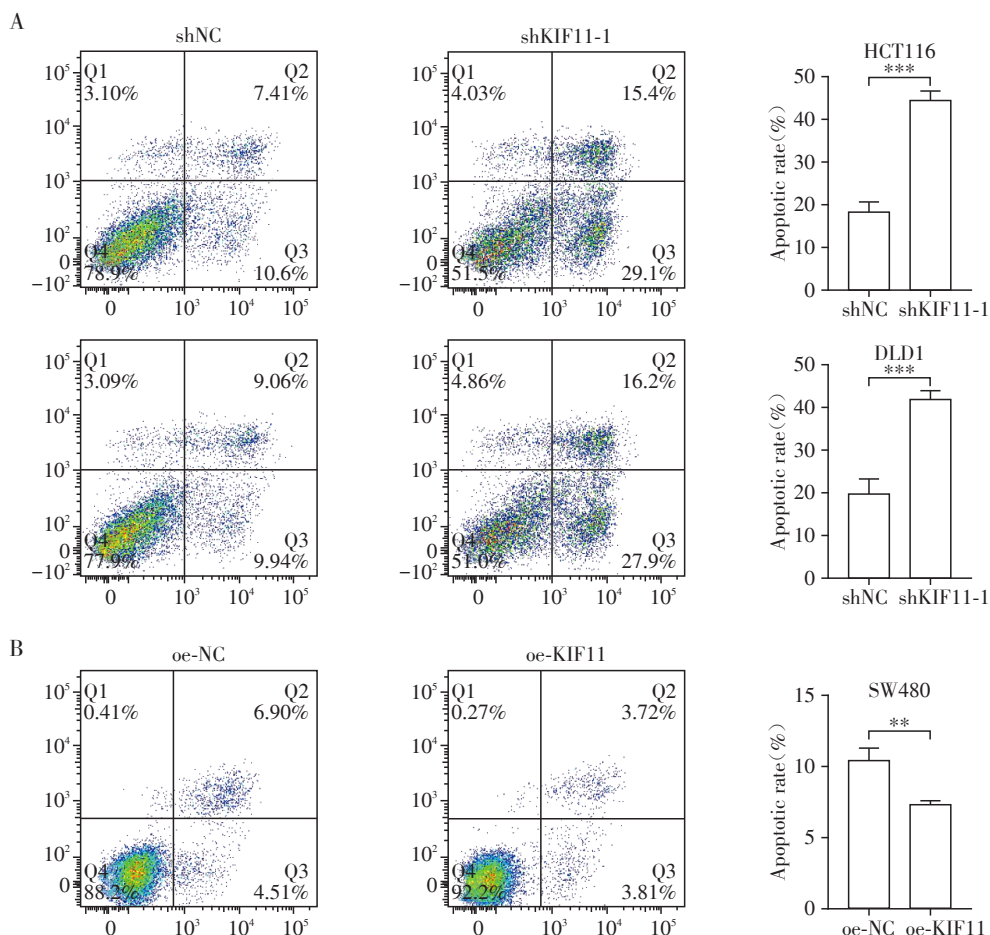
To explore whether KIF11 promotes the progression of CRC *in vivo*, we conducted subcutaneous xenograft experiments. HCT116 cells transfected with shNC or shKIF11-1 were inoculated into mice (five mice per group), and the tumors were compared after 21 days of injection (Figure 4A). The results showed that compared with the control group, the subcutaneous tumors with KIF11 knockdown exhibited lower volume and weight (Figure 4B, C).

2.4 METTL3-regulated m6A modification enhanced



A, B: The effect of KIF11 knockdown on cell proliferation was evaluated by CCK-8 assay in HCT116(A) and DLD1(B) cells. C: The effect of KIF11 overexpression on SW480(C) cell proliferation was assessed using CCK-8 assay. D, E: Colony formation assays were conducted to analyze the impact of KIF11 knockdown on HCT116 and DLD1 cell proliferation (×100, scale bar=100 μm). F, G: The effect of KIF11 overexpression on SW480 cell proliferation was tested *via* colony formation assays. H, I: EdU assays were performed to examine the effect of KIF11 knockdown on HCT116 and DLD1 cell proliferation (×100, scale bar=500 μm). J, K: EdU assays were used to evaluate the effect of KIF11 overexpression on SW480 cell proliferation (×100, scale bar=500 μm). L, M: Transwell migration assays were conducted to determine the effect of KIF11 knockdown on the migration ability of HCT116 and DLD1 cells (×100, scale bar=100 μm). N, O: The impact of KIF11 overexpression on the migration ability of SW480 cells was assessed by transwell assays (×100, scale bar=100 μm). Compared with the shNc group, **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 (*n*=3).

图2 KIF11促进结直肠癌进展
Figure 2 KIF11 promoted colorectal cancer cell progression



A: Flow cytometry assays were performed to observe the percentage change of apoptosis cells after knockdown KIF11. B: Flow cytometry assays were performed to observe the percentage change of apoptosis cells after overexpressing KIF11. ** $P < 0.01$ and *** $P < 0.001$ ($n=3$).

图3 KIF11表达影响CRC细胞凋亡

Figure 3 KIF11 expression affected apoptosis in CRC cells

KIF11 stability through IGF2BP2-dependent mechanisms

To explore potential mechanisms regulating KIF11 expression, we employed the starBase database (<https://rnasysu.com/encori/>) and the STRING website (<https://cn.string-db.org/>) to conduct an enrichment analysis of RNA binding proteins (RBPs) connected with KIF11 mRNA. The results demonstrated a strong correlation between m6A modification and KIF11 mRNA (Figure 5A). Subsequently, we use the SRAMP website (<http://www.cuilab.cn/sramp/>) to confirm the existence of several potentially high-confidence m6A modification sites in the KIF11 mRNA (Figure 5B). The online prediction site (<http://m6a2target.canceromics.org>) revealed that METTL3 mediated the m6A modification of KIF11. According to previous research, METTL3 was highly expressed in CRC and associated

with poor prognosis^[10]. GEPIA database showed that METTL3 was positively correlated with KIF11 expression (Figure 5C). We verified that METTL3 positively regulated KIF11 by qRT-PCR and WB experiments (Figure 5D–G). To verify whether KIF11 mRNA was subject to METTL3-mediated m6A modification, we performed MeRIP-qPCR, and the results revealed that the m6A abundance of KIF11 mRNA was significantly reduced after METTL3 knockdown (Figure 6A). The above results suggest that METTL3 mediates the m6A methylation modification of KIF11. Furthermore, we found that KIF11 mRNA stability was decreased after the knockdown of METTL3 (Figure 6B, C). Overall, METTL3 induced KIF11 m6A modification and stabilized its expression. The current consensus indicates that the m6A modifications function primarily by recruiting “reader” proteins. Earlier research has report-

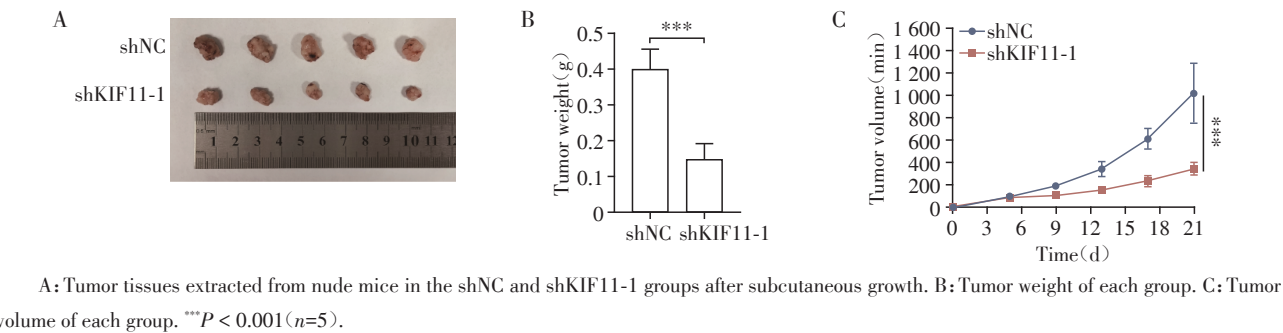


图4 KIF11在体内促进CRC进展
Figure 4 KIF11 promoted CRC progression *in vivo*

ed that the IGF2BPs family is a crucial “reader” of m6A modification, which affects gene expression by regulating the stability of target mRNAs^[24]. Therefore, we used the starBase database to predict the relationship between KIF11 and their expression, and found that the correlation between IGF2BP2 and KIF11 was the highest (Supplementary Figure 2). The RIP-qPCR experiment results demonstrated that IGF2BP2 and KIF11 interacted with each other (Figure 6D). According to qRT - PCR and WB assay results, IGF2BP2 knockdown decreased the mRNA and protein levels of KIF11 (Figure 6E-H). In addition, the RNA stability assay demonstrated that KIF11 mRNA stability decreased after IGF2BP2 knockdown (Figure 6I, J). These findings indicated that IGF2BP2 improved mRNA stability and functions as a “reader” of KIF11 mRNA methylation. Rescue experiments revealed that METTL3 overexpression partially counteracted the reduction of KIF11 expression levels caused by IGF2BP2 silencing (Figure 6K). Taken together, METTL3 - mediated m6A modification enhances KIF11 mRNA stability in an IGF2BP2-dependent manner.

2.5 METTL3 promoted CRC proliferation and migration by regulating KIF11

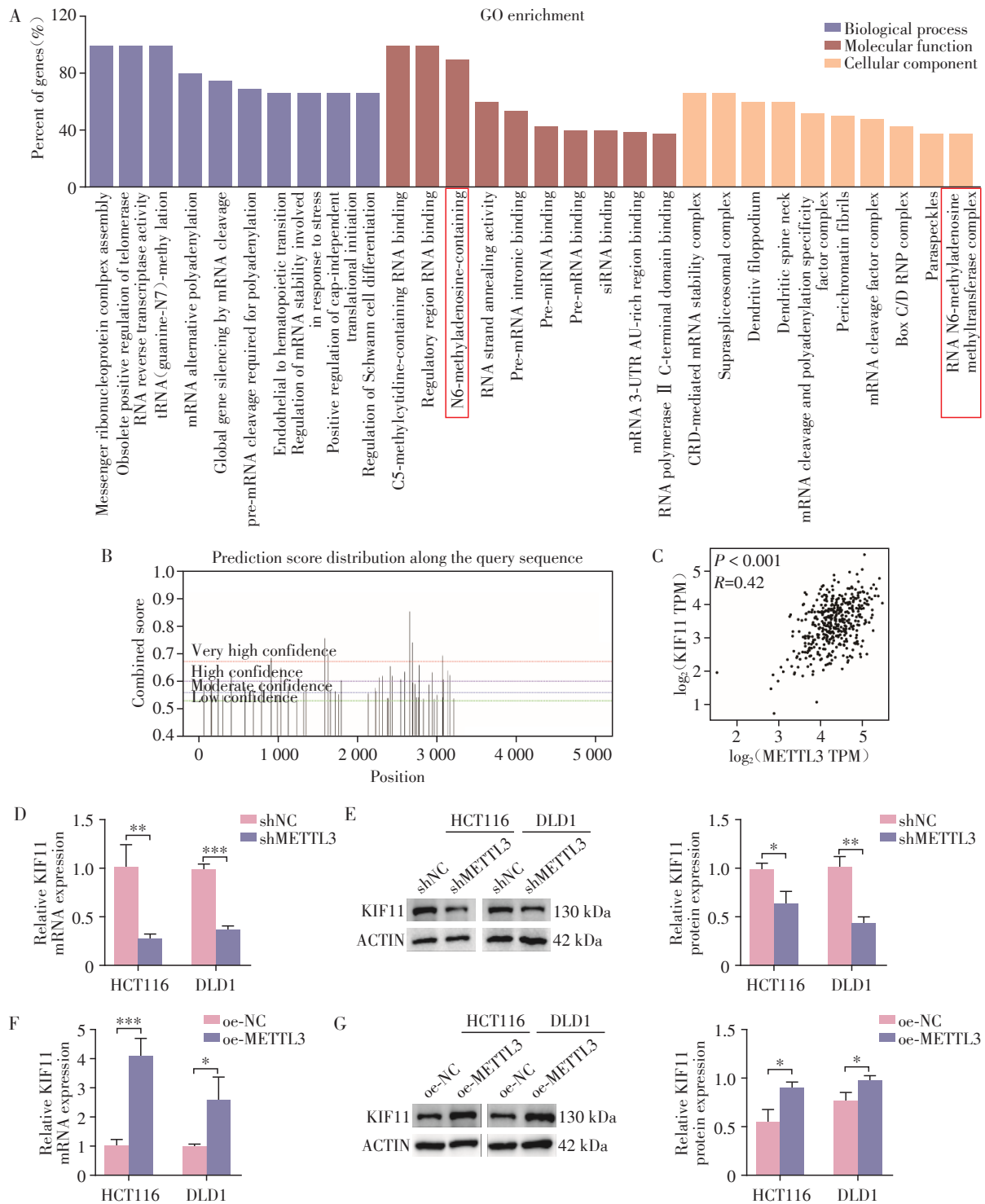
Based on these results, we further investigated the impact and regulatory mechanisms of METTL3 on the proliferation and migration of CRC cells. The results of CCK-8 (Figure 7A, B), colony formation (Figure 7C, D), and EdU (Figure 7E, F) experiments showed that METTL3 silencing inhibited the proliferative ability of CRC cells, while overexpression of KIF11 partially rescued the adverse effect. In addition, Transwell assay results demonstrated that KIF11 overexpression partially elim-

inated the inhibitory effect on migration caused by METTL3 knockdown in CRC cells (Figure 7G, H). Consequently, KIF11 was discovered to be a downstream target of METTL3 to facilitate CRC progression.

2.6 KIF11 promoted CRC progression through the PI3K/AKT signaling pathway

To further explore the possible downstream molecular mechanisms of KIF11, we performed transcriptome sequencing analysis. We found a total of 166 differentially expressed genes (DEGs) between the knock-down and control groups, comprising 114 upregulated genes and 52 downregulated genes in KIF11 knock-down cells ($P < 0.05$, $|\log_2(\text{fold change})| > 1$) (Figure 8A, B). By gene set enrichment analysis (GSEA), we found that KIF11 and PI3K/AKT signaling pathway were closely related (Figure 8C). WB results indicated that the knockdown of KIF11 led to significantly lower phosphorylation levels of PI3K and AKT than that of the NC group, whereas the total protein levels of PI3K and AKT were unaffected (Figure 8D). In addition, we assessed the impact of the METTL3-KIF11 axis on the PI3K/AKT pathway. We overexpressed KIF11 in METTL3 - silenced colorectal cancer cells and found that upregulation of KIF11 partially recovered the phosphorylation level of the PI3K/AKT signaling pathway (Figure 8E).

Among the top 10 down - regulated downstream genes, PROM1 attracted our attention. Previously, Wei *et al*^[25] reported that CD133-p85 interaction activated the PI3K/Akt pathway to promote tumorigenicity of glioma stem cells; Zhu *et al*^[26] found that CD133 promoted the chemoresistance to 5 - fluorouracil in GC cells by activating the PI3K/Akt/p70S6K pathway. So



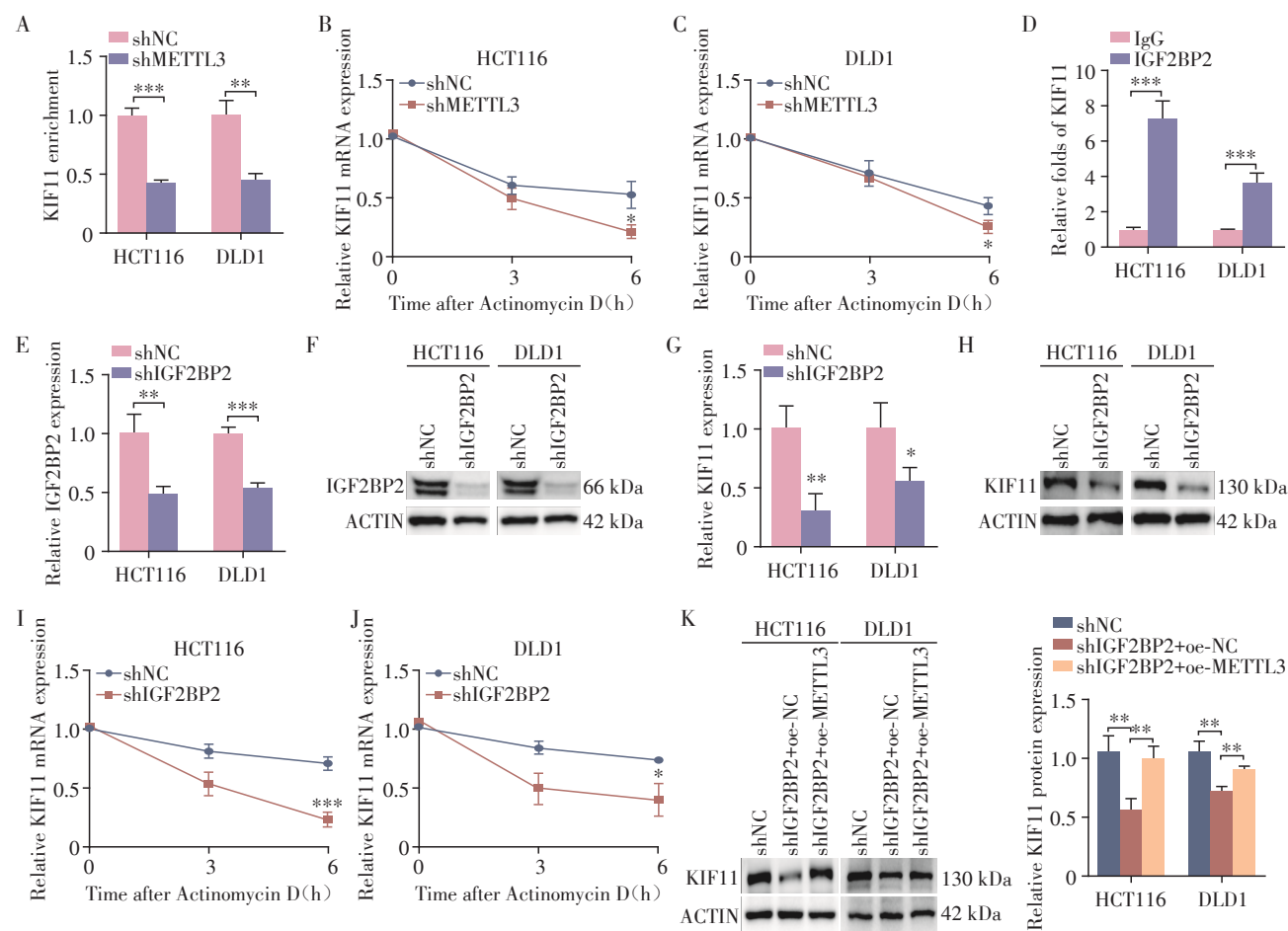
A: GO enrichment showed that KIF11 mRNA was associated with m6A methylation modification ($n=118$). B: SRAMP website predicted the presence of m6A modification sites on KIF11 mRNA. C: GEPIA database showed a positive correlation between METTL3 and KIF11 expression ($n=401$). D, E: The expression level of KIF11 after knockdown of METTL3 was detected by qRT-PCR (D) and WB (E). F, G: The expression level of KIF11 after overexpression of METTL3 was detected by qRT-PCR (F) and WB (G) ($n=3$). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

图5 METTL3对KIF11具有正向调控作用

Figure 5 METTL3 positively regulated KIF11 expression

we hypothesized that KIF11 might promote CRC progression through the PROM1/PI3K/AKT pathway. The results of qRT-PCR and WB demonstrated that PROM1

expression decreased after KIF11 was knocked down, while overexpression of KIF11 produced the opposite effect (Figure 9A–D). CCK - 8, colony formation, and



A: MeRIP-qPCR demonstrated that METTL3 mediated m6A modification of KIF11. B, C: Effect of knockdown of METTL3 on KIF11 mRNA stability. D: The binding of KIF11 to IGF2BP2 was demonstrated by RIP-qPCR. E, F: The knockdown efficiency of IGF2BP2 was verified by qRT-PCR (E) and WB (F). G, H: The expression level of KIF11 after the knockdown of IGF2BP2 was detected by qRT-PCR (G) and WB (H). I, J: Effect of knockdown of IGF2BP2 on KIF11 mRNA stability. K: The expression level of KIF11 after knockdown of IGF2BP2 and overexpression of METTL3 was detected by WB. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ ($n = 3$).

图6 METTL3介导的m6A修饰通过IGF2BP2依赖性机制增强KIF11的稳定性

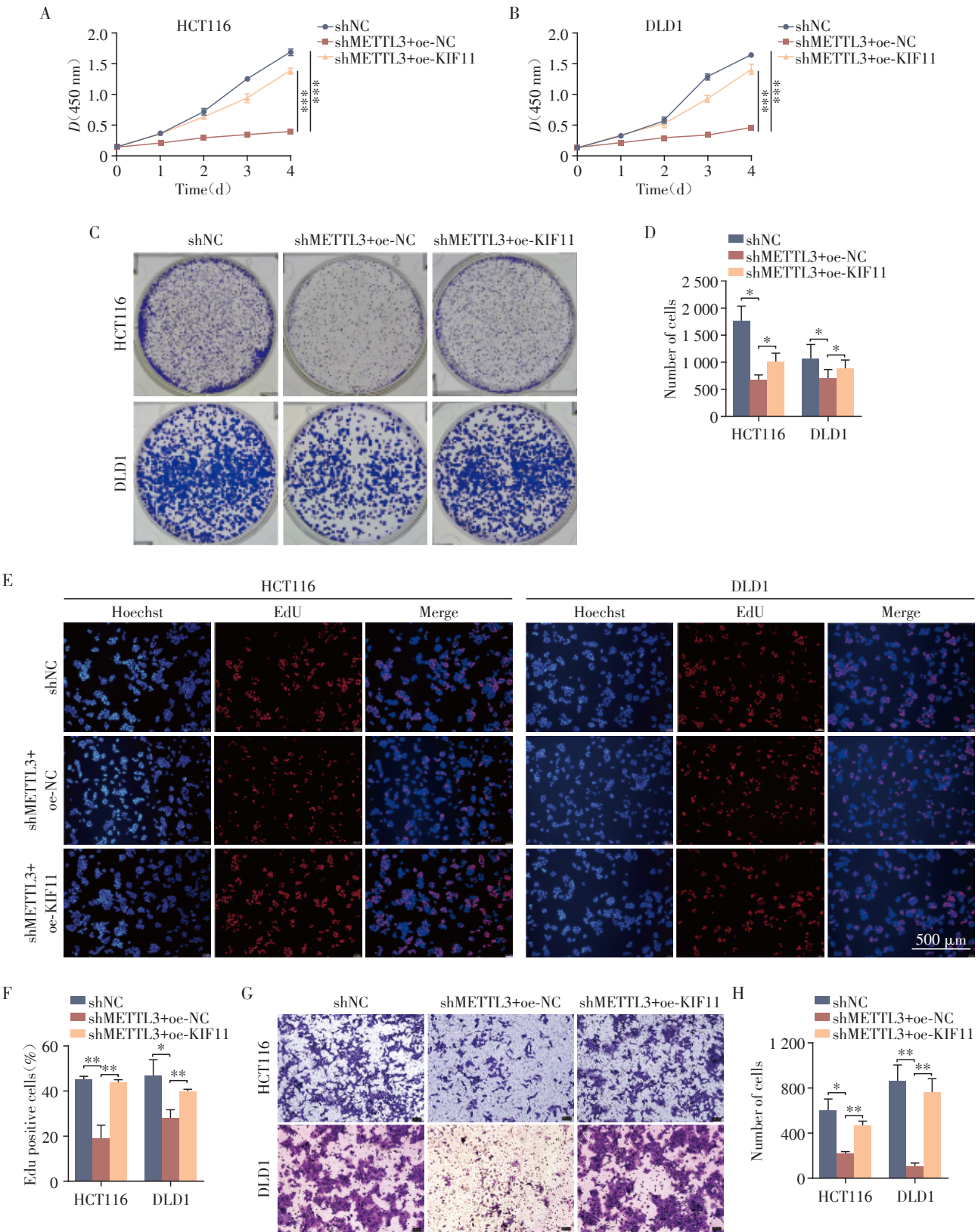
Figure 6 METTL3-regulated m6A modification enhanced KIF11 stability through IGF2BP2-dependent mechanisms

EdU assays showed that KIF11 knockdown had reduced cell proliferation ability, which could be rescued by PROM1 overexpression (Figure 9E–J). Transwell assay demonstrated that overexpression of PROM1 partially rescued the decreased cell migration ability caused by KIF11 knockdown (Figure 10A, B). The WB results revealed that silencing of KIF11 suppressed the activation of the PI3K/AKT pathway, and the overexpression of PROM1 rescued the KIF11 knockdown-mediated inhibition (Figure 10C). These results suggest that KIF11 promotes CRC progression through the PROM1/PI3K/AKT pathway.

3 Discussion

Due to tumor recurrence, metastasis and drug re-

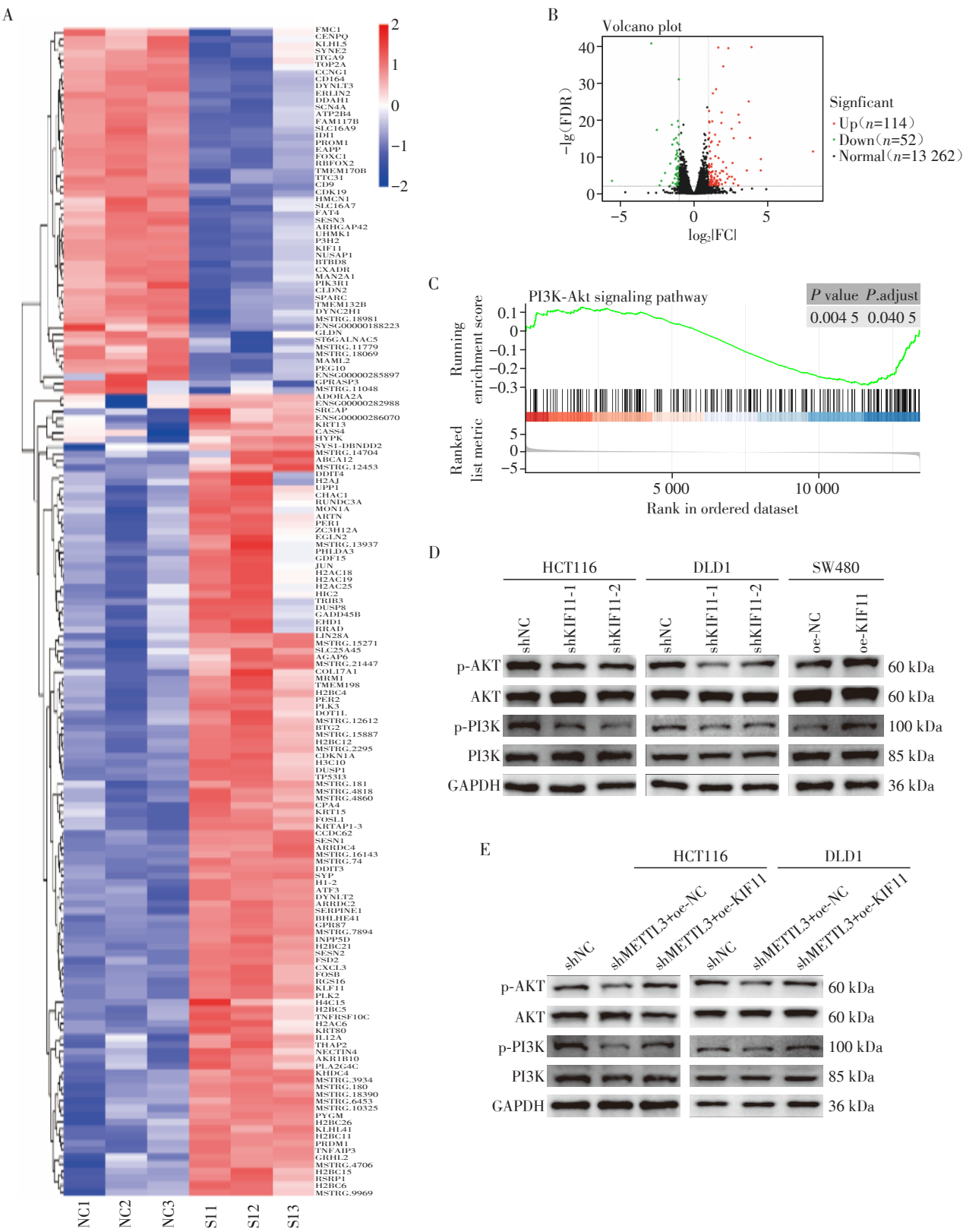
sistance development, the 5-year survival rate of CRC is less than 40%^[27]. The increasing morbidity and mortality suggest that the exploration of novel biomarkers is necessary for the diagnosis and prognosis of colorectal cancer. KIF11 is a member of the kinesin family of proteins, which plays a vital role in the mitotic process^[28–29]. Kinesin family proteins (KIFs) are a group of conserved microtubule-dependent molecular motor proteins that possess ATPase activity and motility properties and are mainly involved in a series of intracellular activities, including cytokinesis, intracellular vesicle and organelle trafficking, and microtubule cytoskeleton reorganization^[30–31]. Studies have shown that the kinesin family is essential to cancer development. For example, KIF20A regulates malignant proliferation and



A, B: CCK-8 assays showed the effects of METTL3 and KIF11 on HCT116(A) and DLD1(B) cell proliferation ability. C, D: Clonogenic assays showed effects of METTL3 and KIF11 on HCT116(A) and DLD1(B) cell proliferation ability. E, F: EdU assays showed effects of METTL3 and KIF11 on HCT116(A) and DLD1(B) cell proliferation ability. (×100, scale bar=500 μm). G, H: The effects of METTL3 and KIF11 on cell migration ability were measured by transwell assay(×100, scale bar=100 μm). **P* < 0.05, ***P* < 0.01, and ****P* < 0.001(*n*=3).

图7 METTL3通过调控KIF11促进CRC增殖和迁移

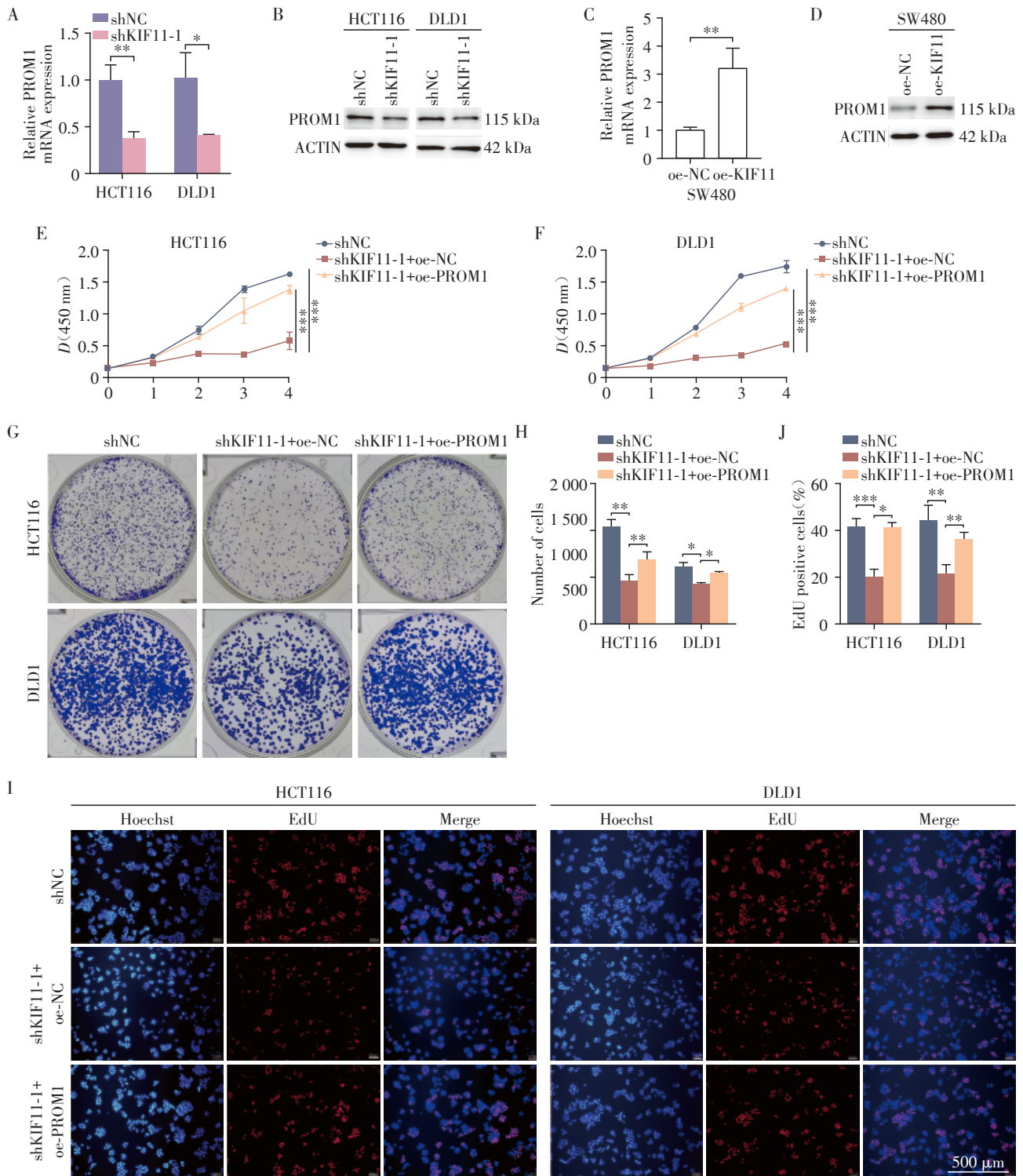
Figure 7 METTL3 promoted CRC proliferation and migration by regulating KIF11



A: Heatmap of the results of cellular RNA-seq analysis. B: Volcano plot of the results of cellular RNA-seq analysis. C: GSEA enrichment analysis showed that KIF11 was associated with PI3K/AKT signaling pathway. D: The expression level of related proteins in the PI3K/AKT signaling pathway after knockdown or overexpression of KIF11 was detected by WB. E: WB was performed to detect the effect of the METTL3/KIF11 axis on the expression level of related proteins in the PI3K/AKT signaling pathway($n=3$).

图8 KIF11通过PI3K/AKT信号通路促进CRC进展

Figure 8 KIF11 promoted CRC progression through the PI3K/AKT signaling pathway



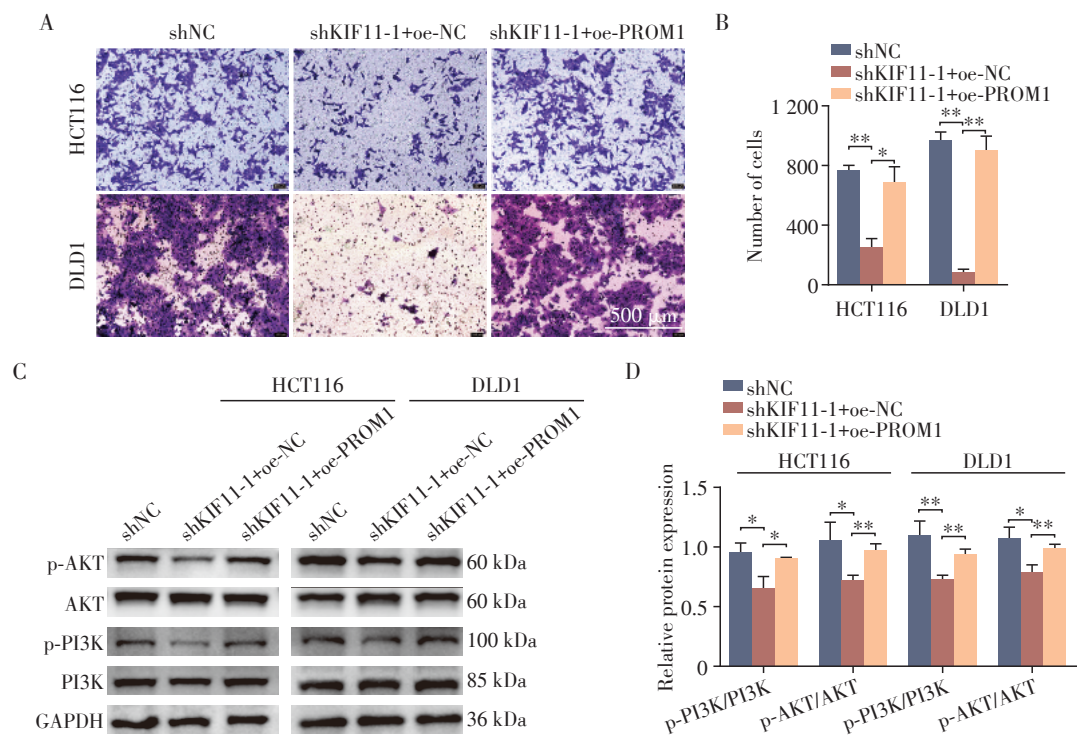
A, B: The expression level of PROM1 after knockdown of KIF11 was detected by qRT-PCR(A) and WB(B). C, D: The expression level of PROM1 after overexpression of KIF11 was detected by qRT-PCR(C) and WB(D). E, F: CCK-8 assays showed that overexpression of PROM1 partially rescued the effect of KIF11 knockdown on HCT116 and DLD1 cell proliferation. G, H: Clone formation assays showed that overexpression of PROM1 partially rescued the effect of KIF11 knockdown on HCT116 and DLD1 cell proliferation. I, J: EdU assays showed that overexpression of PROM1 partially rescued the effect of KIF11 knockdown on HCT116 and DLD1 cell proliferation. ($\times 100$, scale bar=500 μm). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ ($n=3$).

图9 KIF11通过调控PROM1促进CRC增殖

Figure 9 KIF11 promoted CRC proliferation by regulating PROM1

chemotherapy tolerance in CRC through the JAK2/STAT3 signaling pathway^[32]; KIF11 promotes the pro-

gression of hepatocellular carcinoma *via* the OCT4 pathway^[33]. Our study demonstrated that KIF11 was



A, B: Transwell assay showed that overexpression of PROM1 partially rescued the effect of KIF11 knockdown on cell migration ability($\times 100$, scale bar=100 μm). C, D: The effect of the KIF11/PROM1 axis on the expression levels of related proteins in the PI3K/AKT signaling pathway was detected by WB. * $P < 0.05$ and ** $P < 0.01$ ($n=3$).

图 10 KIF11通过调控PROM1促进CRC迁移
Figure 10 KIF11 promoted CRC migration by regulating PROM1

highly expressed in CRC and promoted CRC proliferation and migration.

Studies have shown that KIF11 up-regulation can lead to centrosome fragmentation and chromosome instability through multiple signaling pathways, which leads to exuberant cell division and proliferation, and promotes the progression of tumors, including lung and gastric cancers, and glioblastoma^[34–35]. To explore the molecular mechanisms by which KIF11 regulates CRC development, we utilized databases and found that KIF11 was closely associated with m6A methylation modifications. RNA methylation modification is a kind of modification that widely exists in all types of RNAs, and its leading role is to control the target protein expression by regulating the transcription, stability, and translation efficiency of mRNA. They can regulate the activity of signaling pathways by acting directly or indirectly on key molecules^[36–37]. It was demonstrated that METTL3 mediated the m6A modification of KIF11 and affected its mRNA stability. Moreover, rescue experiments showed that the overexpression of KIF11 partial-

ly restored the tumor suppressor phenotype induced by the knockdown of METTL3. METTL3 is one of the major “writer” proteins, which has been shown to have a dual function in promoting or suppressing cancers. For example, He *et al*^[38] showed that METTL3 was lowly expressed in PTC and acted as a suppressor in regulating neutrophil infiltration; many studies have also indicated that METTL3 is highly expressed in CRC tissues and is strongly associated with poor prognosis. For example, METTL3 promotes CRC development by regulating the m6A-CRB3-Hippo axis^[39]. METTL3 inhibition suppresses cell growth and survival in CRC *via* ASNS downregulation^[40]; It has also been found that METTL3 promotes cellular senescence of CRC *via* modulation of CDKN2B transcription and mRNA stability^[41]. Yang *et al*^[42] reported that METTL3-mediated RanGAP1 recruited YTHDF1 to facilitate the development of CRC *via* the MAPK pathway. Similarly, IGF2BP2 promotes cancer progression by influencing the stability of downstream targets and post-transcriptional regulation. Our study, for the first time, proved

that the m6A methylation of KIF11 mediated by METTL3 was recognized by IGF2BP2, which in turn impacted its expression. Subsequently, through RNA-seq analysis, we found that KIF11 promoted CRC progression by enhancing phosphorylation levels of the PI3K/AKT pathway. Previous studies have shown that KIF11 also enhances the chemosensitivity of CRC to oxaliplatin through p53/GSK3 β signaling^[43]. Aberrant activation of the PI3K/AKT pathway regulates autophagy and EMT levels, induces tumor cell proliferation and migration, and also allows tumor cells to evade apoptosis by creating a dysregulation between anti-apoptotic and pro-apoptotic genes^[44]. Our study demonstrated that METTL3 silencing considerably attenuated the phosphorylation levels of PI3K and AKT, while overexpression of KIF11 in METTL3-knockdown cells partially recovered their phosphorylation levels. These results support the idea that METTL3/IGF2BP2 promotes CRC progression through KIF11 by activating the PI3K/AKT signaling pathway.

Our study also revealed that PROM1 was a downstream target of KIF11. Overexpression of PROM1 in KIF11-knockdown cells could rescue the inhibitory effect of KIF11 on CRC proliferation and migration. In addition, KIF11 was able to promote CRC progression through the PROM1/PI3K/AKT pathway.

Despite these conclusions drawn from our study, several limitations remain. First, while we have established that METTL3 mediates the m6A methylation modification of KIF11, we have not experimentally identified the specific m6A modification sites. Second, although we have recognized the PROM1/PI3K/AKT pathway as a downstream target of KIF11, the precise regulatory mechanisms involved remain to be elucidated. Furthermore, whether KIF11 also promotes tumorigenesis *in vivo* has yet to be confirmed. In addition, we acknowledge as a limitation that multiple testing corrections such as the Bonferroni or Benjamini-Hochberg procedures were not applied in the current analysis. In our future research, we will expand the sample size and use more refined correction strategies to enhance statistical power and further improve the reliability of our research conclusions. Therefore, we will continue our experiments to refine this investigation.

4 Conclusion

In conclusion, our study elucidates a novel mechanism through which KIF11 regulates colorectal cancer. We demonstrate that METTL3/IGF2BP2 modulates KIF11 expression in an m6A-dependent manner, influencing its stability and thereby regulating PROM1 and activating the PI3K/AKT signaling pathway to facilitate CRC progression. These findings underscore the potential of KIF11 as a promising predictive biomarker and an effective therapeutic target, offering new insights into the diagnosis and treatment of CRC.

利益冲突声明:

所有作者声明无利益冲突。

Conflicts of Interests:

The authors declare no conflict of interests.

作者贡献声明:

林书慧完成实验验证和初稿撰写;朱静负责研究构思和实验设计;钱萌森和丁洁完成数据整理与分析;丁洁和李娟负责项目资金获取;罗茜和李杰完成数据收集;王科明和王娟完成论文的修改与审阅。

Author's Contributions:

LIN Shuhui completed the experimental verification and initial draft writing; ZHU Jing was responsible for research conception and experimental design; QIAN Mengsen and DING Jie performed the data organization and analysis; DING Jie and LI Juan managed the project funding acquisition; LUO Qian and LI Jie carried out the data collection; WANG Keming and WANG Juan completed the paper revision and review.

[References]

- [1] BRAY F, LAVERSANNE M, SUNG H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries[J]. CA Cancer J Clin, 2024, 74(3): 229-263
- [2] SIEGEL R L, MILLER K D, FUCHS H E, et al. Cancer statistics, 2021[J]. CA A Cancer J Clin, 2021, 71(1): 7-33
- [3] SINICROPE F A. Increasing incidence of early-onset colorectal cancer [J]. N Engl J Med, 2022, 386(16): 1547-1558
- [4] GUO X H, ZHOU L, WU Y N, et al. KIF11 as a potential pan-cancer immunological biomarker encompassing the disease staging, prognoses, tumor microenvironment, and therapeutic responses [J]. Oxid Med Cell Longev, 2022, 2022: 2764940
- [5] DANG W, BIAN R, FAN Q Q, et al. KIF11 promotes cell proliferation *via* ERBB2/PI3K/AKT signaling pathway in gallbladder cancer [J]. Int J Biol Sci, 2021, 17(2): 514-

- 526
- [6] CHEN D L, WANG J H, LI Y, et al. LncRNA NEAT1 suppresses cellular senescence in hepatocellular carcinoma *via* KIF11 - dependent repression of CDKN2A [J]. Clin Transl Med, 2023, 13(9): e1418
- [7] ZUIDHOF H R, MÜLLER C, KORTMAN G, et al. The m6A demethylase FTO promotes C/EBP β -LIP translation to perform oncogenic functions in breast cancer cells[J]. FEBS J, 2025, 292(10): 2688-2709
- [8] ZHU Z M, HUO F C, SHI C, et al. METTL3 - mediated m6A modification of PAK6 drives cervical cancer progression through activating MAPK14[J]. Int J Biol Macromol, 2025, 309: 142880
- [9] ZOU J, SHI X, WU Z, ZUO S, et al. MRTX1133 attenuates KRAS(G12D)mutated-colorectal cancer progression through activating ferroptosis activity *via* METTL14/ LINC02159/FOXO2 axis [J]. Transl Oncol, 2025, 52: 102235
- [10] ZHOU D, TANG W W, XU Y D, et al. METTL3/YTHDF2 m6A axis accelerates colorectal carcinogenesis through epigenetically suppressing YPEL5[J]. Mol Oncol, 2021, 15(8): 2172-2184
- [11] ZHAO S, SONG P, ZHOU G, et al. METTL3 promotes the malignancy of non-small cell lung cancer by N6 - methyladenosine modifying SFRP2 [J]. Cancer Gene Ther, 2023, 30(8): 1094-1104
- [12] XUE L, LI J, LIN Y H, et al. m6A transferase METTL3-induced lncRNA ABHD11 - AS1 promotes the Warburg effect of non - small - cell lung cancer [J]. J Cell Physiol, 2021, 236(4): 2649-2658
- [13] WANG J Y, CHEN L J, QIANG P. The role of IGF $_2$ BP $_2$, an m6A reader gene, in human metabolic diseases and cancers[J]. Cancer Cell Int, 2021, 21(1): 99
- [14] WANG J Y, ZHU M X, ZHU J H, et al. HES1 promotes aerobic glycolysis and cancer progression of colorectal cancer *via* IGF $_2$ BP $_2$ -mediated GLUT1 m6A modification[J]. Cell Death Discov, 2023, 9(1): 411
- [15] HU X G, PENG W X, ZHOU H X, et al. IGF $_2$ BP $_2$ regulates DANCER by serving as an N6 - methyladenosine reader[J]. Cell Death Differ, 2020, 27(6): 1782-1794
- [16] BARZEGAR BEHROOZ A, SYAHIR A, AHMAD S. CD133: beyond a cancer stem cell biomarker[J]. J Drug Target, 2019, 27(3): 257-269
- [17] ZHU Y L, KONG F F, ZHANG C H, et al. CD133 mediates the TGF - β 1 - induced activation of the PI3K/ERK/ P70S6K signaling pathway in gastric cancer cells[J]. Oncol Lett, 2017, 14(6): 7211-7216
- [18] SHI Q, HE Y, HE S Y, et al. RP11-296E3.2 acts as an important molecular chaperone for YBX1 and promotes colorectal cancer proliferation and metastasis by activating STAT3[J]. J Transl Med, 2023, 21(1): 418
- [19] LIU W J, ZHANG Z L, LUO X T, et al. m6A - mediated LINC02038 inhibits colorectal cancer progression *via* regulation of the FAM172A/PI3K/AKT pathway *via* competitive binding with miR - 552 - 5p[J]. Int J Oncol, 2023, 63(1): 81
- [20] BAKR M, ABD - ELMAWLA M A, ELIMAM H, et al. Telomerase RNA component lncRNA as potential diagnostic biomarker promotes CRC cellular migration and apoptosis evasion *via* modulation of β -catenin protein level[J]. Noncoding RNA Res, 2023, 8(3): 302-314
- [21] BIAN Y B, WANG Y, XU S F, et al. m6A modification of long non - coding RNA HNF1A - AS1 facilitates cell cycle progression in colorectal cancer *via* IGF $_2$ BP $_2$ - mediated CCND1 mRNA stabilization [J]. Cells, 2022, 11(19): 3008
- [22] ZENG H, HOU Y X, ZHOU X Y, et al. Cancer-associated fibroblasts facilitate premetastatic niche formation through lncRNA SNHG5-mediated angiogenesis and vascular permeability in breast cancer [J]. Theranostics, 2022, 12(17): 7351-7370
- [23] MU H Y, CHEN J Z, HUANG W J, et al. OmicShare tools: a zero-code interactive online platform for biological data analysis and visualization[J]. Imeta, 2024, 3(5): e228
- [24] ZHU T Y, HONG L L, LING Z Q. Oncofetal protein IGF2BPs in human cancer: functions, mechanisms and therapeutic potential[J]. Biomark Res, 2023, 11(1): 62
- [25] WEI Y Y, JIANG Y Z, ZOU F, et al. Activation of PI3K/ Akt pathway by CD133-p85 interaction promotes tumorigenic capacity of glioma stem cells [J]. Proc Natl Acad Sci USA, 2013, 110(17): 6829-6834
- [26] ZHU Y L, YU J W, WANG S L, et al. Overexpression of CD133 enhances chemoresistance to 5-fluorouracil by activating the PI3K/Akt/p70S6K pathway in gastric cancer cells[J]. Oncol Rep, 2014, 32(6): 2437-2444
- [27] ZHU M, YU R, LIU Y, et al. LncRNA H19 participates in leukemia inhibitory factor mediated stemness promotion in colorectal cancer cells [J]. Biochem Genet, 2024, 62(5): 3695-3708
- [28] DİEFENBACH R J, MACKAY J P, ARMATI P J, et al. The C-terminal region of the stalk domain of ubiquitous human kinesin heavy chain contains the binding site for kinesin light chain [J]. Biochemistry, 1998, 37(47): 16663-16670
- [29] LUCANUS A J, YIP G W. Kinesin superfamily: roles in breast cancer, patient prognosis and therapeutics[J]. Oncogene, 2018, 37(7): 833-838
- [30] SHARP D J, ROGERS G C, SCHOLEY J M. Microtubule

- motors in mitosis[J]. *Nature*, 2000, 407(6800):41–47
- [31] CAMLIN N J, MC LAUGHLIN E A, HOLT J E. Motoring through: the role of kinesin superfamily proteins in female meiosis [J]. *Hum Reprod Update*, 2017, 23(4): 409–420
- [32] TANG J, XU J, ZHI Z W, et al. MiR - 876 - 3p targets KIF20A to block JAK2/STAT3 pathway in glioma[J]. *Am J Transl Res*, 2019, 11(8):4957–4966
- [33] ZHANG J, WEI Z X, QI X Y, et al. Kinesin family member 11 promotes progression of hepatocellular carcinoma *via* the OCT4 pathway[J]. *Funct Integr Genomics*, 2023, 23(3):284
- [34] VENERE M, HORBINSKI C, CRISH J F, et al. The mitotic kinesin KIF11 is a driver of invasion, proliferation, and self-renewal in glioblastoma[J]. *Sci Transl Med*, 2015, 7(304):304ra143
- [35] SUN R F, HE N, ZHANG G Y, et al. Combined inhibition of KIF11 and KIF15 as an effective therapeutic strategy for gastric cancer[J]. *Curr Cancer Drug Targets*, 2023, 23(4):293–306
- [36] ZOU L B, CHEN W B, ZHOU X M, et al. N6-methyladenosine demethylase FTO suppressed prostate cancer progression by maintaining CLIC4 mRNA stability [J]. *Cell Death Discov*, 2022, 8(1):184
- [37] WANG T Y, KONG S, TAO M, et al. The potential role of RNA N6-methyladenosine in cancer progression[J]. *Mol Cancer*, 2020, 19(1):88
- [38] HE J, ZHOU M X, YIN J, et al. METTL3 restrains papillary thyroid cancer progression *via* m6A/c-Rel/IL-8-mediated neutrophil infiltration [J]. *Mol Ther*, 2021, 29(5):1821–1837
- [39] PAN J S, LIU F, XIAO X L, et al. METTL3 promotes colorectal carcinoma progression by regulating the m6A-CRB3-Hippo axis [J]. *J Exp Clin Cancer Res*, 2022, 41(1):19
- [40] YU Y, HAI Y N, ZHOU H F, et al. METTL3 inhibition suppresses cell growth and survival in colorectal cancer *via* ASNS downregulation [J]. *J Cancer*, 2024, 15(15):4853–4865
- [41] CHEN Z J, ZHOU J W, WU Y, et al. METTL3 promotes cellular senescence of colorectal cancer *via* modulation of CDKN2B transcription and mRNA stability [J]. *Oncogene*, 2024, 43(13):976–991
- [42] YANG R, YANG C, SU D J, et al. METTL3-mediated Ran-GAP1 promotes colorectal cancer progression through the MAPK pathway by recruiting YTHDF1 [J]. *Cancer Gene Ther*, 2024, 31(4):562–573
- [43] ZHOU Y, YANG L P, XIONG L, et al. KIF11 is upregulated in colorectal cancer and silencing of it impairs tumor growth and sensitizes colorectal cancer cells to oxaliplatin *via* p53/GSK3 β signaling [J]. *J Cancer*, 2021, 12(12):3741–3753
- [44] ZUGHAI BI T A, SUHAIL M, TARIQUE M, et al. Targeting PI3K/Akt/mTOR pathway by different flavonoids: a cancer chemopreventive approach [J]. *Int J Mol Sci*, 2021, 22(22):12455

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