N⁶-methyladenosine methyltransferase KIAA1429 elevates colorectal cancer aerobic glycolysis via HK2-dependent manner

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ABSTRACT

Emerging evidence has emphasized the critical roles played by N⁶-methyladenosine RNA (m⁶A) modification in colorectal carcinoma (CRC) initiation and progression. However, the roles and mechanism of m⁶A and KIAA1429 in CRC progression require further clarification. Here, our research aimed to investigate the functions of KIAA1429 in CRC tumorigenesis. Results indicated that KIAA1429 up-regulation closely correlated to the poor prognosis of CRC patients. Bio-functional assays demonstrated that KIAA1429 promoted the aerobic glycolysis, including glucose uptake, lactate production, ATP generation and extracellular acidification rate (ECAR). Mechanistically, KIAA1429 positively up-regulated HK2 level via increasing its mRNA stability by binding the m⁶A site of HK2 mRNA via m⁶A-independent manner. Collectively, our work indicates that KIAA1429 has the potential to promote CRC carcinogenesis by targeting HK2 via m⁶A-independent manner, providing insight into the critical roles of m⁶A in CRC.

Highlights

- KIAA1429 up-regulation closely correlated to the poor prognosis of CRC patients.
- KIAA1429 promoted the aerobic glycolysis.
- Mechanistically, KIAA1429 positively up-regulated HK2 level via increasing its mRNA stability.

1. Introduction

Colorectal carcinoma (CRC) acts as the third most commonly diagnosed cancer and second most common leading causes of cancer-related death worldwide [1,2]. In Asian, CRC acts as one of the top five diagnosed cancers and causes for cancer-related death [3,4]. Although the incidence has declined over the past decade due to the introduction of effective screening program, there is about 50% patients have metastasis when diagnosed [5,6]. Metabolic reprogramming is regarded as an important characteristic for malignant tumor, which significantly regulates the tumorigenesis of CRC. Therefore, it is pressingly necessary to elucidate the mechanisms of CRC metabolic
reprogramming to develop effective strategy against CRC.

\(^6\)methylion of adenosine (m\(^6\)A) in RNA regulates many physiological and disease processes \[7,8\]. In human cancer, m\(^6\)A could regulate the pathophysiological process. Meanwhile, m\(^6\)A modification also critically regulated translation efficiency, mRNA stability, subcellular localization and RNA-protein interactions \[9,10\]. In CRC, the m\(^6\)A and METTL3 levels are both substantially increased in tissues and cells, and METTL3 substantially promotes the proliferation, migration and invasion through activating the Hippo pathway and reducing YAP1 nuclear localization \[11\]. METTL3 facilitates circ_0000677 level through m\(^6\)A modification and SUMO1-mediated SUMOylation regulates METTL3 via regulating circ_0000677/ABCC1 axis \[12\]. Overall, we could conclude that m\(^6\)A play critical functions in CRC.

Emerging research has indicated that m\(^6\)A key regulators play critical roles in human cancer. Especially, the m\(^6\)A methyltransferase exerts its various functions through binding downstream targets, thereby regulating tumorigenesis of CRC.

In present study, we initially investigated the roles and molecular mechanism of KIAA1429 in CRC energy metabolism. KIAA1429 mediated the aerobic glycolysis by mediating HK2 mRNA stability, thereby promoting the proliferation and metastasis of tumorigenesis of CRC. KIAA1429 may act as a novel oncogene with the potential to regulate CRC progression.

2. Materials and methods

2.1. Patients’ samples

This study enrolled 48 CRC patients who underwent surgery at The First Hospital of Jilin University. All procedures had been taken in accordance with guidelines 4\(^{th}\) by Declaration of Helsinki. Tumor tissues and their adjacent normal tissues were obtained at surgery from patients suffering from CRC (Table 1). Patients with chemotherapy or radiotherapy were excluded. During the surgery (resection and/or peripheral lymph node dissection), fresh specimens were directly frozen in liquid nitrogen, and validated by pathological diagnosis. This study was reviewed and approved by the Ethics Committee of The First Hospital of Jilin University (No. JLUFH20190287H). Written informed consent was signed by all patients who participated in the study.

| Table 1. Correlation of KIAA1429 expression with CRC patients’ clinicopathological feature. |
|------------------|------------------|------------------|------------------|------------------|
| Age (years) | Total | High | Low | p_value |
| ≥60 | 22 | 10 | 12 | 0.562 |
| <60 | 26 | 14 | 12 | |
| Gender | | | | |
| Male | 23 | 12 | 11 | 0.772 |
| Female | 25 | 12 | 13 | |
| Differentiation | | | | |
| well/moderate | 30 | 13 | 17 | 0.233 |
| poor | 18 | 11 | 7 | |
| Lymph metastasis | | | | |
| Yes | 12 | 12 | 0 | 0.001 |
| No | 36 | 12 | 24 | |

*p < 0.05 represents statistical difference.

2.2. Cell culture

Normal colonic epithelial cell line (FHC) and CRC cells (SW480, HT29) were provided from Shanghai Cell Bank, Shanghai, China) and then cultured in Dulbecco’s Modified Eagle Medium (DMEM)/high-glucose (HyClone, Logan, Australia) supplemented with 10% fetal bovine serum (FBS, Biological Industries, Cromwell, USA) and penicillin/streptomycin (100 U/mL) at 37°C in incubator with 5% CO\(_2\) under-saturated humidity.

2.3. Cell transfection

The full-length of KIAA1429 was cloned into pcDNA3.1 vector (Hanbio Biotechnology, China) for overexpression transfection. The mock vector without KIAA1429 sequence acted as negative control. Short hairpin RNAs (shRNAs) targeting KIAA1429 and shRNA-NC were synthesized by RiboBio (RiboBio, Guangzhou, China), and the efficiency was detected by qRT-PCR. Lipofectamine 3000 (Invitrogen, Carlsbad, USA) were used to cell transfections. The sequences of siRNAs were listed in Table S1.

2.4. Total RNA isolation and real-time quantitative PCR (qRT-PCR)

Total RNA was isolated from CRC tissues and cells using RNAiso Plus (Takara, Dalian, China).
according to the manufacturer’s instructions. The integrity or purity of extracted RNA was measured using NanoDrop One (Thermo Fisher Scientific, Waltham, USA) using UV spectrophotometer. Reverse transcription was done by Transcriptor First-Strand cDNA Synthesis Kit (Roche, Indianapolis, IN, USA). The PCR was performed on a QuantStudio Real-Time PCR System (Applied Biosystems, Foster City, USA) by SYBR Premix ExTaq II kit (Takara). The qRT-PCR primer sequences were listed as following: KIAA1429, forward, 5'-AAGTGCCCCTGTCTTGGATAG-3', reverse, 5'-GAGCCACCTCAGTATTCACCT-3'; HK2, forward, 5'-ACCAGACCACCTAGTATTCACCT-3', reverse, 5'-GGCCACCTACCTACCTACCTACT-3', Actin, forward, 5'-CTCCATCGGCTCGCTGTCGCTGT-3', reverse, 5'-GCTGTCACCTTCACCGTTCC-3'. The relative quantification data was calculated by the 2^−ΔΔCt method.

2.5. Western blot

CRC cells were harvested and then protein was extracted using and RIPA lysis buffer (Beyotime, cat. P0013B) on ice. Protein quantification was performed by using a BCA Protein Assay Kit (Thermo Fisher, cat.no. A53225). The extracted protein and obtained lysate were centrifuged at 12,000 g at 4°C for 30 min. Protein samples were then subjected to SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene difluoride membranes (PVDF). The PVDF membranes were incubated with primary antibodies (anti-KIAA1429/anti-VIRMA, 1:1000, Cell Signaling Technology, catalog no. 88358) followed by a secondary antibody (β-actin). Finally, bands were detected using Imaging System (Bio-Rad) software was used for the quantitative analysis.

2.6. Glucose uptake and lactate production assay

The glucose, lactate levels and ATP levels were measured using glucose assay kit (Sigma, St-Louis, MO, USA), lactate assay kit (Sigma) and ATP Determination Kit according to the manufacturer protocol (Thermo Fisher Scientific, Cat. A22066) as previously described [13].

2.7. Extracellular acidification rate (ECAR)

For the glycolysis rate, extracellular acidification rate (ECAR) was analyzed using the XF96 Bioenergetic Analyzers (Seahorse Bioscience) according to the manufacturer’s instructions [14]. 1 × 10⁴ adhered cells per well were seeded into 96-well culture microplate and then washed with assay medium (unbuffered DMEM supplemented with 2 mM L-glutamine, pH = 7.4). Then the microplates were loaded into the Seahorse Analyzer. Glucose (10 mM), oxidative phosphorylation inhibitor oligomycin (1.0 μM) and the glycolytic inhibitor 2-deoxyglucose (2-DG, 50 mM) were sequentially injected at indicated time points. All measurements were normalized to cell number.

2.8. m⁶A quantification analysis

The m⁶A quantification analysis was performed using m6A RNA methylation quantification kit (ab185912; Abcam) following the manufacturer's protocol [15]. Total RNA samples (400 ng) of each group were used to determine the m⁶A percentage. The m⁶A levels was colorimetrically quantified by absorbance at 450 nm wavelength, and the calculation was performed based on the standard curve.

2.9. RNA stability assay

In KIAA1429 knockdown and overexpression cells, as well as their control transfection, were cultured in six-well plates. Cells were treated with actinomycin D (Act D, 2 μg/mL) at indicated time, as 0, 4 and 8 h. Then, cells were collected and total RNA were isolated. qRT-PCR was performed to detect the relative level of HK2 mRNA as mentioned previously.

2.10. RNA immunoprecipitation (RIP) assay

RIP assay was performed to detect the molecular interaction within HK2 mRNA and KIAA1429 using an EZ-Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (Millipore, Billerica, MA, USA) according to the manufacturer’s instructions. CRC cells with 90% confluence was lysed using complete RIP lysis buffer with RNase
Inhibitor (Millipore) and protease inhibitor. Cell extract (100 μl) was incubated with RIP buffer containing anti-KIAA1429 antibody-conjugated magnetic beads. Normal mouse anti-IgG antibody (Cell Signaling Technology) acts as the negative control.

2.11. Methylated RNA immunoprecipitation (MeRIP)-qPCR

MeRIP-PCR was performed to examine the m\(^6\)A modification of HK2 gene using Magna MeRIP Kit (Cat# CR203146; Millipore, Massachusetts, USA) according to the manufacturer’s instructions as previously described [16]. CRC cells were washed by ice-cold PBS twice and then subsequently harvested for collection by centrifugation at 4°C 1500 rpm for 5 min. Removing the supernatant, CRC cells were mixed with 100 μL RIP lysis buffer and incubated with the lysate on ice for 5 min. Cell preparation was stored at −80°C. Anti-m\(^6\)A antibody (5 μg) was coated to magnetic beads and for rotation for 30 min. The bead was washed with RIP buffer twice and resuspended in RIP immunoprecipitation buffer (900 μL) mixed with cell lysate (100 μL) by centrifugation at 14,000 rpm at 4°C for 10 min. After overnight 4°C, beads were washed and the extraction was analyzed by qRT-PCR.

2.12. Animal in vivo assay

BALB/c nude mice (5 weeks old, 10 mice) were provided from Slac Laboratory Animal Center (Shanghai, China). All animal experiments were approved by the Ethical Committee of The First Hospital of Jilin University (No. JLUFH20190287A).

2.13. Statistical analysis

Statistical analyses were performed using SPSS version 22.0. The significance of the differences was determined using one-way ANOVA or Student’s t-test. The correlations between the two groups were calculated using Spearman’s correlation coefficient. Kaplan–Meier analysis was employed to calculate survival rate, and the survival probabilities differences were estimated by log-rank test. P value<0.05 was considered to determine statistical significance.

3. Results

KIAA1429 up-regulation closely correlated to the poor prognosis of CRC patients. Bio-functional assays demonstrated that KIAA1429 promoted the aerobic glycolysis, including glucose uptake, lactate production, ATP generation and extracellular acidification rate (ECAR). Mechanistically, KIAA1429 positively up-regulated the level of HK2 via increasing its mRNA stability via m6A-independent manner by binding the m6A site in 3‘-UTR of HK2 mRNA.

3.1. KIAA1429 was related to CRC patients’ poor prognosis

Clinically, we found that, in the CRC tissue samples, expression of KIAA1429 elevated as comparing to the normal controls (Figure 1a). Moreover, in the CRC cell lines (SW480, HT29), expression of KIAA1429 analogously elevated as comparing to the normal cells (Figure 1b). The CRC tissue samples were divided into high/low group according to the median value (Figure 1c). Survival analysis using Kaplan–Meier analysis and log-rank test found that the CRC patients with high KIAA1429 level indicated the poor survival (Figure 1d). Taken together, the above results clearly demonstrated that KIAA1429 acted as potential oncogene and was positively associated with CRC poor prognosis.

3.2. KIAA1429 accelerated the aerobic glycolysis and malignant phenotype of CRC cells

In following functional analysis, KIAA1429 expression was enhanced using plasmids transfection (SW480), and KIAA1429 expression was also silenced using shRNA transfection (HT29), which was detected using RT-PCR (Figure 2a). Moreover, the transfection efficient was detected using western blot (Figure 2b). For the glycolytic test, glucose uptake analysis revealed that, in KIAA1429 overexpression, glucose uptake quantitation was up-regulated and, in KIAA1429 silencing, glucose uptake quantitation was silenced (Figure 2c). Lactate analysis found that
KIAA1429 overexpression promoted the lactate quantitation, while the KIAA1429 silencing repressed it (Figure 2d). ATP analysis demonstrated that KIAA1429 overexpression promoted the ATP production and KIAA1429 silencing repressed it (Figure 2e). Extracellular acidification rate (ECAR) analysis found that in KIAA1429 overexpression, ECAR quantitation was up-regulated and, in KIAA1429 silencing, ECAR quantitation was repressed (figure 2f, 2g). Taken together, the above results clearly demonstrated that KIAA1429 accelerated the aerobic glycolysis and malignant phenotype of CRC cells.

3.3. HK2 acted as a target of KIAA1429 in CRC

Given that aerobic glycolysis is involved in several key enzymes, including HK2, LDHA et al, we found that the dysregulation of KIAA1429 remarkably regulated the level of HK2 in CRC cells (Figure 3a). Therefore, we assumed that HK2 might act as a target of KIAA1429 in CRC. Gene alignment analysis revealed that the m6A motif of KIAA1429 on the HK2 genomic location was AUGGACC (Figure 3b). In the predictive analytics using SRAMP (http://www.cuilab.cn/sramp), we found that the potential m6A modification site on HK2 genomic location concentrated on the 3’-UTR of HK2 genome (Figure 3c). In the analysis of database TCGA, cohort analysis illustrated that the expression of HK2 elevated in the CRC patients’ cohort (Figure 3d). For the in vivo animal analysis, subcutaneous xenograft results indicated that KIAA1429 knockdown repressed that tumor in vivo growth (Figure 3e, 3f). Taken together, the above results clearly demonstrated that HK2 acted as a target of KIAA1429 in CRC.

3.4. KIAA1429 strengthened the stability of HK2 mRNA via m6A-dependent manner

The quantitative analysis of m6A in CRC cells was calculated and the results showed that KIAA1429 overexpression promoted the m6A quantitation and the KIAA1429 silencing repressed
the m\textsuperscript{6}A modification (Figure 4(a)). RIP assay revealed that KIAA1429 closely correlated with HK2 mRNA (Figure 4(b)). MeRIP-PCR analysis found that the KIAA1429 overexpression increased the m\textsuperscript{6}A modification level on HK2 mRNA, while the KIAA1429 silencing repressed the m\textsuperscript{6}A modification level on HK2 mRNA (Figure 4(c)). RNA stability analysis indicated that KIAA1429 overexpression up-regulated the RNA level of HK2 mRNA, while the KIAA1429 silencing repressed the RNA level of HK2 mRNA (Figure 4(d)). Taken together, the above results clearly demonstrated that KIAA1429 strengthened the stability of HK2 mRNA via m\textsuperscript{6}A-dependent manner.

4. Discussion
Recently, the incidence of malignant tumor CRC has increased worldwide [17]. Emerging evidence
suggests that N^6-methyladenosine (m^6A) plays critical roles in tumor progression. Therefore, efforts that aid the implementation of m^6A for CRC treatment strategies are urgently required.

Here, our research found that m^6A methyltransferase KIAA1429 up-regulated in the CRC tissue and cells. Clinically, the up-regulation of KIAA1429 closely correlated to the poor prognosis of CRC. In the bio-functional in vitro assays, KIAA1429 promoted the CRC aerobic glycolysis, including glucose uptake, lactate production, ATP generation and extracellular acidification rate (ECAR). Besides, the knockdown of KIAA1429 repressed the aerobic glycolysis. The function of KIAA1429 has been preliminarily identified by our study, which is involved in the tumor energy metabolism.

Figure 3. HK2 acted as a target of KIAA1429 in CRC. (a) RT-PCR analysis detected the HK2 mRNA levels in CRC cells upon KIAA1429 overexpression (KIAA1429, vector) and KIAA1429 knockdown (sh-KIAA1429, sh-NC). (b) The m^6A motif of KIAA1429 on the HK2 genomic location was AUGGACC. (c) The predictive analytics using SRAMP (http://www.cuilab.cn/sramp) revealed the potential m^6A modification site on HK2 genomic location concentrated on the 3'-UTR of HK2 genome. (d) Cohort analysis of database TCGA illustrated the expression of HK2 elevated in the CRC patients’ cohort. (e) Tumor volume and (f) weight were calculated in subcutaneous xenograft results upon KIAA1429 knockdown. *p < 0.05.
Energy metabolism, especially aerobic glycolysis (also known as Warburg effect), plays critical role in CRC tumorigenesis. For example, OTUB2 negatively regulates PKM2 ubiquitination and the depletion of OTUB2 reduces the glucose consumption, lactate production and cellular ATP production, exhibiting attenuated proliferation and migration and increased sensitivity to chemotherapy drugs for CRC [18]. In CRC, NDRG2 inhibits the cellular proliferation, reduces glucose uptake and decreases key glycolysis enzymes expression through promoting the occupancy of transcription factor Mondo A on TXNIP promoter and the suppression of C-myc [19]. The literature shows that aerobic glycolysis acts as important oncogenic element for CRC.

N6-methyladenosine (m6A), one of the common epitranscriptomic modifications in eukaryotic mRNA, is an emerging and increasing
field for cancer [20,21]. The m^6^A could be installed by m^6^A methyltransferase complex, containing methyltransferase-like 3 (METTL3), METTL14 and Wilms tumor 1-associated protein (WTAP). Besides, m^6^A was removed by fat mass and obesity-associated protein (FTO) and alkB homologue 5 (ALKBH5) [22,23]. In CRC, researchers found that the m^6^A modification level increases and METTL3 level was substantially elevated in CRC tissues. Moreover, CRC patients with a high m^6^A level exhibit shorter overall survival. The knockdown of METTL3 substantially inhibits CRC proliferation, migration and invasion [11]. Moreover, m^6^A readers YTHDF1 promotes CRC cells' growth and lung/liver metastasis in vivo. Besides, YTHDF1 binds to the m^6^A sites of ARHGEF2 mRNA to enhance the translation of ARHGEF2. ARHGEF2 acts as a key target of YTHDF1 [24]. The current findings showed that m^6^A participates in the CRC progression.

In present research, we found that m^6^A ‘writer’ KIAA1429 elevated in the CRC, which was closely correlated to the clinical poor prognosis. Besides, aerobic glycolysis key enzyme HK2 acts as a downstream of KIAA1429, and KIAA1429 enhances the stability of HK2 via a m^6^A-dependent manner (Figure 5). In CRC, research has reported that KIAA1429 acts as a potential prognostic marker [25]. In several human cancer, KIAA1429 has been reported to regulate their tumorigenesis. For example, KIAA1429 significantly promotes the migration and invasion of breast cancer cells and knockdown of KIAA1429 suppresses the cell migration, invasion, and epithelial-mesenchymal transition (EMT) progress. Besides, KIAA1429 could bind to the motif in the SMC1A mRNA 3’-UTR to directly enhance SMC1A mRNA stability [26]. These results collectively suggest that KIAA1429 plays an important tumor role in cancer progression.

**Conclusion**

In summary, our findings demonstrated that m^6^A and KIAA1429 levels significantly increased in CRC. KIAA1429 promoted the CRC progression and might act as an oncogenic biomarker for CRC patients. Mechanistically, KIAA1429 regulates the tumorigenesis of CRC by regulating the m^6^A-HK2-dependent aerobic glycolysis manner. In conclusion, our work provides potential therapeutic strategies for CRC.

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References

[1] Aiello P, Sharghi M, Mansourkhani SM, et al. Medicinal Plants in the Prevention and Treatment of Colon Cancer. Oxid Med Cell Longev. 2019;2019:2075614.

[2] Dhillon AS, Ibraheim H, Green S, et al. Curriculum review: serrated lesions of the colorectum. Frontline Gastroenterol. 2020;11(3):243–248.

[3] Grothey A, Prager G, Yoshino T. The Mechanism of Action of Regorafenib in Colorectal Cancer: a Guide for the Community Physician. Clin Adv Hematol Oncol. 2019;17(12):1–19.

[4] Khanjani N, Mirzaei S, Nasrolahi H, et al. Insufficient lymph node assessment in gastric adenocarcinoma. J Egypt Natl Canc Inst. 2019;31(1):2.

[5] Lopez-Lopez V, Gómez-Ruiz AJ, Eshmuninov D, et al. Surgical oncology in patients aged 80 years and older is associated with increased postoperative morbidity and mortality: a systematic review and meta-analysis of literature over 25 years. Surg Oncol. 2020;33:81–95.

[6] Masuishi T, Taniguchi H, Kawakami T, et al. Impact of tumour growth rate during preceding treatment on tumour response to regorafenib or trifluridine/tipiracil in refractory metastatic colorectal cancer. ESMO open. 2019;4(6):e000584.

[7] Gu C, Shi X, Dai C, et al. RNA m(6)A Modification in Cancers: molecular Mechanisms and Potential Clinical Applications. Vol. 1(3). Innovation (New York, N.Y.); 2020. p. 100066.

[8] Li Q, He W, Wan G. Methyladenosine Modification in RNAs: classification and Roles in Gastrointestinal Cancers. Front Oncol. 2020;10:586789.

[9] Shriwas O, Mohapatra P, Mohanty S, et al. The Impact of m6A RNA Modification in Therapy Resistance of Cancer: implication in Chemotherapy, Radiotherapy, and Immunotherapy. Front Oncol. 2020;10:612337.

[10] Vasic R, Gao Y, Liu C, et al. The role of RNA epigenetic modification in normal and malignant hematopoiesis. Current stem cell reports. 2020;6(4):144–155

[11] Pan J, Liu F, Xiao X, et al. METTL3 promotes colorectal carcinoma progression by regulating the m6A-CRB3-Hippo axis. J Exp Clin Cancer Res. 2022;41(1):19.

[12] Liu Q, Huang Q, Liu H, et al. SUMOylation of METTL3 facilitates CRC progression by promoting circ_0000677 to regulate ABCC1 in an m6A-dependent manner. J Gastroenterol Hepatol. 2022;37(4):700–713.

[13] Zhang X, Wang S, Wang H, et al. Circular RNA circNRIPI1 acts as a microRNA-149-5p sponge to promote gastric cancer progression via the AKT1/mTOR pathway. Mol Cancer. 2019;18(1):20.

[14] Chen Z, Zuo X, Zhang Y, et al. MiR-3662 suppresses hepatocellular carcinoma growth through inhibition of HIF-1α-mediated Warburg effect. Cell Death Dis. 2018;9(5):549.

[15] Ma JZ, Yang F, Zhou CC, et al. METTL14 suppresses the metastatic potential of hepatocellular carcinoma by modulating N(6)-methyladenosine-dependent primary MicroRNA processing. Hepatology. Vol. 65. Baltimore Md.: Hepatology. 2017:529–543

[16] Li XC, Jin F, Wang BY, et al. The m6A demethylase ALKBH5 controls trophoblast invasion at the maternal-fetal interface by regulating the stability of CYR61 mRNA. Theranostics. 2019;9(13):3853–3865.

[17] Omofoye OA, Binello E. Intraventricular metastases from rectal carcinoma: case report and literature review. J Biomed Res. 2019;34(4):318–322.

[18] Yu S, Zang W, Qiu Y, et al. Deubiquitinating OTUB2 exacerbates the progression of colorectal cancer by promoting PKM2 activity and glycolysis. Oncogene. 2022;41(1):46–56.

[19] Hu J, Feng L, Ren M, et al. Colorectal Cancer Cell Differentiation Is Dependent on the Repression of Aerobic Glycolysis by NDRG2-TXNIP Axis. Dig Dis Sci. 2021. 10.1007/s10620-021-07188-8

[20] Guan Q, Lin H, Miao L, et al. Functions, mechanisms, and therapeutic implications of METTL14 in human cancer. J Hematol Oncol. 2022;15(1):13.

[21] Liu L, Li H, Hu D, et al. Insights into N6-methyladenosine and programmed cell death in cancer. Mol Cancer. 2022;21(1):32.

[22] Liu Q. Current Advances in N6-Methyladenosine Methylation Modification During Bladder Cancer. Front Genet. 2022;12:825109.

[23] Peng L, Long T, FeiLi QX. Emerging role of m(6)A modification in cardiovascular diseases. Cell Biol Int. 2022. 10.1002/cbin.11773

[24] Wang S, Gao S, Zeng Y, et al. N6-Methyladenosine Reader YTHDF1 Promotes ARHGEF2 Translation and RhoA Signaling in Colorectal Cancer. Gastroenterology. 2021;162(4):1183–1196.

[25] Ma L, Lin Y, Sun SW, et al. KIAA1429 is a potential prognostic marker in colorectal cancer by promoting the proliferation via downregulating WEE1 expression in an m6A-independent manner. Oncogene. 2022;41(5):692–703.

[26] Zhang X, Dai XY, Qian JY, et al., SMC1A regulated by KIAA1429 in m6A-independent manner promotes EMT progress in breast cancer, Molecular therapy. 2022;Nucleic acids. 27:133–146.