European Review for Medical and Pharmacological Sciences 2020; 24: 2380-2386

ADPGK-AS1 promotes the progression of colorectal cancer *via* sponging miR-525 to upregulate FUT1

H.-Y. JIANG, Z.-J. WANG

Department of Gastroenterology, Beijing University of Chinese Medicine Third Affiliated Hospital, Beijing, China

Abstract. – OBJECTIVE: We sought to uncover the potential role of long non-coding RNA (lncRNA) ADPGK-AS1 in colorectal cancer (CRC).

PATIENTS AND METHODS: ADPGK-AS1 levels in 58 pairs of CRC tissues and paracancerous tissues and 30 normal colorectal tissues were determined. The *in vitro* level of ADPGK-AS1 in CRC cell lines was tested as well. The regulatory effects of ADPGK-AS1 on the proliferative, migratory, and invasive properties of HCT116 and SW480 cells were assessed. Using a Dual-Luciferase reporter gene assay, the interaction among ADPGK-AS1/miR-525/FUT1 was identified. Finally, potential influences of the regulatory loop ADPGK-AS1/miR-525/FUT1 on the phenotypes of CRC cells were explored.

RESULTS: ADPGK-AS1 was upregulated in CRC tissues and cells. Knockdown of ADPGK-AS1 attenuated the proliferative, migratory, and invasive abilities of CRC cells. Meanwhile, miR-525 was confirmed to be the target of ADPGK-AS1 and FUT1 was the downstream gene binding miR-525. The regulatory loop ADPGK-AS1/miR-525/FUT1 was found to aggravate the malignant progression of CRC.

CONCLUSIONS: ADPGK-AS1 is upregulated in CRC. The regulatory loop ADPGK-AS1/miR-525/FUT1 exacerbates the progression of CRC by promoting the proliferation, migration, and invasion of tumor cells.

Key Words:
CRC, ADPGK-AS1, MiR-525, FUT1.

Introduction

The incidence and mortality of colorectal cancer (CRC) have increased in recent years. It has been reported that the incidence of CRC was 83.4/100,000, ranking fourth among all malignancies in the United States. Unhealthy diet, obesity, and smoking are risk factors leading to the increased incidence of CRC in the Asian population[1,2]. With the improvement of the economy, dietary changes, and aging of the population, CRC has become a public health problem. So far, surgery combined with postoperative chemotherapy and radiotherapy is the preferred treatment for CRC[3]. It is necessary to diagnose and intervene against CRC as early as possible. However, the 5-year survival of CRC is only approximately 30%, clarifying the molecular mechanisms of CRC could be significant to improve the clinical outcomes of affected patients.

Long non-coding RNA (lncRNAs) were considered to be byproducts of transcription without or almost no protein-encoding functions. With in-depth studies, lncRNAs are been found to be critical regulators responsible for mediating cellular behaviors[4]. Multiple lncRNAs have been discovered to be closely associated with CRC progression. LncRNA ADPGK-AS1 is a tumor-associated gene. In breast cancer, ADPGK-AS1 affects the proliferative and migratory properties, as well as EMT[5]. High levels of ADPGK-AS1 predict a poor prognosis in patients with gastric cancer[6].

In this paper, ADPGK-AS1 was found to be upregulated in CRC. Potential influences of ADPGK-AS1 on the malignant phenotypes of CRC were clarified. Our findings may provide novel directions for the clinical treatment of CRC.

Patients and Methods

Patients and Samples

CRC tissues (n=58), paracancerous tissues (n=58), and normal colorectal tissues (n=30) were collected from CRC patients undergoing surgical resection at Beijing University of Chinese Medicine Third Affiliated Hospital between January 2017 and January 2019. Among the 58 enrolled patients, 29 were in stage T1+T2 and the remain-

Corresponding Author: Zaijian Wang, MD; e-mail: seagull310@163.com

ing were in stage T3+T4. Twenty-nine out of 58 CRC patients suffered lymphatic metastasis. Informed consent was obtained prior to tissue collection and analyses. This investigation was approved by the Ethics Committee of Beijing University of Chinese Medicine Third Affiliated Hospital. All patients provided written informed consents. This study was conducted in accordance with the Declaration of Helsinki.

Cell Culture and Transfection

Normal colorectal cells (NCM480) and CRC cells (HCT116, HT29, SW480, and LoVo) provided by American Type Culture Collection (ATCC; Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 100 μg/mL streptomycin and 100 IU/mL penicillin. Cells in good condition were transfected with related plasmids (GenePharma, Shanghai, China) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The sequences of ADPGK-AS1 siRNA were 5'-GUGCACAACACGAGAGUUA-3'.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

TRIzol (Invitrogen, Carlsbad, CA, USA) was applied for isolating cellular RNA, which was quantified using a spectrometer. RNA was reversely transcribed into cDNA using the PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). SYBR Premix Ex Taq™ (TaKaRa, Otsu, Shiga, Japan) was utilized for qRT-PCR with 40 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 90 s. Primer sequences were as follows: ADPGK-AS1: F: 5'-GATGTCGACACAAGCGCTAC-3'; R: 5'- AGGTCAGGAGCCGAGAGAAT-3'; FUT1: F: 5'-CTTCCTGCTAGTCTGTGTCCT-3', R: 5'-ATTGGGGTAGACAGTCCAGGT-3'.

Cell Counting Kit (CCK-8) Assay

Cells were seeded into a 96-well plate with 2×10^3 cells per well. At the appointed time points, 10 μL of Cell Counting Kit-8 solution (CCK-8; Dojindo Molecular Technologies, Kumamoto, Japan) was added in each well. The absorbance at 450 nm of each sample was recorded.

5-Ethynyl-2'-Deoxyuridine (EdU) Assay

Cells were seeded in a 96-well plate with 1×10^5 cells per well. They were labeled with EdU solution in the dark for 30 min, and stained with Hoechst 33342 for another 30 min. Images of EdU-labeled cells, Hoechst-labeled nucleus, and merged one were taken under a fluorescence microscopy.

Transwell Assay

Cell suspensions of 1×10^6 cells/mL were prepared. 100 μL of suspension was applied in the upper chamber of transwell dishes (Corning, Corning, NY, USA). In the bottom chamber, 500 μL of medium containing 20% FBS was applied. After 48 h of incubation, cells that had migrated to the bottom chamber were fixed in methanol for 15 min, stained with crystal violet for 20 min, and counted using a microscope. Cell invasion assay was performed in a similar manner, with additional precoating of the filter insert with diluted Matrigel (1:9) and 50 μL of FN.

Bioinformatics Prediction

Potential binding miRNAs to ADPGK-AS1 were predicted on Starbase and miR-525, with the highest score, was selected. Target genes of miR-525 were predicted by TargetScan, miRBase and miRDB, and FUT1 was finally selected.

Dual-Luciferase Reporter Gene Assay

Wild-type and mutant-type vectors were constructed based on binding sequences. Cells inoculated in a 96-well plate were co-transfected with 80 ng wild-type/mutant-type vector and 50 pmol/L miR-525 mimics/NC for 48 h. Subsequently, cells were lysed and the supernatant was collected for measuring relative luciferase activity (Promega, Madison, WI, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 (SPSS IBM, Armonk, NY USA) statistical software was used for data analysis. All data were expressed as mean ± SD (standard deviation). The paired two-tailed *t*-test was used for comparing differences between two groups. Spearman correlation test was applied for comparing expression correlation between two genes. $p<0.05$ was considered to be statistically significant.

Results

Upregulation of ADPGK-AS1 in CRC

Compared with normal colorectal tissues, ADPGK-AS1 was markedly upregulated in CRC tissues (Figure 1A). Similarly, the expression of

ADPGK-AS1 was higher in CRC tissues than in paracancerous tissues (Figure 1B). Clinical data of enrolled CRC patients were collected. It is shown that ADPGK-AS1 level remained high in CRC patients with stage T3+T4 compared to those with stage T1+T2 (Figure 1C). A greater abundance of ADPGK-AS1 was observed in CRC patients diagnosed with metastasis (Figure 1D).

Knockdown of ADPGK-AS1 Suppressed the Proliferative and Metastatic Abilities of CRC

The *in vitro* level of ADPGK-AS1 was higher in CRC cells than in normal colorectal cells (Figure 2A); it was the most enhanced in HCT116 and SW480 cells. Here, transfection of si-ADPGK-AS1 effectively inhibited the ADPGK-AS1 level in HCT116 and SW480 cells (Figure 2B). After knockdown of ADPGK-AS1, the viability of CRC cells markedly decreased (Figure 2C). EdU assay yielded the same result, indicating that knockdown of ADPGK-AS1 attenuated the proliferative ability of CRC (Figure 2D). Moreover, the transwell assay revealed that the migratory and invasive abilities of ADPGK-AS1 knockdown CRC cells were suppressed (Figure 2E, 2F).

ADPGK-AS1 Sponged MiR-525 to Upregulate FUT1

Using Starbase, potential miRNAs capable of binding ADPGK-AS1 were predicted and miR-525 was selected for further analysis. In CRC tissues, miR-525 was downregulated (Figure 3A). Its level was negatively correlated to that of ADPGK-AS1 in CRC (Figure 3B). Based on their binding sequences, wild-type and mutant-type ADPGK-AS1 vectors were constructed (Figure 3C). Luciferase activity markedly decreased after cotransfection of ADPGK-AS1 WT and miR-525 mimic, implicating the potential binding of ADPGK-AS1 with miR-525 (Figure 3D). Consistent with this conclusion is the observation that the *in vitro* level of miR-525 was upregulated in ADPGK-AS1 knockdown CRC cells (Figure 3E).

Using the same method, FUT1 was predicted to be the downstream gene of miR-525, and found to be upregulated in CRC tissues (Figure 3F). A negative correlation was identified between the expression levels of miR-525 and FUT1 (Figure 3G). Furthermore, decreased luciferase activity after cotransfection of FUT1 WT and miR-525 mimic indicated their binding interaction (Figure 3H, 3I). Remarkably, overexpression of miR-525 inhibited FUT1 level in

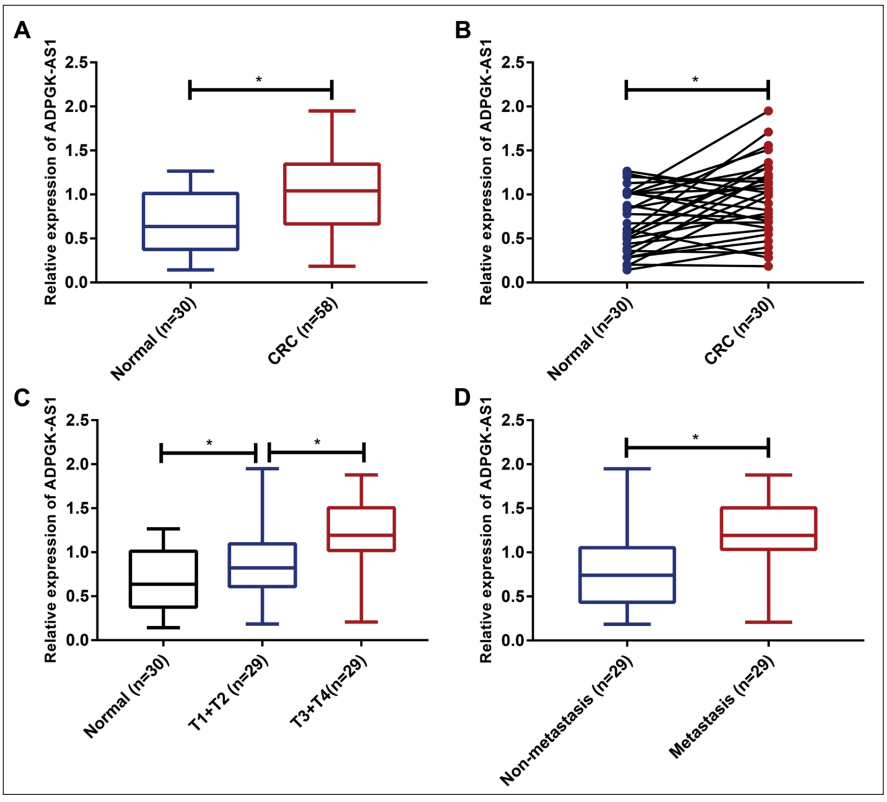

Figure 1. Upregulated ADPGK-AS1 in CRC. **A,** ADPGK-AS1 levels in 58 CRC tissues and 30 normal colorectal tissues. **B,** ADPGK-AS1 levels in 30 paired CRC tissues and paracancerous ones. **C,** ADPGK-AS1 levels in normal colorectal tissues and CRC tissues with T1+T2 or T3+T4. **D,** ADPGK-AS1 levels in CRC patients either with metastasis or not.

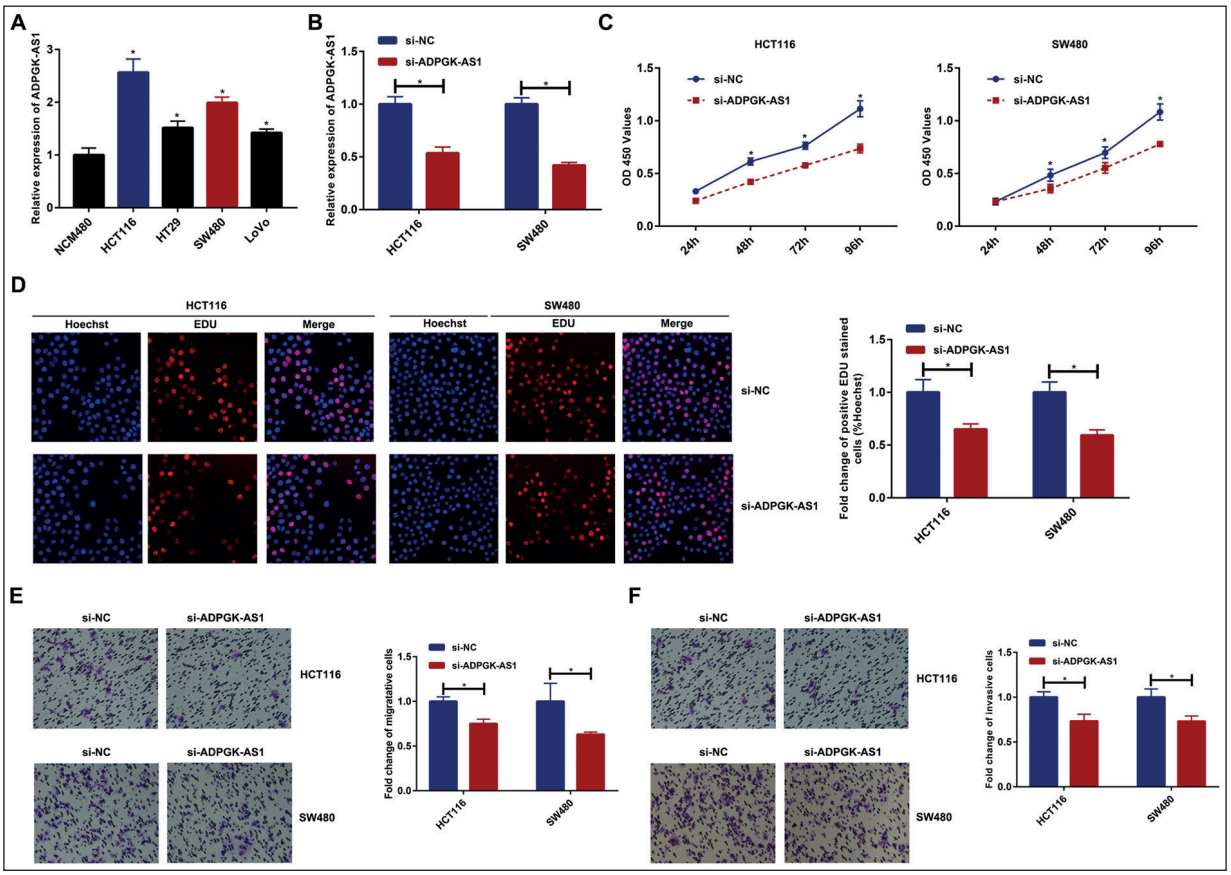

Figure 2. Knockdown of ADPGK-AS1 suppressed proliferative and metastatic abilities in CRC. **A,** ADPGK-AS1 levels in normal colorectal cells (NCM480) and CRC cells (HCT116, HT29, SW480 and LoVo). **B,** Transfection efficacy of si-ADPGK-AS1 in HCT116 and SW480 cells. **C,** Viability in HCT116 and SW480 cells transfected with si-NC or si-ADPGK-AS1. **D,** Images of Hoechst-labeled cells, EdU-labeled cells and merged ones in HCT116 and SW480 cells transfected with si-NC or si-ADPGK-AS1 (magnification ×40). **E,** Migratory ability in HCT116 and SW480 cells transfected with si-NC or si-ADPGK-AS1 (magnification ×40). **F,** Invasive ability in HCT116 and SW480 cells transfected with si-NC or si-ADPGK-AS1 (magnification ×40).

CRC cells (Figure 3J). Based on the above findings, we infer the existence of the interaction among ADPGK-AS1/miR-525/FUT1, whereby ADPGK-AS1 sponged miR-525 to upregulate FUT1 expression.

ADPGK-AS1/MiR-525/FUT1 Regulatory Loop Promoted the Progression of CRC

Rescue experiments were conducted to clarify the biological role of the ADPGK-AS1/miR-525/FUT1 regulatory loop in the progression of CRC. Decreased viability (Figure 4A) and EdU-positive ratio (Figure 4B) of ADPGK-AS1 knockdown CRC cells were partially reversed by knockdown of miR-525 or overexpression of FUT1. Transfection of the miR-525 inhibitor or pcDNA3.1-FUT1 partially abolished the inhibitory effects of downregulated ADPGK-AS1 on the migratory (Figure 4C) and invasive (Figure 4D) properties. Thus, ADPGK-AS1 was confirmed to exacerbate the progression of CRC by sponging miR-525 to upregulate FUT1 expression.

Discussion

CRC is a highly prevalent cancer of the digestive system, contributing to increased mortality and morbidity in recent years[7]. The 5-year survival of CRC patients with lymphatic metastasis or distant metastasis is less than 50%. Metastasis and recurrence are two major causes of CRC death[8]. The molecular mechanisms underlying the high mortality and poor prognosis of CRC patients remain unclear[9]. Currently, molecular targeted therapy is beneficial for the clinical treat-

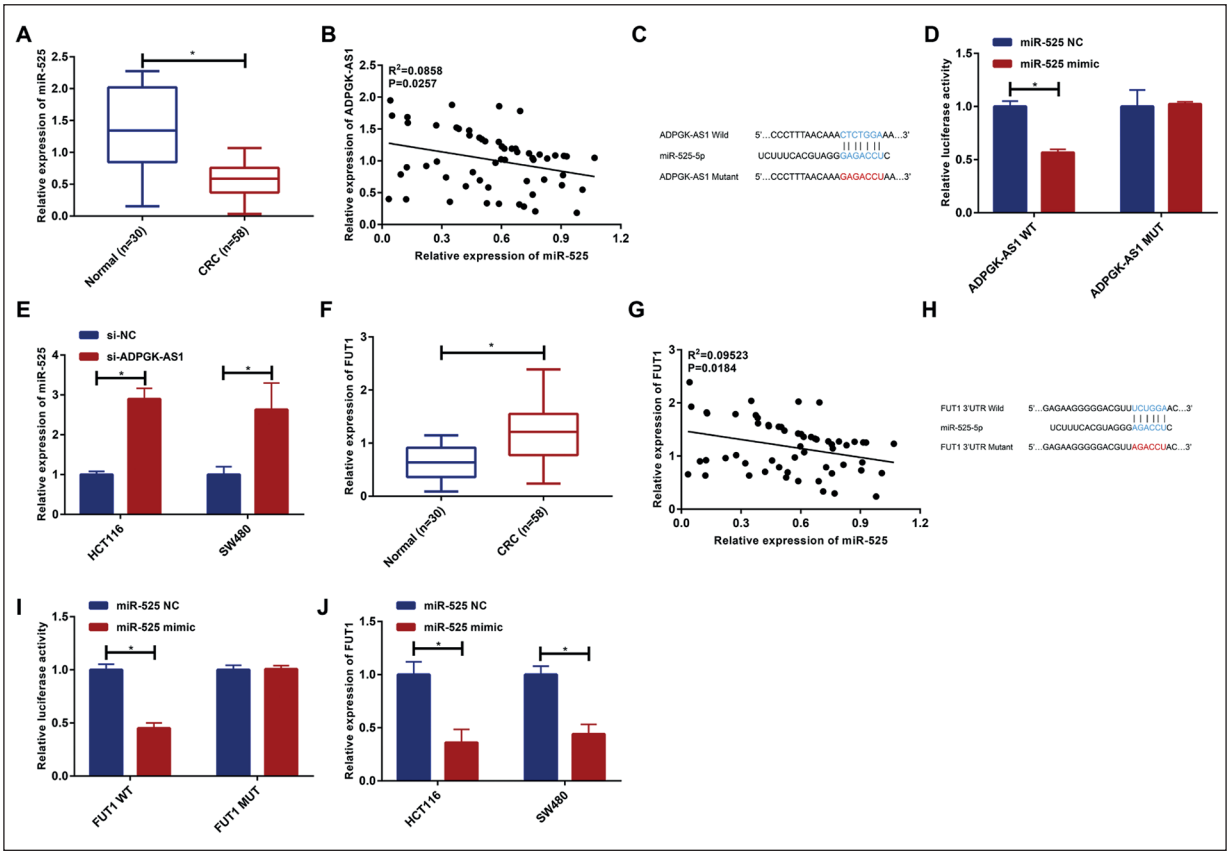

Figure 3. ADPGK-AS1 sponged miR-525 to upregulate FUT1. **A,** MiR-525 levels in 58 CRC tissues and 30 normal colorectal tissues. **B,** A negative correlation between expression levels of miR-525 and ADPGK-AS1 in CRC tissues. **C,** Binding sequences between miR-525 and ADPGK-AS1. **D,** Luciferase activity after co-transfection of ADPGK-AS1 WT/ADPGK-AS1 MUT and miR-525 mimic/NC. **E,** MiR-525 level in HCT116 and SW480 cells transfected with si-NC or si-ADPGK-AS1. **F,** FUT1 levels in 58 CRC tissues and 30 normal colorectal tissues. **G,** A negative correlation between expression levels of miR-525 and FUT1 in CRC tissues. **H,** Binding sequences between miR-525 and FUT1. **I,** Luciferase activity after co-transfection of FUT1 WT/FUT1 MUT and miR-525 mimic/NC. **J,** FUT1 level in HCT116 and SW480 cells transfected with NC or miR-525 mimic.

ment of tumors as a result to the advanced medical technologies. The development of effective and sensitive treatments targeting tumor diseases contributes to improving the clinical outcome of CRC patients.

Only 1% genes in the human genome are transcribed into RNAs, while the majority are noncoding RNAs without known biological functions[10]. LncRNAs are commonly transcribed in eukaryotic cells, presenting critical biological functions[11,12]. They are widely involved in the regulation of cellular behavior by mediating gene expressions, subcellular distribution, and complex formation[13-15]. Recently, the ceRNA theory proposed that lncRNAs serve as miRNA sponges to affect downstream gene functions. In CRC tissues, lncRNA PVT-1 is upregulated, and positively correlated to rate of invasion and lymphatic metastasis[16]. LncRNA MALAT1 promotes CRC cells to proliferate and metastasize[17]. LncRNA PLAT1 is upregulated in CRC[18] and considered as an effective hallmark for 5-year survival. Serving as an MYC enhancer, lncRNA CCAT1-L is upregulated in CRC tissues[19]. Our findings pointed out that ADPGK-AS1 was upregulated in CRC tissues and cell lines, which stimulated CRC cells to proliferate and metastasize.

Significant roles of fucosyltransferases (FUTs) in the occurrence and development of tumors have been highlighted[20-25]. FUTs are capable of triggering angiogenesis, cell adhesion, and invasiveness of tumor cells. Moreover, FUTs are critical in homing and colonization of stem cells. Here, we have demonstrated that FUT1 was the direct target of miR-525. Collectively, ADPGK-AS1/miR-525/FUT1 regulatory loop was

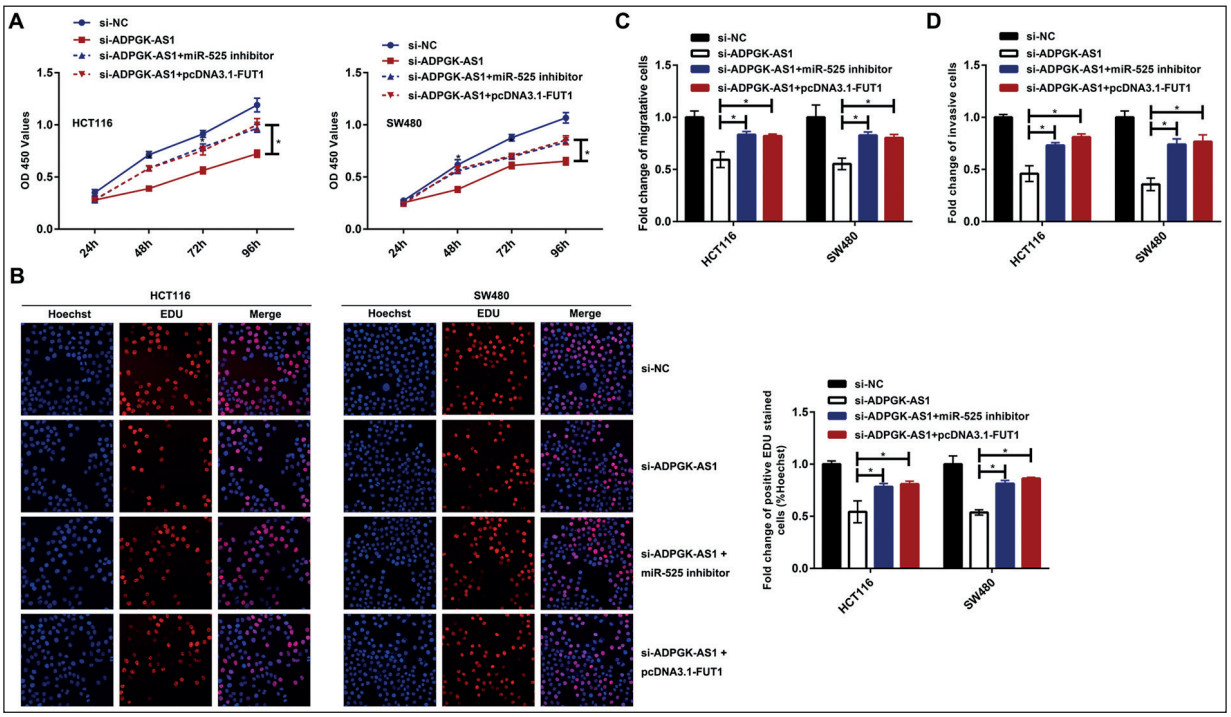

Figure 4. ADPGK-AS1/miR-525/FUT1 regulatory loop promoted the progression of CRC. HCT116 and SW480 cells were transfected with si-NC, si-ADPGK-AS1, si-ADPGK-AS1 + miR-525 inhibitor or si-ADPGK-AS1 + pcDNA3.1-FUT1. **A,** Cell viability; **B,** EdU-positive ratio (magnification ×40); **C,** Migratory cell number; **D,** Invasive cell number.

demonstrated to be responsible for aggravating the malignant progression of CRC. However, *in vivo* experiments are lacked to validate the biological function of ADPGK-AS1 in CRC.

Conclusions

In summary, ADPGK-AS1 is upregulated in CRC. The regulatory loop ADPGK-AS1/miR-525/FUT1 exacerbates the progression of CRC by promoting the proliferation and metastasis of tumor cells.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.

2) Zheng R, Zeng H, Zhang S, Chen T, Chen W. National estimates of cancer prevalence in China, 2011. Cancer Lett 2016; 370: 33-38.

3) Yan Y, Grothey A. Molecular profiling in the treatment of colorectal cancer: focus on regorafenib. Onco Targets Ther 2015; 8: 2949-2957.

4) Zhou JF, Wang HG, Ma TH, Yan W, Wu SN, Shi YT, Yang XZ. Long noncoding RNA LINC01510 is highly expressed in colorectal cancer and predicts favorable prognosis. Eur Rev Med Pharmacol Sci 2018; 22: 7710-7715.

5) Yang J, Wu W, Wu M, Ding J. Long noncoding RNA ADPGK-AS1 promotes cell proliferation, migration, and EMT process through regulating miR-3196/OTX1 axis in breast cancer. In Vitro Cell Dev Biol Anim 2019; 55: 522-532.

6) Huang Z, Yang H. Upregulation of the long noncoding RNA ADPGK-AS1 promotes carcinogenesis and predicts poor prognosis in gastric cancer. Biochem Biophys Res Commun 2019; 513: 127-134.

7) Yan L, Spitznagel EL, Bosland MC. Soy consumption and colorectal cancer risk in humans: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2010; 19: 148-158.

8) McRee AJ, Goldberg RM. Optimal management of metastatic colorectal cancer: current status. Drugs 2011; 71: 869-884.

9) Kushlinskii NE, Gershtein ES, Korotkova EA, Prokov VV. Prognostic role of tumor-associated

proteases in colorectal cancer. Bull Exp Biol Med 2013; 154: 365-369.

10) Abbas Q, Raza SM, Biyabani AA, Jaffar MA. A review of computational methods for finding non-coding RNA genes. Genes (Basel) 2016; 7: 113.

11) Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet 2009; 10: 155-159.

12) Umlauf D, Fraser P, Nagano T. The role of long non-coding RNAs in chromatin structure and gene regulation: variations on a theme. Biol Chem 2008; 389: 323-331.

13) Caley DP, Pink RC, Trujillano D, Carter DR. Long noncoding RNAs, chromatin, and development. ScientificWorldJournal 2010; 10: 90-102.

14) De Lucia F, Dean C. Long non-coding RNAs and chromatin regulation. Curr Opin Plant Biol 2011; 14: 168-173.

15) Spitale RC, Tsai MC, Chang HY. RNA templating the epigenome: long noncoding RNAs as molecular scaffolds. Epigenetics 2011; 6: 539-543.

16) Takahashi Y, Sawada G, Kurashige J, Uchi R, Matsumura T, Ueo H, Takano Y, Eguchi H, Sudo T, Sugimachi K, Yamamoto H, Doki Y, Mori M, Mimori K. Amplification of PVT-1 is involved in poor prognosis via apoptosis inhibition in colorectal cancers. Br J Cancer 2014; 110: 164-171.

17) Zheng HT, Shi DB, Wang YW, Li XX, Xu Y, Tripathi P, Gu WL, Cai GX, Cai SJ. High expression of lncRNA MALAT1 suggests a biomarker of poor prognosis in colorectal cancer. Int J Clin Exp Pathol 2014; 7: 3174-3181.

18) Eldai H, Periyasamy S, Al QS, Al RM, Muhammed MS, Deeb A, Al SE, Afzal M, Johani M, Yousef Z, Aziz MA. Novel genes associated with colorectal cancer are revealed by high resolution cytogenetic analysis in a patient specific manner. PLoS One 2013; 8: e76251.

19) Xiang JF, Yin QF, Chen T, Zhang Y, Zhang XO, Wu Z, Zhang S, Wang HB, Ge J, Lu X, Yang L, Chen LL. Human colorectal cancer-specific CCAT1-L lncRNA regulates long-range chromatin interactions at the MYC locus. Cell Res 2014; 24: 513-531.

20) Liu J, Zheng M, Qi Y, Wang H, Liu M, Liu Q, Lin B. Lewis(y) antigen-mediated positive feedback loop induces and promotes chemotherapeutic resistance in ovarian cancer. Int J Oncol 2018; 53: 1774-1786.

21) Zhao R, Qin W, Qin R, Han J, Li C, Wang Y, Xu C. Lectin array and glycogene expression analyses of ovarian cancer cell line A2780 and its cisplatin-resistant derivate cell line A2780-cp. Clin Proteomics 2017; 14: 20.

22) Gao N, Liu J, Liu D, Hao Y, Yan L, Ma Y, Zhuang H, Hu Z, Gao J, Yang Z, Shi H, Lin B. c-Jun transcriptionally regulates alpha 1, 2-fucosyltransferase 1 (FUT1) in ovarian cancer. Biochimie 2014; 107 Pt B: 286-292.

23) Tan KP, Ho MY, Cho HC, Yu J, Hung JT, Yu AL. Fucosylation of LAMP-1 and LAMP-2 by FUT1 correlates with lysosomal positioning and autophagic flux of breast cancer cells. Cell Death Dis 2016; 7: e2347.

24) Duell EJ, Bonet C, Munoz X, Lujan-Barroso L, Weiderpass E, Boutron-Ruault MC, Racine A, Severi G, Canzian F, Rizzato C, Boeing H, Overvad K, Tjonneland A, Arguelles M, Sanchez-Cantalejo E, Chamosa S, Huerta JM, Barricarte A, Khaw KT, Wareham N, Travis RC, Trichopoulou A, Trichopoulos D, Yiannakouris N, Palli D, Agnoli C, Tumino R, Naccarati A, Panico S, Bueno-de-Mesquita HB, Siersema PD, Peeters PH, Ohlsson B, Lindkvist B, Johansson I, Ye W, Johansson M, Fenger C, Riboli E, Sala N, Gonzalez CA. Variation at ABO histo-blood group and FUT loci and diffuse and intestinal gastric cancer risk in a European population. Int J Cancer 2015; 136: 880-893.

25) Lai TY, Chen IJ, Lin RJ, Liao GS, Yeo HL, Ho CL, Wu JC, Chang NC, Lee AC, Yu AL. Fucosyltransferase 1 and 2 play pivotal roles in breast cancer cells. Cell Death Discov 2019; 5: 74.