Arginine ADP-ribosyltransferase 1 Regulates Glycolysis in Colorectal Cancer via the PI3K/AKT/HIF1α Pathway*

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[Abstract] Objective: Arginine ADP-ribosyltransferase 1 (ART1) is involved in the regulation of a diverse array of pathophysiological processes, including proliferation, invasion, apoptosis, autophagy and angiogenesis of colorectal cancer (CRC) cells. However, how ART1 regulates glycolysis in CRC remains elusive. Methods: To elucidate the role of ART1 in glycolysis in CRC, we assessed the protein level of ART1, hypoxia-inducible factor 1α (HIF1α), and glucose transporter type 1 (GLUT1) in 61 CRC tumor tissue specimens obtained from patients with different 2-[18F]fluoro-2-deoxy-D-glucose ([18F-FDG]) uptake as analyzed by PET/CT before surgery. Colon adenocarcinoma CT26 cells with ART1 knockdown and overexpression were established, respectively, and the molecular mechanism underlying the effect of ART1 on glycolysis in CRC was determined both in vivo and in vitro. Results: The expression of ART1 and GLUT1 was significantly associated with FDG uptake (P=0.037 and P=0.022, respectively) in CRC tissues. Furthermore, the expression of hexokinase 2 (HK2) and lactate dehydrogenase (LDH) was upregulated in ART1-overexpressed CT26 cells, but was downregulated in ART1-knockdown CT26 cells. The volume and weight of subcutaneously transplanted tumors were markedly increased in the ART1-overexpressed BALB/c mice group and decreased in the ART1-knockdown group. In CT26 cells, the overexpression of ART1 promoted the expression levels of HK2 and LDH, and knockdown of ART1 suppressed them in the CT26 tumors. In both normal and hypoxic conditions, ART1 expression was associated with the protein level of phospho-serine/threonine kinase (p-AKT), HIF1α, and GLUT1 but not with that of AKT in CT26 cells and subcutaneous transplanted tumors. Conclusion: ART1 plays a crucial role in the elevation of glucose consumption in CT26 cells and may regulate GLUT1-dependent glycolysis in CRC via the PI3K/AKT/HIF1α pathway.

Key words: arginine ADP-ribosyltransferases 1; colorectal cancer; Warburg effect; hypoxia-inducible factor; glucose transporter type 1

Colorectal cancer (CRC) is one of the most prevalent malignant neoplasms, and its mortality ranks third amongst all malignancies[1]. CRC affects both men and women equally, and despite marked advances in the understanding of CRC carcinogenesis, a lack of cancer-specific therapy continues to exist currently. Many cancer cells, including those of CRC, exhibit altered glucose metabolism and are characterized by an enhanced uptake of glucose and an increased conversion of glucose to lactate in cancer cells. In fact, this occurs even under adequate oxygen tension, which is described as aerobic glycolysis or the Warburg effect[2]. Aerobic glycolysis is associated with the overexpression of many key factors, including hypoxia-inducible factor (HIF) family members, glucose transporter type 1 (GLUT) family members, hexokinases (HKs) and lactate dehydrogenases (LDHs)[2–4]. An increasing number of studies have indicated an association of several oncogenes and tumor suppressors such as phosphatase and tensin homolog (PTEN), phosphoinositide 3-kinase (PI3K)/serine/threonine kinase (AKT), p53, and miR124 with the regulation of altered metabolism and the development of CRC[5–8]. Although the Warburg effect has been extensively investigated, little is known about the subsequent mechanisms underlying this metabolic reprogramming in CRC.
Adenosine diphosphate (ADP) ribosylation, an enzyme-catalyzed post-translational protein modification, is essential for the regulation of a diverse array of pathophysiological processes. Arginine ADP-riboseyltransferase 1 (ART1), an arginine-specific ADP-riboseyltransferase, catalyzes the transfer of ADP-ribose moiety to arginine residues on an acceptor protein using nicotinamide adenine dinucleotide (NAD⁺) as a substrate, thus eliciting changes in the activities and functions of the acceptor proteins. In this context, Xiao et al. found that ART1 silencing promoted the inhibition of cell survival and induced apoptosis via the PI3K/AKT/NF-κB signaling pathway in murine colon adenocarcinoma CT26 cells. Moreover, Tang et al. suggested that the suppression of ART1-mediated starvation-induced autophagy inhibited the growth and augmented the apoptosis of CT26 cells. Besides, ART1 has been indicated to exhibit a crucial role in the proliferation and invasion of CT26 cells in vivo. These lines of evidence strongly suggested that ART1 functions as an essential factor in the regulation of several biological processes in CRC cells.

We previously revealed that ART1 might play a crucial role in aerobic glycolysis in CRC. Although ART1 has been suggested to exert its effects on glycolysis under high-glucose conditions and energy metabolism, and to promote angiogenesis in CRC cells through the PI3K/AKT pathway, little is known about how ART1 regulates glycolysis in CRC. Therefore, in this study, we evaluated CRC patients with different glucose consumption by PET/CT. Furthermore, we also analyzed the mechanism underlying the regulation of glycolysis in CRC via ART1.

1 MATERIALS AND METHODS

1.1 Ethics Statement

The study was approved by the Ethics Committee of the Affiliated Hospital of Southwest Medical University (Luzhou, China). Each patient provided written informed consent. All experimental protocols were approved by the same committee.

1.2 Tumor Specimens

A total of 61 tumor tissue specimens were collected from patients with CRC who were screened with 18F-fluoro-2-deoxy-D-glucose (18F-FDG) PET/CT and under-went surgery at the Affiliated Hospital of Southwest Medical University, Luhou, China, between 2012 and 2015. These patients included 33 men and 28 women, with an average age of 62.36 (34–88) years. PET/CT images were reviewed by two experienced nuclear medicine physicians, and the maximum standardized uptake value (SUVmax) was noted.

Since a proper cut-off value for FDG uptake is study-dependent, in the present study, we defined 10 as the cut-off to distinguish between high and low FDG uptake. Pathological assessment of CRC was performed according to the World Health Organization (WHO) 2019 classification system for CRC by two experienced pathologists at the Department of Pathology, and pathological characteristics were recorded.

1.3 Cell Lines and Animals

The murine colon adenocarcinoma CT26 cell line was kindly provided by Professor Yu-quan WEI (Sichuan University, Chengdu, Sichuan, China). Cells were cultured in RPMI 1640 medium (Gibco, Invitrogen, China) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin/streptomycin at 37°C with 5% CO₂ in a humidified atmosphere.

ART1-short hairpin RNA (shRNA), ART1 overexpression, and control vector CT26 cells were constructed, and the efficiency of ART1 overexpression and knockdown was described as in our previous study. The animals were cared for in accordance with the guide for the care and use of laboratory animals in China. All procedures and animal experiments were approved by the Laboratory Animal Ethics Committee of the Affiliated Hospital of Southwest Medical University, Luhou, China. BALB/c-nude mice (6–8 weeks of age and weighing 18–22 g) were acquired from the animal experimental center of Southwest Medical University. The animals were housed under specific pathogen-free controlled conditions of 25±1°C temperature and 60%±10% humidity with a 12 h light/dark cycle and with free access to standard rodent diet and water ad libitum for one week.

1.4 Immunohistochemistry Assay

The collected tissue specimens were washed with 1% ice-cold saline and fixed in 10% paraformaldehyde and then embedded in paraffin. Sections were then cut at 4 μm thick and stained with hematoxylin and eosin (H&E) for routine histopathological examination. For immunohistochemistry (IHC), 4 μm paraffin sections were deparaffinized in 100% xylene and rehydrated in an ethanol gradient. Antigen retrieval was performed by heating the tissue sections at 95–98°C in citrate buffer in a microwave oven for 15 min. The endogenous peroxidase activity was blocked by 3% H2O2/methanol for 10 min and 5% goat serum for 30 min at 37°C. Subsequently, the specimens were incubated with primary antibodies overnight at 4°C. The polyclonal antibodies to ART1 (AP2311a; Abgent, Inc., USA), HIF1α, and GLUT1 (AF1837 and AF1015, respectively, Beyotime, China) were used at 1:1000, 1:500, and 1:500 dilutions, respectively. Then, the sections were washed thrice with PBS and incubated with a biotin-labeled secondary anti-rabbit antibody and avidin-biotin-peroxidase or an anti-goat antibody and avidin-biotin-peroxidase for 30 min at 37°C. The immunostaining was carried out by staining with 3′-diaminobenzidine chromogen (DAB) counter-
stained with hematoxylin, followed by dehydration and mounting, and finally examination of the sections under a microscope. For the negative control group, PBS was used instead of the primary antibody. The negative case was defined as 0–10% immunoreactive cancer cells, and the positive case was defined as ≥10%.

1.5 RNA Extraction and qRT-PCR

Total RNA from transfected CT26 cells or tissues was extracted using a total RNA extraction kit (DP431, Tiangen Biochemical Technology Co., Ltd, China) following the manufacturer’s protocol. The RNA quality was determined using an AmoyDx SMA4000 Bioanalyzer. For reverse transcription, cDNA was synthesized using the Promega M-MLV reverse transcriptase (M1701, Promega, USA) according to the manufacturer’s protocol. The qRT-PCR was performed with the Promega GoTag qPCR kit (A6001, Promega, USA) on a Roche quantitative PCR system. The qRT-PCR cycle profile was performed at 95°C for 3 min to activate DNA polymerase, followed by 45 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 40 s. The β-actin was used as an internal reference, and the relative gene expression as fold change was calculated using the 2ΔΔCT method. All experiments were repeated three times for each gene.

1.6 Western Blotting Analysis

The cultured transfected CT26 cells or tissues were collected after being washed with PBS. The total protein was extracted with RIPA lysis buffer (P0013K, Beyotime, China) supplemented with a protease inhibitors cocktail on ice for 30 min. Protein concentration was quantified using the BCA method. An equal amount of protein was separated on 6% or 10% sodium dodecyl sulfate-polyacrylamide (SDS-PAGE), and then the proteins were electrophoretically transferred onto polyvinylidene fluoride (PVDF) membranes. Skimmed milk (5%) was used to block heterogenetic antigen on the membranes for 1 h. Subsequently, the membrane was incubated with primary antibodies against HK2 (1:500; AF7080, Beyotime, China), LDH (1:500; AF1660, Beyotime, China), AKT, p-AKT Thr308 (1:1000; BS2987 and BS4009, respectively, Bioworld Technology, Co., Ltd., USA), HIF1α (1:1000), GLUT1 (1:500), ART1 (1:1000), and β-actin (1:1000; BA2305, Boster Biological Technology, Ltd., China) at 4°C overnight, followed by incubation with secondary antibodies (1:5000 dilutions) for 30 min at room temperature. Secondary antibodies, peroxidase-conjugated anti-goat or rabbit IgG, were then added and incubated for 30 min at room temperature. The protein bands were visualized using the enhanced chemiluminescence detection BeyoECL Plus kit (Beyotime, China), and subsequently imaged and analyzed.

1.7 Lactic Acid Measurement

The supernatant was collected from cell culture by centrifugation. The amount of lactic acid in the supernatant was determined using a lactic acid test kit (A019-2, Nanjing Jiancheng Bioengineering Institute, China), according to the manufacturer’s protocol. Absorbance was measured at 530 nm.

1.8 Cobalt Chloride (CoCl₂) Treatment

The four groups of cells were seeded in 6-well plates and cultured in RPMI 1640 medium. For the induction of hypoxia, 2 mL of fresh medium with a final concentration of 100 μmol/L CoCl₂ was added to each well until cells reached 70% confluence. Fresh medium with no CoCl₂ served as a control. All the cells were incubated for 24 h before further experiments.

1.9 Xenograft Tumor Mouse Model

BALB/c female mice at 6–8 weeks old and weighing 20–25 g were randomly divided into 4 groups: (1) ART1 shRNA transfection group (ART1 SiRNA), (2) ART1 full-length CDS transfection group (ART1 CDS), (3) empty vector group (vector control), and (4) the untransfected group (WT). CT26 cells were harvested from each of the four cell groups; a 200 μL single-cell suspension containing 2×10⁶ cells was injected subcutaneously into the right forelimb of Balb/c mice to establish the CT26 transplanted tumor model. The tumor volume of each mouse was determined (in cubic millimeter) by the following formula: \( V = \frac{ab^2}{2} \), where \( a \) refers to the maximum diameter and \( b \) to the minimum diameter. All mice were sacrificed after 14 days, and tumors were carefully excised and prepared for routine pathological procedures.

1.10 Statistical Analysis

Data are expressed as the mean±standard deviation (SD), and obtained from three independent experiments. The association between clinical characteristics was evaluated using a \( \chi^2 \) test. A Student’s t-test was used to compare the mean between each group. All statistical analyses were performed using the SPSS 19.0 software (SPSS, Inc., USA). All tests were two-tailed, and a \( P \) value less than 0.05 was considered significant.

2 RESULTS

2.1 Association of ART1 with Glycolysis in CRC Cells

To elucidate the association between ART1 and glycolysis in CRC, an IHC assay was performed to compare the expressions of ART1, HIF1α, and GLUT1 in 61 CRC tissues from patients who were evaluated with \( ^{18} \)F-FDG PET/CT. Cases with high FDG uptake exhibited significantly higher expression of ART1 and GLUT1 than those with low FDG uptake (fig. 1A–1C). ART1 and HIF1α were predominantly localized in the cytoplasm of cancer cells, and GLUT1 was mainly expressed in the cytoplasm and cell membrane (fig. 1D–1I). There was a significant association between FDG uptake and ART1 expression in CRC cases (\( P=0.037 \)). GLUT1 expression was also significantly...
associated with FDG uptake ($P=0.022$); however, HIF1α expression did not exhibit a significant association ($P=0.349$). Pearson’s analysis indicated that ART1 expression showed a significant correlation with HIF1α expression and GLUT1 expression, respectively (table 1).

2.2 ART1 Regulates Glycolysis by Promoting the Expression of Glycolytic Rate-limiting Enzymes in CT26 Cells

Functional colorimetric validation revealed that the lactic acid production was significantly enhanced in ART1-overexpressed CT26 cells but decreased in ART1-knockdown CT26 cells in both normal and CoCl₂ induced hypoxic conditions (fig. 2A and 2B). Both the mRNA and protein expression levels of HK2 and LDH were found to be significantly upregulated in ART1-overexpressed CT26 cells and downregulated in ART1-knockdown CT26 cells (fig. 2C–2F), which was consistent with the changes of lactic acid content in cells. However, there was no significant difference between the two controls cells.

2.3 ART1 Promotes the Growth of Subcutaneous Transplanted CT26 Tumors in BALB/c Mice

Compared with subcutaneously transplanted vector-control and untransfected CT26 tumors, ART1-silenced CT26 tumors exhibited significantly lower uptake of FDG (fig. 3A and 3B). The volume and weight of subcutaneous transplanted tumors were markedly increased in the ART1-overexpressed group and decreased in the ART1-knockdown group (fig. 3C–3E). In CT26 cells, overexpression of ART1 promoted the protein expression of HK2 and LDH, while knockdown of ART1 inhibited the protein expression in the tumors (fig. 3F).

2.4 ART1 Influences the PI3K-AKT-Regulated HIF1α-GLUT1 Pathway In Vitro

Under both normal and hypoxic conditions, the mRNA levels of HIF1α and GLUT1 were considerably upregulated in ART1-overexpressed CT26 cells and downregulated in ART1-knockdown cells (fig. 4A and 4B) compared to the controls. In ART1-overexpressed CT26 cells, the protein levels of p-AKT, HIF1α, and
Table 1 Association between the expression of ART1, HIF1α and GLUT1 and clinicopathologic parameters of patients with colorectal cancers

| Factors                        | Gender | n     | Expression of ART1 | Expression of HIF1α | Expression of GLUT1 |
|--------------------------------|--------|-------|--------------------|---------------------|--------------------|
|                                | Male   | 33    | 22 (66.7)          | 17 (51.5)           | 20 (60.6)          |
|                                | Female | 28    | 19 (67.9)          | 14 (50)             | 22 (78.6)          |
| Age (years old)                | >60    | 35    | 22 (62.9)          | 16 (45.7)           | 23 (65.7)          |
|                                | ≤60    | 26    | 19 (73.1)          | 15 (57.7)           | 19 (73.1)          |
| Differentiation                | I + II | 45    | 32 (71.1)          | 23 (51.1)           | 30 (66.7)          |
|                                | III    | 16    | 9 (56.3)           | 8 (50)              | 12 (75)            |
| Tumor location                 | Colon  | 41    | 28 (68.3)          | 21 (51.2)           | 29 (70.7)          |
|                                | Rectum | 20    | 13 (65)            | 10 (50)             | 13 (65)            |
| Tumor size (cm)                | <5     | 37    | 24 (64.9)          | 18 (48.6)           | 25 (67.6)          |
|                                | ≥5     | 24    | 17 (70.8)          | 13 (54.2)           | 17 (70.8)          |
| Tumor type                     | Non-mucinous | 54  | 36 (66.7)          | 26 (48.1)           | 36 (66.7)          |
|                                | Mucinous | 7   | 5 (71.4)           | 2 (28.6)            | 6 (85.7)           |
| TNM stage                      | I + II | 28    | 17 (60.7)          | 14 (50.0)           | 16 (57.1)          |
|                                | III+IV | 33    | 24 (72.7)          | 17 (51.5)           | 26 (78.8)          |
| T category                     | I + II | 10    | 6 (60)             | 5 (50)              | 7 (70)             |
|                                | III+IV | 51    | 35 (68.6)          | 26 (51)             | 35 (68.6)          |
| N category                     | N0     | 32    | 21 (65.6)          | 15 (46.9)           | 20 (62.5)          |
|                                | N1-2   | 29    | 20 (69.0)          | 16 (55.2)           | 22 (75.9)          |
| M category                     | M0     | 51    | 32 (62.7)          | 27 (52.9)           | 32 (62.7)          |
|                                | M1     | 10    | 9 (90)             | 4 (40)              | 10 (100)           |
| AJCC stage                     | I + II | 28    | 17 (60.7)          | 14 (50)             | 16 (57.1)          |
|                                | III+IV | 33    | 24 (72.7)          | 17 (51.5)           | 26 (78.8)          |

The association between clinicopathologic variables was evaluated using a χ2 test. Significance analysis was two-tailed, and P<0.05 was considered statistically significant. *P<0.05

GLUT1 were enhanced under both normal and hypoxic conditions compared to the controls, but the protein level of total AKT was not altered in either condition. Moreover, in ART1-downregulated cells, the protein levels of p-AKT were reduced under both normal and hypoxic conditions; however, the protein level of HIF1α and GLUT1 were suppressed in the normal condition compared to the hypoxic condition (fig. 4C–4D).

Compared with subcutaneously transplanted control tumors in BALB/c mice, the expression levels of p-AKT, HIF1α, and GLUT1 were augmented in ART1-overexpressed CT26 tumors and reduced in ART1-knockdown tumors (fig. 4E).

3 DISCUSSION

ART1 is an ectoenzyme that is a member of the ecto-ART family proteins, which catalyzes ADP-ribosylation by transferring an ADP-ribose moiety from NAD+ to an amino acid residue. ART1 thus plays a significant role in protein modification and participates in a variety of biological processes within the cell. Studies have demonstrated that ART1 is involved in apoptosis, cell proliferation, and the migration and angiogenesis of tumor cells [10, 12, 14]. Moreover, ART1 plays a crucial role in regulating the Warburg effect in CRC. Tumors with high uptake of FDG exhibited high expression of ART1, indicating that the expression level of ART1 may reflect the degree of hypoxia in the tumor.

Aerobic glycolysis or the Warburg effect is a hallmark of metabolic phenotypes of cancer, which is characterized by an increased metabolism of glucose to lactate despite the presence of sufficient...
oxygen\textsuperscript{[2]}. In this study, lactate content was enhanced in ART1-overexpressed cells and reduced in ART1-knockdown cells compared to control cells in both normal and hypoxic conditions. This result indicates that ART1 promotes the efficiency of glycolysis in tumor cells, as the rate of glycolysis is crucial for cellular lactate accumulation\textsuperscript{[16]}. Moreover, the mRNA and protein levels of HK2 and LDH were also altered corresponding to the level of lactate in cells, suggesting that ART1 also regulates the rate of glycolysis, which is achieved by regulating the expression of key glycolytic enzymes.

To elucidate the association between changes in ART1 in CT26 cells and glycolysis in vivo, ART1-overexpressed and ART1-knockdown CT26 cells were subcutaneously transplanted into BALB/c mice. The results indicated an increase in the volume and weight of subcutaneously transplanted ART1-overexpressed CT26 tumor tissues and a decrease in tumor weight of ART1-knockdown CT26 tumor tissues in BALB/c mice. Uptake of FDG was also significantly altered only in ART1-knockdown CT26 tumors. However, the protein levels of HK2 and LDH were upregulated in ART1-overexpressed tissues and downregulated in ART1-knockdown tumors. Taken together, these results demonstrated that ART1 might affect not only the growth and development of transplanted tumors but also regulate glycolysis of the tumor in vivo\textsuperscript{[12]}.

The PI3K/AKT pathway is dysregulated in tumors and is frequently involved in the regulation of many cellular processes, including the proliferation, migration, and survival of tumors cells. AKT represents one of the primary effectors in the pathway, and increased AKT\textsuperscript{Thr308} phosphorylation implies an augmentation of the PI3K pathway\textsuperscript{[17]}. Accumulating studies have suggested that activation of AKT promotes the phosphorylation of glycogen synthase-3 (GSK3), inhibits HIF1\textalpha degradation, and regulates HIF1\textalpha gene transcription\textsuperscript{[18]}. In CRC, HIF1\textalpha overexpression has been associated with aggressive biological tumor behavior. However, HIF1\textalpha expression was not significantly correlated with age, tumor location, sex, TNM, or pathological stage\textsuperscript{[19]}, which
was also confirmed in the present study. We used ART1-overexpressed and ART1-knockdown CT26 cells to evaluate the expression of AKT and HIF1α. We found that the expression of AKT did not change with the expression of ART1 in both normal and hypoxic conditions; however, the expression of p-AKT and HIF1α changed with ART1. Besides, ART1-overexpressed cells exhibited increased expression of p-AKT and HIF1α, and ART1-knockdown cells exhibited a reduction in expression, suggesting that by regulating the activation of AKT, ART1 plays a crucial role in HIF1α-dependent glycolysis in CT26 cells.

GLUT1 was found to be overexpressed in hypoxic cells as one of the HIF1α-induced genes [20, 21]. In CRC, overexpression of both GLUT1 and HIF1α was observed by IHC analyses [19, 22]. In the present study, we also confirmed that 50.8% and 68.9% of CRC tissues exhibited high expression of HIF1α and GLUT1, respectively. We further suggested that the patients with high uptake of FDG exhibited a significantly higher rate of GLUT1 than those with low uptake of FDG. This may be because GLUT1 contributes to the avid uptake of glucose even when the glucose is insufficient due to the apparent malignant growth of tumors [23]. Furthermore, Jun et al. [24] examined 515 CRC cases and reported that GLUT1 expression distinctly varied between men and women, tumor type, well-differentiated and less-differentiated groups, and N tumor stage. A recent study by Martins et al. [25] also revealed that GLUT1 expression was associated with poorly-differentiated tumors. However, in our results, GLUT1 expression was not significantly correlated with differentiation, but with M tumor stage and FDG uptake. This may be attributed to the fact that the study was conducted on a low sample size of only 16 CRC cases with poorly differentiated tumors. Furthermore, GLUT1 expression was markedly correlated with FDG uptake, suggesting GLUT1 as an indicator of glycolytic rate in CRC. Notably, in CT26 cells, ART1-overexpressed cells exhibited an increased level of GLUT1,

Fig. 3 ART1 enhanced proliferation and glycolysis of subcutaneous transplanted CT26 tumors in BALB/c mice
A: representative PET/CT images of FDG uptake in BALB/c mice; B: the SUV$_{max}$ analysis of FDG uptake in BALB/c mice; C–E: representative image of subcutaneous transplanted CT26 tumors (C), statistical analysis of tumor volumes (D), and tumor weights (E); F: expression of HK2 and LDH in the subcutaneously transplanted CT26 tumors. A Student’s t-test was used to compare the mean between groups. *P<0.05, **P<0.01

[Image of PET/CT images, SUV$_{max}$ analysis, tumor volumes, tumor weights, and protein expression]
and ART1-knockdown cells exhibited a reduced level, revealing that ART1 could regulate glucose metabolism by the HIF1α-GLUT1 pathway. In previous studies, it was indicated that the activation of HIF1α could be upregulated by PI3K/AKT activation and downregulated by PI3K/AKT inhibition[26]. However, inhibition of AKT reduced the expression of HIF1α but did not significantly change ART1 expression[14], suggesting that the AKT pathway was located downstream of ART1. In the present study, overexpression of ART1 increased the expression of both p-AKT and HIF1α, while downregulation of ART1 decreased the respective expression, which confirmed previous findings. We therefore speculated that ART1 could promote phosphorylation of AKT and subsequent HIF1α-GLUT1 activity. Thus, these data suggest that ART1 regulates HIF1α-GLUT1 activation through the PI3K/AKT pathway in CRC.

In conclusion, this study demonstrated that ART1 could promote the glycolysis of CRC tumors under both normal and hypoxic conditions by promoting the expression of HK2 and LDH. Overall, the findings of this study suggested that ART1 plays a crucial role in the elevation of glucose consumption in CT26 cells and may regulate GLUT1-dependent glycolysis in CRC via the PI3K/AKT/HIF1α pathway.

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Conflict of Interest Statement

The authors declare that they have no conflicting of any interest.
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