Circ-BANP Contributes to Cell Carcinogenesis and Aerobic Glycolysis by Regulating MAPK1 Through miR-874-3p in Colorectal Cancer

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Abstract

Background

Circular RNA-BTG3 associated nuclear protein (circ-BANP) was identified to involve in cell proliferation of colorectal cancer (CRC). The aerobic glycolysis is a key metabolism mediating cancer progression. However, the role of circ-BANP on aerobic glycolysis in CRC remains unknown.

Methods

The expression of circ-BANP, microRNA (miR)-874-3p, and mitogen-activated protein kinase 1 (MAPK1) mRNA was detected using quantitative real-time polymerase chain reaction. Cell viability and invasion were measured by cell counting kit-8 assay or transwell assay. Glucose consumption and lactate production were assessed by a glucose and lactate assay kit. XF Extracellular Flux Analyzer was used to determine extracellular acidification rate (ECAR).

Western blot was used to analyze the levels of hexokinase-2 (HK2), pyruvate kinase M2 (PKM2), MAPK1, proliferating cell nuclear antigen (PCNA), Cyclin D1, N-cadherin, E-cadherin, hypoxia inducible factor-1α (HIF-1α), glucose transport protein 1(GLUT1), and c-Myc. The interaction between miR-874-3p and circ-BANP or MAPK1 was confirmed by dual luciferase reporter assay. In vivo experiments were conducted through the murine xenograft model.

Results

Circ-BANP was up-regulated in CRC tissues and cell lines. Circ-BANP knockdown suppressed CRC cell proliferation, invasion and aerobic glycolysis in vitro as well as inhibited tumor growth in vivo. Circ-BANP was a sponge of miR-874-3p and performed anti-tumor effects by binding to miR-874-3p in CRC cells. Subsequently, we confirmed MAPK1 was a target of miR-874-3p and circ-BANP indirectly regulated MAPK1 expression by sponging miR-874-3p.

After that, we found MAPK1 overexpression partially reversed circ-BANP deletion-mediated inhibition on cell carcinogenesis and aerobic glycolysis in CRC.

Conclusion

Circ-BANP accelerated cell carcinogenesis and aerobic glycolysis by regulating MAPK1 through miR-874-3p in CRC, suggesting a promising therapeutic strategy for CRC treatment.

Highlights

1. Circ-BANP is up-regulated in CRC tissues and cell lines.
2. Circ-BANP knockdown suppresses CRC cell proliferation, invasion and aerobic glycolysis in vitro as well as inhibits tumor growth in vivo.
3. MiR-874-3p directly binds to circ-BANP and MAPK1 3′-UTR.
4. Circ-BANP indirectly regulates MAPK1 expression by serving as a sponge of miR-874-3p.
5. Circ-BANP deletion-induced anticancer effects can be remarkably attenuated by miR-874-3p inhibition or MAPK1 re-expression.

**Background**

Colorectal cancer (CRC) ranks as the fourth highest leading cause of cancer-related mortality with almost 700,000 people died every year [1]. To our knowledge, early stage of CRC patients can be cured with surgery [2]. Unfortunately, conventional treatment, such as surgical resection, chemotherapy or radiotherapy, is not effective for patients with advanced CRC [3]. Although ongoing improvements to treatment, like immunotherapy and targeted therapy, there have been no definitive improvements in the prognosis of CRC [4]. Thus, further investigations on the molecular understanding of the underlying pathogenesis in CRC to develop mechanism-based therapeutic approaches are necessary. The pathogenic characteristics of malignancy include sustained cell proliferation, highly invasive potential, as well as abnormal metabolism, which are significant biological features in the clinical process of tumor growth [5–7]. The metabolism of cancer cells is more metabolically active compared with the normal cells. They often have a higher proliferation rate and can able to promote cell metastasis [8]. Most tumor cells prefer to take glycolysis as the main source of adenosine triphosphate (ATP) regardless of oxygen availability, this phenomenon is often defined as aerobic glycolysis (or Warburg effect), which characterizes by higher rates of glucose consumption and lactate production even under normoxia [9, 10].

Increasing evidence reveals that metabolic alteration is one of the most consistent hallmarks of cancer, and have critical effects on tumor progression, besides that the aberrant expression of glycolysis-associated molecules also contributes to tumorigenesis [11, 12]. Therefore, targeting these characteristics of tumor cells may be a potential way for cancer therapeutic interventions. Circular RNAs (circRNAs) are one of covalently closed RNA transcripts without a 5′ cap and a 3′ polyadenylated tail, which make them more stable to resistant exonuclease degradation compared with their linear RNAs decay [13]. In recent, emerging evidence has identified that circRNAs are novel master controllers in regulating malignant cancer physiological or pathological cellular processes, including cell proliferation, growth, invasion, angiogenesis, metabolism, and so on [14–17]. In CRC, many circRNAs have also been identified to involve in the tumorigenesis. For example, circ-CBL.11 inhibited CRC cell proliferation via directly interacting with microRNA (miR)-6778-5p [18]. CircRNA hsa_circRNA_102958 contributed to cell proliferation and metastasis in CRC by regulating CDC25B expression through miR-585 [19]. Therefore, circRNAs are important regulators for CRC development and progression. CircRNA-BTG3 associated
nuclear protein (BANP) is a novel identified functional circRNA. A recent study showed circ-BANP was elevated in CRC tissues, and circ-BANP deletion could inhibit cell proliferation in CRC [20]. However, the role of circ-BANP in aerobic glycolysis of CRC remains unknown. In this study, we focused on the roles of circ-BANP on CRC cell proliferation, invasion and aerobic glycolysis, and explored the molecular mechanism underlying circBANP in CRC tumorigenesis.

Materials And Methods

Patients and specimens

Tumor samples and paratumor specimens were obtained from 56 paired CRC patients who underwent surgical resection at China-Japan Union Hospital of Jilin University and immediately stored at −80 °C until further analysis. All patients did not receive any preoperative treatment and were diagnosed by histopathological examination. This study was permitted by the Ethics Committee of China-Japan Union Hospital of Jilin University and all subjects had signed the written informed consents.

Cell culture and transfection

Human CRC cell line HCT116 and SW620, and normal colon (FHC) cells were obtained from Shanghai Academy of Life Science (Shanghai, China) and grown in the Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Gibco) with at 37 °C in a humidified atmosphere with 5% CO2. Lentiviral plasmids encoding circ-BANP-specific short hairpin RNA (shRNA) (sh-circ-BANP, 5′-UGC UAU CAU UUU CCA GGG-3′) and shRNA scramble control (sh-NC) were designed and produced by Invitrogen (Carlsbad, CA, USA). HCT116 and SW620 cells were transfected with lentivirus LV-3 (pGLVH1/GFP + Puro) vector and selected with puromycin (2–3 μg/mL) for 2 weeks to obtain sh-circ or sh-NC stably expressed cell lines. The small interfering RNA (siRNA) sequences targeting circ-BANP covalent closed junction (si-circ-BANP, 5′-UCA UUG UUG AGU AUU ACU GUA-3′), siRNA negative control (si-NC), pcDNA3.1-circ-BANP overexpression vector (circ-BANP), and pcDNA3.1 empty vector (pcDNA), pcDNA3.1-MAPK1 overexpression vector (MAPK1) were also purchased from Invitrogen. The miR-874-3p mimics, miR-874-3p inhibitor, and their corresponding negative control (miR-NC and inhibitor NC) were produced by RIBOBIO (Guangzhou, China). HCT116 and SW620 cells were plated into six-well culture plates (1 × 10^5 cells/well) at a confluence of 50 to 60%, and then plasmid, mimics, inhibitor and negative control were transfected into cells using Lipofectamine 2000 transfection reagent (Invitrogen) according to the manufacturer’s instructions.
**Quantitative real-time polymerase chain reaction**

(qRT-PCR)

Total RNA was isolated using TRizol reagent (Invitrogen) with the standard procedure. Subsequently, extracted RNA was incubated with Rnase R (Epicentre, Madison, WI, USA), followed by interaction with RNeasy MinElute Cleanup Kit (Qiagen, Valencia, CA, USA). After that, total RNA was reversely transcribed into complementary DNA (cDNA) by using the Prime Script RT Master Mix (Takara, Dalian, China), and then qRT-PCR was carried out on the ABI7500 system using SYBR Green methods. The following thermocycling conditions were used for the qPCR: Initial denaturation at 95 °C for 5 min; 40 cycles of 95 °C for 10 s, and annealing at 60 °C for 30 s. A melt curve step from 65-95 °C was performed in increments of 0.5 °C per 5 s. Relative transcription expression was analyzed by $2^{-\Delta \Delta Ct}$ method and normalized by glyceraldehyde 3-phosphate dehydrogenase (GADPH) or U6 small nuclear B noncoding RNA (U6). The specific primer sequences were presented as follow: circ-BANP: F 5′-CAG GAC GGT CAG CGT CGT -3′, R 5′-GGC ACA GCG TTG CTA ATG AC-3′; MAPK1: F 5′-ACC AAC CTC TCG TAC ATC GG-3′, R 5′-GGG CTG ATT TTC TTG ATA GC-3′; miR-874-3p: F 5′-GAA CTC CAC TGT AGC AGA GAT GGT -3′, R 5′-CAT TTT TTC CAC TCC TCT TCT CTC -3′; GADPH: F 5′-GAT ATT GTT GCC ATC AAT GAC-3′, R 5′-TTG ATT TTG GAG GGA TCT CG-3′; U6: F 5′-CTC GCT TCG GCA AGACA-3′, R 5′-ACG CTT CAC GAA TTT GCG T-3′.

**Cells viability assay**

Transfected HCT116 and SW620 (1 × 104 cells/well) were seeded on the wells of a 96-well plate and cultured in DMEM including 10% FBS for 72h. Then 10 μL cell counting kit-8 (CCK-8) solution (Beyotime, Shanghai, China) was supplemented into per well and incubated for another 2 h. Finally, the optical density (OD) at 450 nm was determined by a microplate reader in the indicated time to assess the cell viability.

**Transwell assay**

Transfected cells (4 × 10^5) with serum-free DMEM were seeded on the top chambers, which membranes were pre-coated with matrigel (BD Biosciences, San Jose, CA, USA). Then the lower chambers were filled with 500 μL serum-DMEM. After incubation for 24h, cells on the lower face of the membranes were fixed and stained.

Finally, invaded cells in 5 randomly selected fields were counted with a microscope.

**Measurements of glucose and lactate levels**

After transfection for 48h, cells were maintained in a 6-well plate. 24 h later, supernatants of cell culture media were collected to detect the levels of glucose and lactate using a glucose and lactate assay kit
(Sigma, St Louis, MO, USA) according to the manufacturer's protocol. Levels were assessed based on the standard curve and normalized by the protein concentration of samples.

**Measurement of extracellular acidification rate (ECAR)**

ECAR was determined according to the XF Glycolysis Stress Test protocol on a Seahorse XFe24 Extracellular Flux Analyzer (Agilent Technologies, Santa Clara, CA, USA). Transfected HCT116 and SW620 cells (8 x 10^3 cells/well) were plated in a glucose-free Seahorse Assay plates. Following baseline examinations, per well were sequentially added with 25 mM glucose, 1 μM oligomycin, and 50 mM glucose analog 2-deoxyglucose (2-DG) at indicated time points. Finally, ECAR were analyzed using the Seahorse XFe24 Extracellular Flux Analyzer and normalized to total protein concentration on each well.

**Western blot**

Total protein was extracted using RIPA buffer containing a proteinase and phosphatase inhibitor cocktail. Then isolated protein was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, shifted onto a polyvinylidene fluoride membranes, and blocked with 5% non-milk. Afterwards, the membranes were incubated with primary antibodies against hexokinase-2 (HK2) (1:2000, ab104836, Abcam, Cambridge, MA, USA), pyruvate kinase M2 (PKM2) (1:3000, ab137852, Abcam), MAPK1 (1:1000, ab241580, Abcam), proliferating cell nuclear antigen (PCNA) (1:5000, ab29, Abcam), Cyclin D1 (1:10,000, ab134175, Abcam), N-cadherin (N-cad) (1:5000, ab18203, Abcam), E-cadherin (E-cad) (1:5000, ab15148, Abcam), hypoxia inducible factor-1α (HIF-1α) (1:1000, ab1, Abcam), glucose transport protein 1(GLUT1) (1:10,000, ab115730, Abcam), c-Myc (1:1000, ab32072, Abcam), and GAPDH (1:10,000, ab181602, Abcam), followed by interaction with HRP-conjugated secondary antibody (1:1000, ab9482, Abcam). Finally, protein bands were visualized using the chemiluminescence chromogenic substrate (Beyotime, Beijing, China).

**Dual-luciferase reporter assay**

The circ-BANP and MAPK1 3' UTR containing the wild-type (WT) or mutant (MUT) binding sites of miR-874-3p were cloned into the pmiR-RB-Report (Promega, Shanghai, China), respectively. Subsequently, HCT116 and SW620 cells (3 x 10^4 cells) were seeded in a 24-well plate individually and co-transfected with 200 ng of WT or MUT pmiR-RB-Report-circ-BANP/MAPK1 3' UTR with 10 nM miR-18b-5p mimics or
miR-NC using Lipofectamine™ 2000 (Invitrogen). Finally, a dual luciferase assay kit (Promega) was used to analyze the luciferase activity as described earlier [21].

**Xenograft experiments in vivo**

Five-week-old BALB/c nude mice (N = 6) were obtained from Jinan Pengyue Animal Center (Jinan, China). This study was approved by the Animal Research Committee of China-Japan Union Hospital of Jilin University and was undertaken according to the guidelines of the National Animal Care and Ethics Institution. HCT116 cells (1 × 10^5) stably transfected with lentivirus containing sh-circ-BANP or sh-NC were subcutaneously injected into the flanks of the nude mice. After 7 days following the inoculation, the tumor size was examined 3 days and the tumor volume was calculated. After 21 days, all mice were sacrificed, and tumor masses were weighed and harvested for further molecular analysis.

**Statistical analysis**

Data were expressed as the mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 7 software (GraphPad Inc., San Diego, CA, USA). Significant differences between different groups were analyzed using Student’s t-test or one-way analysis of variance (ANOVA). The correlation analysis was performed using Spearman’s correlation test. The P value less than 0.05 indicated statistically significant.

**Results**

**Circ-BANP is up-regulated in CRC tissues and cell lines**

To explore the potential biological functions of circ-BANP involved in CRC carcinogenesis, the expression of circ-BANP was detected. qRT-PCR analysis showed circ-BANP was greatly up-regulated in CRC tissues compared with the normal tissues (Fig. 1a). Similarly, the high expression of circ-BANP was also investigated in CRC cell lines compared to the normal FHC cell line (Fig. 1b).

Thus, circ-BANP might be a potential regulator in CRC progression.

**Circ-BANP knockdown suppresses proliferation, invasion and aerobic glycolysis of CRC cells**

Tumor cell proliferation, and invasion are pivotal steps in tumorigenesis. First, HCT116 and SW620 cells were transfected with si-circ-BANP or si-NC to conduct functional experiments, qRT-PCR analysis showed a significant reduction of circ-BANP expression in HCT116 and SW620 cells after transfection (Fig. 2a). After that, CCK-8 assay showed circ-BANP knockdown suppressed the proliferation of HCT116 and SW620 cells (Fig. 2b). Transwell assay indicated the invaded cells were markedly reduced by circ-BANP
deletion (Fig. 2c). Besides that, it was also observed that circ-BANP knockdown elevated the level of E-cad, but decreased the levels of PCNA, CyclinD1, and N-cad in HCT116 and SW620 cells, further confirming that knockdown of circ-BANP suppressed tumor cell proliferation and invasion in vitro (Fig. 2d, e). Afterwards, we investigated whether circ-BANP might affect aerobic glycolysis in CRC. We found the circ-BANP silencing significantly reduced glucose consumption (Fig. 2f) and lactate production (Fig. 2g) in HCT116 and SW620 cells. Moreover, ECAR assay showed that circ-BANP silencing significantly reduced glycolysis, glycolytic capacity and glycolytic reserve in both HCT116 and SW620 cells (Fig. 2h); meanwhile, western blot analysis showed the protein levels of HK2, PKM2 and GLUT1 were markedly down-regulated in circ-BANP-decreased HCT116 and SW620 cells (Fig. 2i, j). All these results indicated that circ-BANP knockdown decreased the rate of aerobic glycolysis in CRC cells, thus could influence the proliferation of CRC. Importantly, the effects of circ-BANP on nontumor cells were investigated. When we elevated the levels of circ-BANP in normal FHC cells (Additional file 1: Fig. S1 A), we found that circ-BANP overexpression could induce FHC cell proliferation and invasion (Additional file 1: Fig. S1). Taken together, circ-BANP played roles in CRC progression via regulating cell proliferation, invasion and aerobic glycolysis.

**Circ-BANP is a sponge of miR-874-3p and performs carcinogenic effects by binding to miR-874-3p**

We further explored the underlying molecular mechanism of circ-BANP in CRC cell tumorigenesis. According to the search from StarBase database, miR-874-3p was predicted to contain the putative binding sites of circ-BANP (Fig. 3a). Then a dual-luciferase reporter assay was performed and declined luciferase activity in HCT116 and SW620 cells co-transfected with WT-circ-BANP and miR-874-3p confirmed the interaction between circ-BANP and miR-874-3p (Fig. 3b). After that, the expression of miR-874-3p was measured and we found miR-874-3p was down-regulated in CRC tissues and cell lines compared with the controls (Fig. 3c, d), and a negative correlation between miR-874-3p and circ-BANP was observed (Fig. 3e). Besides that, after increasing the level of circ-BANP through transfecting circ-BANP overexpression vector into HCT116 and SW620 cells (Fig. 3f), we discovered that the expression of miR-874-3p was increased by circ-BANP down-regulation, but was decreased by circ-BANP up-regulation (Fig. 3g). These results indicated that circ-BANP targetedly suppressed miR-874-3p expression in CRC cells. Based on the relationship of miR-874-3p and circ-BANP, we hypothesized miR-874-3p might implicate in circ-BANP deletion-induced repression on cell tumorigenicity. Firstly, the interference efficiency of miR-874-3p inhibitor was confirmed, we found the transfection of miR-874-3p inhibitor markedly reduced miR-874-3p...
expression in HCT116 and SW620 cells (Fig. 3h). After that, si-NC, si-circ-BANP, si-circ-BANP + inhibitor NC, or si-circ-BANP + miR-874-3p inhibitor was respectively transfected into HCT116 and SW620 cells to conduct rescue assay. Results showed miR-874-3p down-regulation could partially attenuate circ-BANP deletion-mediated inhibition on HCT116 and SW620 cell proliferation and invasion (Fig. 3i–l).

Additionally, miR-874-3p inhibition also reversed circ-BANP silence-induced suppression on aerobic glycolysis, as illustrated by the increase of glucose consumption (Fig. 3m), lactate production (Fig. 3n) and ECAR (Fig. 3o), as well as the elevation of HK2, PKM2 and GLUT1 protein in HCT116 and SW620 cells (Fig. 3p, q). Altogether, these data indicated circ-BANP knockdown repressed proliferation, invasion and aerobic glycolysis of CRC cells by sponging miR-874-3p.

MAPK1 overexpression partially reverses circ-BANP deletion mediated inhibition on cell proliferation, invasion and aerobic glycolysis in CRC

MAPK1 is a target of miR-874-3p and is negatively regulated by miR-874-3p

Immediately, the molecular mechanism underlying circ-BANP/miR-874-3p axis was then elucidated. Based on the prediction of StarBase program, we found miR-874-3p contained the binding sites of MAPK1 (Fig. 4a). Subsequently, the dual-luciferase reporter assay showed a great reduction of luciferase activity in HCT116 and SW620 cells co-transfected with WT-MAPK1 and miR-874-3p, suggesting the direct interaction between MAPK1 and miR-874-3p (Fig. 4b). Subsequently, MAPK1 expression was found to be increased in CRC tissues at mRNA and protein levels (Fig. 4c, d), and was negatively correlated with miR-874-3p (Fig. 4e). Besides, the level of MAPK1 was also elevated in CRC cells (Fig. 4f, g). After that, HCT116 and SW620 cells were transfected with miR-874-3p mimics or miR-NC to increase the level of miR-874-3p (Fig. 4h). Then western blot analysis showed miR-874-3p up-regulation decreased MAPK1 expression, while miR-874-3p down-regulation increased MAPK1 expression in HCT116 and SW620 cells (Fig. 4i). Therefore, we confirmed that miR-874-3p targetedly inhibited MAPK1 expression in CRC cells.

Circ-BANP indirectly regulates MAPK1 expression by serving as a sponge of miR-874-3p

Based on the above results, the relationship between circ-BANP and MAPK1 was investigated. Western blot analysis indicated the expression of MAPK1 was up-regulated by miR-874-3p inhibitor, while this promotion was rescued by the following circ-BANP deletion in HCT116 and SW620 cells (Fig. 5a, b). Thus, we identified the regulatory network circ-BANP/miR-874-3p/MAPK1 in CRC cells.

MAPK1 overexpression partially reverses circ-BANP deletion mediated inhibition on cell proliferation, invasion and aerobic glycolysis in CRC
According to the regulatory relationship between circ-BANP and MAPK1, we wanted to know whether MAPK1 involved in the effects of circ-BANP on CRC tumorigenesis. Firstly, pcDNA and MAPK1 were transfected into HCT116 and SW620 cells, then MAPK1 was found to be elevated in HCT116 and SW620 cells, indicating the successful transfection (Fig. 6a, b). Next, si-circ-BANP, si-NC, si-circ-BANP + pcDNA, or si-circ-BANP + MAPK1 was respectively transfected into HCT116 and SW620 cells to perform rescue assay. Results exhibited that MAPK1 overexpression partially reversed circ-BANP deletion-mediated inhibition on cell proliferation, invasion (Fig. 6c–f) and aerobic glycolysis (Fig. 6g–j). These data revealed that circ-BANP deletion exerted anti-tumor effects by regulating MAPK1 in CRC cells.

The circ-BANP/miR-874-3p/MAPK1 axis influence cell aerobic glycolysis via regulating c-Myc or HIF-1α in CRC

It has been reported that both Myc and HIF-1α regulate glycolysis [22–24]. As shown in Fig. 7a, b, we observed that circ-BANP knockdown reduced the levels of c-Myc or HIF-1α in HCT116 and SW620 cells, which were rescued by miR-874-3p down-regulation or MAPK1 up regulation. Therefore, we verified that the circ-BANP/miR-874-3p/MAPK1 axis could regulate cell aerobic glycolysis via regulating c-Myc or HIF-1α in CRC.

Circ-BANP deletion suppresses tumor growth and aerobic glycolysis in vivo

We further explored the regulatory effects of circ-BANP in vivo. We discovered that circ-BANP silence suppressed tumor volume and weight in sh-circ-BANP group (Fig. 8a–c). Besides that, the expression of circ-BANP and MAPK1 was down-regulated, whereas miR-874-3p was up-regulated in tumor tissues collected from sh-circ-BANP group (Fig. 8d, e). Importantly, it was also observed that circ-BANP knockdown reduced the levels of HK2 and PKM2 in the tissues (Fig. 8f). Therefore, circ-BANP deletion restrained tumor growth and aerobic glycolysis in vivo by down-regulating MAPK1 through sponging miR-874-3p.

Discussion

Currently, it has become clear that altered energy metabolism is essential for the rapid growth of cancer cells, which is recognized as one of hallmarks of cancer cells [25]. Unlike normal cells, cancer cells grow and duplicate rapidly by unlimited cell division, thus showing a high-energy requirement. Glycometabolism is the pivotal point of energy metabolism, and it has been reported that cancer cells prefer to take ATP by aerobic glycolysis regardless of oxygen [26]. The metabolic alteration of aerobic glycolysis has been verified in a wide range of cancer contexts and shown to involve in tumor cell proliferation, migration and invasion in many cancers, including CRC [12, 27, 28]. For example, FOXC1 contributed to cell proliferation in CRC by enhancing the aerobic glycolysis through binding to FBP1 [29]. STK25 suppressed aerobic glycolysis to repress cell proliferation in CRC by regulating GOLPH3-mTOR pathway [30]. Pim1 promoted CRC cell growth and tolerance to glucose starvation through enhancing the
aerobic glycolysis [31]. Thus, the intervention of aerobic glycolysis may be a potential way for CRC treatment.

Numerous studies have indicated that altered metabolic pathways in cancers are strictly controlled by noncoding RNAs [17, 32]. For example, miR-885-5p inhibited aerobic glycolysis by targeting HK2 silencing in liver cancer [33]. Long noncoding RNA (IncRNA) PCGEM1 enhanced the glucose uptake through promoting aerobic glycolysis in prostate cancer cells [34]. Besides that, circRNAs also involves in the regulation of aerobic glycolysis in several cancers. For instance, circ-FOXP1 accelerated carcinogenesis and aerobic glycolysis by interacting with PKLR in gallbladder cancer [35]. CircRNA hsa_circRNA_100290 contributed to cell growth by regulating glycolysis through miR-378a/GLUT1 in oral squamous cell carcinoma [36]. Circ-NRIP1 positively regulated glycolysis to promote gastric cancer by regulating miR-149-5p-AKT1/mTOR axis [37]. However, the relationship between circRNAs and aerobic glycolysis in CRC remains unknown.

In this study, we found circ-BANP was up-regulated in CRC tissues and cell lines, and circ-BANP knockdown suppressed CRC cell proliferation, which was consistent with the previous study [20]. Additionally, we also found circ-BANP knockdown not only inhibited cell proliferation but also suppressed invasion in vitro as well as tumor growth in vivo. Immediately, the effects of circ-BANP on aerobic glycolysis in CRC were explored. We found knockdown of circ-BANP decreased glucose consumption, lactate production and ECAR. GLUT1, as facilitative-type glucose transporters, are highly expressed in a number of types of cancer and are involved in cellular glucose consumption [38]. Aberrant tyrosine kinase signaling is a key driver of oncogenesis and tumor growth in numerous different cancers, which functions to enhance the aerobic glycolysis in cancer metabolism through elevating glycolysis and lactate production. PKM2 is a glycolytic target of tyrosine kinase signaling where phosphorylation of it induces increased glycolytic rate and cancer cell proliferation [39, 40]. HK2 is a major player in both the aerobic glycolysis and cancer cell immortalization, which is both highly elevated in rapidly growing cancers and is bound to mitochondrial voltage dependent anion channels (VDAC), thus elevating glycolysis [41]. In this study, we also found that the levels of GLUT1, PKM2, and HK2 were decreased by circ-BANP down-regulation in CRC. Altogether, circ-BANP knockdown suppressed CRC cell glycolysis, suppressing the proliferation and invasion of CRC cells in vitro and in vivo.

Accumulating investigations have revealed that circRNAs can directly regulate transcription by interacting with mRNAs or IncRNAs, or sponging microRNA (miR

NAs) [42]. Therefore, we hypothesized that circ-BANP might regulate cancer tumorigenesis and metabolism by binding to miRNAs or other targets. MiR-874-3p is a well-recognized tumor suppressor, which has been reported to inhibited tumor progression in several cancers [43–45]. In addition, miR-874-3p inhibition was found to promote chemoresistance by inactivating Hippo signaling pathway in CRC [46], indicating the regulatory roles of miR-874-3p in CRC. MAPK1 is a member of the MAP kinase family, which has been
revealed to implicate in various cellular biological processes, such as cell growth, metastasis, and apoptosis in CRC by serving as a target of miRNAs or some signal transduction pathways [47, 48]. In this study, miR-874-3p was confirmed to directly bind to circ-BANP and MAPK1 3'UTR, circ-BANP deletion could significantly relieve miR-874-3p inhibition-mediated increase on MAPK1 by serving as a sponge. In addition, circ-BANP deletion-induced inhibition on cell proliferation, invasion and aerobic glycolysis could be remarkably attenuated by miR-874-3p inhibition or MAPK1 re-expression. Thus, a circ-BANP/miR-874-3p/MAPK1 axis in CRC tumorigenesis was identified.

Based on above results, we confirmed that the circ-BANP/miR-874-3p/MAPK1 axis could regulate glycolysis in CRC via GLUT1, PKM2, and HK2, thus affecting tumor cell proliferation and invasion. However, how these molecules are regulated by this axis remain unclear. Previous studies have reported that both Myc and HIF-1α regulate glycolysis [22, 23]. HIF-1α is usually activated as a consequence of mutations in tumor suppressor genes (e.g., P53, P21) and contributes in part to the overexpression of glucose transporters and several glycolytic enzymes such as HK2, PFK, PKM, and LDH, thereby increasing glucose metabolism [10]. The oncogene c-Myc is also known to be dysregulated in a variety of human cancers, it stimulates glycolysis and alters mitochondrial metabolism through exogenous glutamine metabolism, besides that, c-Myc, in essence, drive expression of all genes involved in glycolysis and many in glutaminolysis, such as PKM2 and GLUT1 [23]. In this study, we found the expression of c-Myc and HIF-1α could be impacted by the circ-BANP/miR-874-3p/MAPK1 axis. Thus, we illustrated that circ-BANP triggered glycolysis through HIF-1α or c-Myc pathway via regulating miR-874-3p/MAPK1 axis. Nevertheless, certain questions are still need further study. First, the effects of circ-BANP on other malignant phenotypes of CRC cells were not proven in the present study and require investigation. Next, more detailed mechanistic link how glycolysis is regulated by circ-BANP still need explored. In addition, the alternative targets of circ-BANP upon increasing glycolysis merit investigation.

**Conclusion**

In conclusion, this study highlighted a key role of circ-BANP in cancer metabolic reprogramming. Circ-BANP knockdown could suppress aerobic glycolysis by regulating MAPK1 through miR-874-3p in CRC, thus impeding tumor growth, which suggested a promising therapeutic strategy for CRC treatment.

**Declarations**

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**Availability of data and materials**
The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Authors’ contribution

Conceptualization and Methodology: Kexin Shen and Hanyi Zha; Formal analysis and Data curation: Wentao Zhang and Haishan Zhang; Validation and Investigation: Yunxin Zhang and Wentao Zhang; Writing - original draft preparation and Writing - review and editing: Yunxin Zhang, Kexin Shen and Haishan Zhang; Approval of final manuscript: all authors.

Ethics approval and consent to participate

The present study was approved by the ethical review committee of China-Japan Union Hospital of Jilin University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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