Circular RNA circRNF20 promotes breast cancer tumorigenesis and Warburg effect through miR-487a/HIF-1α/HK2

Lili Cao¹, Min Wang¹, Yujin Dong¹, Bo Xu¹, Ju Chen², Yu Ding³, Shusheng Qiu³, Liang Li³, Elena Karamfilova Zaharieva⁴, Xinwen Zhou⁵ and Yanbin Xu³

Abstract
Compelling evidence has demonstrated the potential functions of circular RNAs (circRNAs) in breast cancer (BC) tumorigenesis. Nevertheless, the underlying mechanism by which circRNAs regulate BC progression is still unclear. The purpose of present research was to investigate the novel circRNA circRNF20 (hsa_circ_0087784) and its role in BC. circRNA microarray sequencing revealed that circRNF20 was one of the upregulated transcripts in BC samples. Increased circRNF20 level predicted the poor clinical outcome in BC specimens. Functionally, circRNF20 promoted the proliferation and Warburg effect (aerobic glycolysis) of BC cells. Mechanistically, circRNF20 harbor miR-487a, acting as miRNA sponge, and then miR-487a targeted the 3'-UTR of hypoxia-inducible factor-1α (HIF-1α). Moreover, HIF-1α could bind with the promoter of hexokinase II (HK2) and promoted its transcription. In conclusion, this finding illustrates the vital roles of circRNF20 via the circRNF20/miR-487a/HIF-1α/HK2 axis in breast cancer progress and Warburg effect, providing an interesting insight for the BC tumorigenesis.

Introduction
Breast cancer (BC) is emerging as the leading cause of cancer-related deaths and the second highest mortality in women worldwide. In the last few decades, outstanding advances have been achieved for breast cancer treatments, however, the morbidity and mortality are still high. Existing research shows that the initiation factors inducing BC are complicated, including hormone disorders, heredity, environment. Besides factors above, epigenetic mutations are also important factors in the tumorigenesis of BC. Therefore, it is critical to identify the molecular mechanisms involved in the proliferation and progression genesis in BC. Moreover, more valuable coping strategies and precise targeting therapies are pressing challenges with significant clinical implications.

Circular RNAs (circRNAs) are group of RNA transcripts and covalently-closed loops formed by back-splicing. Generally, circRNAs are derived from the exon or intron of gene precursor mRNA (pre-mRNA). The 5’ and 3’ end of exon or intron are jointed to construct the closed loop. The alternative splicing for pre-mRNA transcript could yield diverse circRNAs subtype. In human cancer, circRNA could diffuse to modify the tumor progression by post-transcriptional regulation. For example, circRNA circ-ABCB10 is significantly upregulated in BC tissue and circ-ABCB10 knockdown suppresses the proliferation and increased apoptosis of breast cancer cells by sponging miR-127. In human cancer, circRNA could diffusely modify the tumor progression by post-transcriptional regulation. For example, circRNA circ-ABCB10 is significantly upregulated in BC tissue and circ-ABCB10 knockdown suppresses the proliferation and increased apoptosis of breast cancer cells by sponging miR-127. circRNA circ-Ccnb1 is decreased in BC, which inhibits the p53 mutations function through H2AX and represses BC tumor growth. circRNA circYAP is overexpressed in BC and remarkably suppresses proliferation, migration and colony formation of cells. Besides, circYAP significantly decreases YAP protein.

Correspondence: Yanbin Xu (xuyanbin.zibo.edu@aliyun.com)
¹Department of Oncology, Zibo Central Hospital, Zibo 255020, China
²Department of Ultrasound, Zibo Central Hospital, Zibo 255020, China
Full list of author information is available at the end of the article.
These Author contributed equally: Lili Cao, Min Wang
Edited by A. Finazzi-Agrò

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however does not affect YAP mRNA level. As a consequence, emerging evidences support the critical roles of circRNA for BC.

Warburg effect (aerobic glycolysis) is a distinctive cellular metabolic manner in cancer cells, exhibiting an increasing rate of glucose uptake and lactic acid fermentation in aerobic environment. Unlike normal cells which undergo both oxidative phosphorylation and glycolysis, cancer cells are inclined to glycolysis in anoxic or aerobic microenvironment. CircRNAs have been identified to modulate the Warburg effect of human cancers. In neuroblastoma, CUX1-generated circRNA (circ-CUX1) promotes expression of enolase 1, glucose-6-phosphate isomerase, and phosphoglycerate kinase 1, and binds to EWSR1 to facilitate its interaction with MAZ, contributing to the aerobic glycolysis and neuroblastoma progression. In hepatocellular carcinoma, circRNA MAT2B upregulates the expression levels of PKM2 through sponging miR-338-3p, promoting the glycolysis and HCC progression.

In our study, we performed the circRNA high-throughput sequencing and revealed the circRNA profile in BC tissue. Finally, we identified a novel circRNA circRNF20 (hsa_circ_0087784) and investigated its biological roles in the BC progress and Warburg effect. circRNF20 is a 499 bp transcript originated from RNF20 gene exon-3, exon-4, and exon-5. circRNF20 could target the miR-487a/HIF-1α in BC phenotype. Moreover, HIF-1α could bind with the HK2 promoter, initiate HK2 expression and eventually promote BC progression. Overall, these findings indicate that circRNF20 might act as novel biomarkers for BC prognosis and promising therapeutic targets.

Materials and methods

Clinical tissue samples

BC tissue specimens (fifty causes) and their corresponding adjacent normal tissue were collected during the surgery. None of chemotherapy or radiotherapy was administrated before surgical excision. Tissue samples were snap-frozen in liquid nitrogen for further analysis. All tissue specimens received a postoperative histopathological diagnosis by pathologist. The overall survival (OS) was identified as the time from tumor excision to either the last follow-up visit or death of BC patients. The relationship between circRNF20 level and clinical characteristic was listed in Table 1. The Written consent was obtained from each BC patient before surgery. The study had been approved by the ethnic committee of the Zibo Central Hospital.

Cell lines

BC cell lines (MCF-7, MDA-MB-468, MDA-MB-231, MDA-MB-453) and normal human breast epithelial cell (MCF-10A) were provided from American Type Culture Collection (ATCC, USA). Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Corning, USA) with 10% fetal bovine serum (FBS, Gibco, USA), 2 mM glutamine, 100 units/mL penicillin/streptomycin at 5% CO2 cell culture under 37 °C.

Oligonucleotides transfections

Short hairpin oligonucleotides (shRNAs) and control oligonucleotides, overexpression vectors and control vectors targeting circRNF20 were synthesized by Gene-Seed Biotechnology Co., Ltd (Guangzhou, China). miR-487a mimics and inhibitor were synthesized by RiboBio (Guangzhou, China). Transfections of expression plasmids in GC cells were performed using the Lipofectamine 2000 kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer’s instructions. All sequences were presented in Table S1.

RNA isolation and real-time PCR

Total RNA was extracted from the BC tissue samples using RNeasy Mini Kit (QIAGEN, Hilden, Germany) was used according to the manufacturer’s protocol. Subsequently, isolated RNA was detected at the ratio of A260/A280 for quantitation, and then converted into cDNA using Promega reverse transcription kit. Quantitative real-time PCR was performed in the ABI7500 system using SYBR Green methods. The relative gene expression was measured and normalized to beta-actin by the 2−ΔΔct method. The primers used in the study were displayed in Supplement Table S1.
**RNA Sanger sequencing**

Total RNA was extracted using TRIZOL reagent according to the standard method. RNA Sanger sequencing was performed BGI Genomics Co., Ltd (Shenzhen, China).

**Cellular proliferation and apoptosis assay**

Cell proliferation was measured using CCK-8 assays. In brief, $1 \times 10^3$ cells were seeded in 96-well plates, and incubated with $10 \mu$L of CCK-8 assay solution (Dojindo, Tokyo, Japan) in each well. After the solution administration, the absorbance value was detected using enzyme immunoassay analyzer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 450 nm. Cellular apoptosis assay was detected using flow cytometry. In brief, cells were resuspended in Annexin-binding buffer (5 μL Annexin V-FITC, 1 μL PI) at room temperature for 15 min in the dark using APC Annexin V Apoptosis Detection Kit (BioLegend, San Diego, CA, USA). FITC and PI fluorescence were analyzed by flow cytometry (FACScan, BD Biosciences).

**Lactate production, glucose uptake, and ATP levels**

The glucose level was quantified by glucose assay kit (Sigma-Aldrich, St. Louis, MO, USA). The lactate level was detected using the Lactate Assay kit (BioVision, Mountain View, CA, USA). The ATP level was determined with CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, MI, USA).

**Western blotting analysis**

Total protein was extracted from using RIPA buffer added with proteinase and phosphatase inhibitor cocktail. Protein extraction (30 mg) was loaded to 10% SDS-PAGE and then transferred onto the polyvinylidene fluoride (PVDF) membranes. Primary antibodies were incubated with the membranes, including anti-HK2 (Abcam, ab209847, 1:1000), anti-HIF-1α (Abcam, ab51608, 1:1000). beta-actin was used as normalized control.

**Luciferase assay**

The sequences including miR-487a binding sites (wild-type, mutant) in the circRNF20 and HIF-1α 3'-UTR were subcloned into psiCHECK2 Dual-luciferase vector (Promega, Madison, WI, USA). The vectors were co-transfected with miR-487a mimics or control (NC) into 293T cells. After 48 h transfection, luciferase activities between the Firefly and Renilla (control) luciferase activities were measured using the Dual Light System following the instruction.

**RNA-fluorescence in situ hybridization (RNA-FISH)**

circRNF20 probes were designed and synthesized by GenePharma (Shanghai, China). The hybridization was performed overnight with probes using fluorescent in situ hybridization kit according to the manufacturer’s protocol (GenePharma, Shanghai, China). Fluorescence images were captured using confocal laser-scanning microscope (LSM 780, Zeiss, Germany).

**Chromatin immunoprecipitation (ChIP) assay**

ChIP Assay Kit (Millipore, Bedford, MA) was used according to manufacturer’s instructions. In brief, the cross-linked chromatin was sonicated into fragments and then immunoprecipitated using HIF-1α antibody. IG was used as negative control. DNA fraction was analyzed by qRT-PCR. The primers sequences for ChIP-PCR were listed in Table S1.

**In vivo tumorigenesis assay**

Male nude mice (4–5 weeks old) were provided by Slac Laboratory Animal Center (Shanghai, China) and maintained in microisolator cages. Mice were subcutaneously inoculated with $1 \times 10^7$ MDA-MB-231 cells (0.1 mL) that stably transfected with shRNA for circRNF20. The animal assay was conducted according to the institutional guidelines and approved by the Animal Ethics Committee of Zibo Central Hospital. The mice tumor size was measured every three days for volume calculated using the formula: length × width ²/2. The weight was measured after three weeks.

**Statistical analysis**

Statistical analysis was performed using SPSS software (SPSS, Chicago, Illinois, USA). Survival curves for BC patients were generated using the Kaplan-Meier method, and the difference was analyzed by log-rank test. The optimal cut-off value of the relative expression of circRNF20 in BC was determined by ROC curve analysis in Cutoff Finder (http://molpath.charite.de/cutoff/). Comparison between groups was calculated by Student’s $t$-test. Data were presented as mean ± standard deviation (SD) from three independent experiments. $P$-value less than 0.05 was considered statistically significant.

**Results**

circRNF20 is upregulated in the BC tissue and cells

To identify the potential dysregulated circRNAs in the BC, we performed circRNA expression profile in BC tissue and adjacent normal tissue. Heat map showed that there were hundreds of upregulated circRNAs and downregulated circRNAs (Fig. 1a, Fig. S1). Sanger sequencing illustrated the joint site of circRNF20. The joint site was converged by the 3′-end of exon 5 and 5′-end of exon 3 (Fig. 1b). Schematic diagram illustrated the formation of circRNF20 originated from RNF20 pre-mRNA (exon 3, 4, 5) (Fig. 1c). RT-PCR revealed that the expression of circRNF20 was upregulated in the BC cell lines (Fig. 1d). RNA stability
assay found that circular transcript circRNF20 was much more stable than the linear mRNA transcript in BC cells under treatment with transcription inhibitor (actinomycin D) (Fig. 1e). In the administration of RNase R or control, RT-PCR showed the expression of circRNF20 and RNF20 mRNA in MDA-MB-231 cells. Linear form of RNF20 was significantly decreased under the RNase R treatment, however circRNF20 resist to RNase R digestion (Fig. 1f). In the BC tissue specimens clinical analysis, circRNF20 expression was closely correlated with lymph node metastasis and tumor size (Table 1). RT-PCR showed that circRNF20 was over-expressed as comparing to the normal tissue (Fig. 1g). Prognosis analysis calculated by Kaplan-Meier and log-rank test unveiled that the higher circRNF20 expression indicated the lower survival rate of BC patients (Fig. 1h). These results suggest that circRNF20 is upregulated in the BC tissue and cells, and indicated the prognosis marker.

Fig. 1 circRNF20 is upregulated in the BC tissue and cells. a Heat map showed the upregulated circRNAs and downregulated circRNAs. b Sanger sequencing illustrated the joint site of circRNF20 3'-end of exon 5 and 5'-end of exon 3. c Schematic diagram illustrated the formation of circRNF20 originated from RNF20 pre-mRNA (exon 3, 4, 5). d RT-PCR revealed the expression of circRNF20 in the BC cell lines. e RNA stability assay found the expression of circRNF20 or linear mRNA transcript in MDA-MB-231 cells under treatment with transcription inhibitor (actinomycin D). f RT-PCR showed the expression of circRNF20 and RNF20 mRNA in MDA-MB-231 cells administered with RNase R or Mock control. g RT-PCR showed the circRNF20 level in the BC tissue. h Prognosis analysis calculated by Kaplan-Meier and log-rank test unveiled the survival rate of BC patients with higher or lower circRNF20 expression. **p < 0.01 vs. control.
Fig. 2 circRNF20 promotes the progress and glycolysis of BC. 

a Stable oligonucleotides transfections were constructed, including circRNF20 knockdown (sh-circRNF20) in MDA-MB-231 cells and circRNF20 overexpression (circRNF20 OE) in MCF-7 cells.

b CCK-8 proliferative assay showed the proliferation with the transfection of circRNF20 knockdown and circRNF20 overexpression.

c Flow cytometry apoptosis assay indicated the apoptotic rate of MDA-MB-231 and MCF-7 cells.

d Relative glucose uptake was detected in BC cells.

e Relative lactate production was detected in BC cells.

f Relative ATP level was detected in BC cells.

g, h In vivo mice heterograft assay unveiled the tumor growth in mice injection using MDA-MB-231 cells transfected with circRNF20 knockdown. **p < 0.01 vs. control. *p < 0.05 vs. control.
circRNF20 promotes the progress and glycolysis of BC

In order to investigate the biological roles of circRNF20, we constructed the circRNF20 knockdown (sh-circRNF20) in MDA-MB-231 cells and circRNF20 overexpression (circRNF20 OE) in MCF-7 cells (Fig. 2a). CCK-8 proliferative assay showed that circRNF20 knockdown repressed the proliferation and circRNF20 overexpression promoted it (Fig. 2b). Flow cytometry apoptosis assay indicated that circRNF20 knockdown increased the apoptosis and circRNF20 overexpression reduced it (Fig. 2c). Subsequently, the Warburg effect correlated analysis indicated that circRNF20 knockdown reduced the glucose uptake, lactate production and ATP level as comparing to the blank control (Fig. 2d–f). In vivo mice heterograft assay unveiled that circRNF20 knockdown (MDA-MB-231 cells) inhibited the tumor growth (Fig. 2g, h). These data illustrated that circRNF20 promotes the progress and glycolysis of BC.

circRNF20 acts as the sponge of miR-487a

The subcellular location of circRNF20 was found to be primarily distributed in the cytoplasm, more than nucleus (Fig. 3a). The cytoplasmic location of circRNF20 indicated the potential post-transcriptional regulation. Online bioinformatic prediction (CircInteractome, https://circinteractome.nia.nih.gov) and ENCORI (http://starbase.sysu.edu.cn/) indicated that miR-487a functioned as the target of circRNF20 with complementary binding sites. Luciferase reporter assay indicated that circRNF20 closely combined with miR-487a (Fig. 3b). RNA-Fluorescence In Situ Hybridization (RNA-FISH) unveiled that circRNF20 and miR-487a were both primarily located in the cytoplasm (Fig. S2). In BC cells (MDA-MB-231, MCF-7), RT-PCR illustrated that circRNF20 knockdown increased the miR-487a expression and circRNF20 overexpression reduced the miR-487a (Fig. 3c). Moreover, miR-487a level was found to be downregulated in the BC cell lines (Fig. 3d). In conclusion, these findings suggest that circRNF20 acts as the sponge of miR-487a.

circRNF20/miR-487a targets HIF-1α in BC cells

Online bioinformatic prediction (http://starbase.sysu.edu.cn/) inspired that miR-487a could also target the 3′-UTR of HIF-1α mRNA (Fig. 4a). Luciferase reporter assay unveiled that miR-487a combined with HIF-1α mRNA wild type at molecular level (Fig. 4b). In BC cells, RT-PCR showed that circRNF20 knockdown reduced the HIF-1α mRNA level, and the circRNF20 overexpression could enhance the HIF-1α mRNA (Fig. 4c). Moreover, miR-487a
overexpression decreased the HIF-1α level and miR-487a repression upregulated the HIF-1α level (Fig. 4d). Western blot analysis showed that circRNF20 overexpression rescued the protein generation (Fig. 4e). Correlation analysis by Spearman’s rank correlation coefficient showed that circRNF20 was positively correlated with the HIF-1α in the BC patients samples (Fig. 4f). These findings suggest that circRNF20/miR-487a targets HIF-1α in BC cells.

**HIF-1α facilitates the transcription of HK2**

Previous findings illustrated that circRNF20 regulated the Warburg effect, therefore, there could be a connection with the circRNF20/miR-487a/HIF-1α and glycolysis in BC. For the potential targets, we found that HIF-1α was positively correlated with the HK2 expression (Fig. 5a).

The bioinformatics analysis indicated that HIF-1α had the putative Hypoxia Response Elements (HRE) (CGTG) on the upstream promoter region of HK2 (Fig. 5b). Chro-matin immunoprecipitation (ChIP) results indicated that HIF-1α could bind with the site 1 of HK2 promoter (Fig. 5c). Luciferase reporter assay indicated that HIF-1α targeted the wild type of site 1, instead of mutant sequence (Fig. 5d). Western blot results suggested that enhanced HIF-1α expression could upregulated the HK2 protein (Fig. 5e). RT-PCR indicated that enhanced HIF-1α expression might increase the HK2 mRNA in BC cells (Fig. 5f). Overall, these data suggest that HIF-1α facilitates the transcription of HK2.

**Discussion**

The roles of circular RNA (circRNA) have been identified in the human cancer, including breast cancer (BC)\(^9\).
Multiple pathological factors participate in the tumor progression of BC, moreover, circRNA could regulate the series of tumor phenotype, including metastasis and Warburg effect. Warburg effect, also known as the aerobic glycolysis, provides the major energy for the BC tumor microenvironment. Warburg effect is characterized by a vital metabolic event involved in proliferation, invasion, and migration in BC.

CircRNA is a group of transcript generated by the back splicing without the 5’-end and 3’-end. In present study, we selected the novel circRNA using the circRNA microarray in the BC tissue samples. CircRNA circRNF20 is a novel circRNA (hsa_circ_0087784). CircRNF20 is a 499 bp transcript originated from RNF20 gene exon-3, exon-4, and exon-5. In clinical investigation, circRNF20 is highly expressed in the BC samples, and its overexpression is correlated with the poor prognosis of BC patients. In the cellular experiments, results indicated that circRNF20 could promote the proliferation and reduce the apoptosis of BC cells. Moreover, circRNF20 promote the glucose uptake, lactate production and ATP level in BC cells. In other words, circRNF20 may function as a promoting factor in the BC Warburg effect (Fig. 6).

More and more researches have illustrated the critical roles of circRNAs in the BC. For example, circRNA circ-ABCB10 is significantly upregulated in BC tissue and cells.
cancer Individualized diagnosis, treatment and transformation, Zibo Central Hospital, Zibo 255020, China. 4Department of Experimental Oncology, Medical College of Soochow University, Suzhou 215123, China

Author details
1Department of Oncology, Zibo Central Hospital, Zibo 255020, China. 2Department of Ultrasound, Zibo Central Hospital, Zibo 255020, China. 3Department of Breast and Thyroid Surgery, Zibo Key laboratory of Breast cancer Individualized diagnosis, treatment and transformation, Zibo Central Hospital, Zibo 255020, China. 4Department of Experimental Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima 734-8553, Japan. 5School of Radiation Medicine and Protection, Medical College of Soochow University, Suzhou 215123, China

Conflict of interest
The authors declare that they have no conflict of interest.

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References
1. Thompson, B. et al. Breast cancer disparities among women in underserved communities in the USA. Curr. Breast Cancer Rep. 10, 131–141 (2018).
2. Ashoor, A., Monti, S. & Pezzella, M. Fibromatosis, a benign breast disease mimicking carcinoma: a case report. Int. J. Surg. Case Rep. 41, 392–397 (2017).
3. Roumeliotis, G. A., Dostaler, G. & Boyd, K. U. Complementary and alternative medicines and patients with breast cancer: a case of mortality and systematic review of patterns of use in patients with breast cancer. Plast. Surg. 25, 279–283 (2017).
4. Galluzzi, L. et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ. 25, 486–541 (2018).
5. Marinko, T. Pericardial disease after breast cancer radiotherapy. Radiol. Oncol. 53, 1–5 (2018).
6. Gurnapu, S., Pupo, E., Franzolin, G., Langetti, L. & Tamagnone, L. Sema4C/ PlexinB2 signaling controls breast cancer cell growth, hormonal dependence and tumorigenic potential. Cell Death Differ. 25, 1259–1275 (2018).
7. Xie, Z., Zhang, Y., Jin, C. & Fu, D. Gemcitabine-based chemotherapy as a viable option for treatment of advanced breast cancer patients: a meta-analysis and literature review. Oncotarget 9, 7148–7161 (2018).
8. Wu, H., Ma, X., Li, Z. & Feng, H. Hibernoma in the clavicular fossa: a case report and literature review. Radiol. Case Rep. 13, 47–50 (2018)

Fig. 6: circRNF20/miR-487a/HIF-1α/HK2 axis promotes the glycolysis in BC cells.
9. Kowalczyk, L., Bartsch, R., Singer, C. F. & Farr, A. Adverse Events of Trastuzumab Emtansine (T-DM1) in the treatment of HER2-positive breast cancer patients. Breast Care 12, 401–408 (2017).
10. Holdt, L. M., Kohlmaier, A. & Teupser, D. Circular RNAs as therapeutic agents and targets. Front. Physiol. 9, 1262 (2018).
11. Chen, Y., Wei, S., Wang, X., Zhu, X. & Han, S. Progress in research on the role of circular RNAs in lung cancer. World J. Surgical Oncol. 16, 215 (2018).
12. Wu, J. et al. Emerging epigenetic regulation of circular RNAs in human cancer. Mol. Ther. Nucleic Acids 16, 589–596 (2019).
13. Liang, H. F., Zhang, X. Z., Liu, B. G., Jia, G. T. & Li, W. L. Circular RNA circ-ABC10 promotes breast cancer proliferation and progression through sponging miR-1271. Am. J. Cancer Res. 7, 1566–1576 (2017).
14. Fang, L. et al. Enhanced breast cancer progression by mutant p53 is inhibited by the circular RNA circ-Ccnb1. Cell Death Differ. 25, 2195–2208 (2018).
15. Wu, N. et al. Translation of yes-associated protein (YAP) was antagonized by its circular RNA via suppressing the assembly of the translation initiation machinery. Cell Death Differ. 26, 2758–2773 (2019).
16. Li, H. et al. Therapeutic targeting of circ-CUX1/EWSR1/MAZ axis inhibits glycolysis and neuroblastoma progression. EMBO Mol. Med. 11, e10835 (2019).
17. Li, Q. et al. Circular RNA MAT2B promotes glycolysis and malignancy of hepatocellular carcinoma through the miR-338-3p/PKMI axis under hypoxic stress. Hepatology 70, 1298–1316 (2019).
18. Dong, W. et al. The RNA-binding protein RBM3 promotes cell proliferation in hepatocellular carcinoma by regulating circular RNA SCD-circRNA 2 production. ElBioMedicine 45, 155–167 (2019).
19. Liang, Y. et al. Targeting the circBMMP2/miR-553/USP4 axis as a potent therapeutic approach for breast cancer. Mol. Ther. Nucleic Acids 17, 347–361 (2019).
20. Xu, F. et al. miR-885-5p negatively regulates Warburg effect by silencing hexokinase 2 in liver cancer. Mol. Ther. Nucleic Acids 18, 308–319 (2019).
21. Wu, Z., Wu, J., Zhao, Q., Fu, S. & Jin, J. Emerging roles of aerobic glycolysis in breast cancer. Clin. Transl. Oncol. 1–16 (2019). https://doi.org/10.1007/s12094-019-02187-8.
22. Tucker, J. D., Lu, P. J., Xiao, X. & Lu, Q. L. Overexpression of mutant FKRP restores functional glycosylation and improves dystrophic phenotype in FKRP mutant mice. Mol. Ther. Nucleic Acids 11, 216–227 (2018).
23. Sun, Q. et al. Phosphoglyceric acid mutase-1 contributes to oncogenic mTOR-mediated tumor growth and confers non-small cell lung cancer patients with poor prognosis. Cell Death Differ. 25, 1160–1173 (2018).
24. Liu et al. Germline BAP1 mutations induce a Warburg effect. Cell Death Differ. 24, 1694–1704 (2017).
25. Wu, J. et al. CircRAK3 sponges miR-3607 to facilitate breast cancer metastasis. Cancer Lett. 430, 179–192 (2018).
26. Zhao, W. et al. Splicing factor derived circular RNA circUHRF1 accelerates oral squamous cell carcinoma tumorigenesis via feedback loop. Cell Death Differ. (2019). https://doi.org/10.1038/s41418-019-0423-5.
27. Feng, Y. et al. Circular RNA circ0005276 promotes the proliferation and migration of prostate cancer cells by interacting with FUS to transcriptionally activate XIAP. Cell Death Dis. 10, 792 (2019).
28. Wang, Y. et al. Sox2 promotes hypoxia-induced breast cancer cell migration by inducing NEDD9 expression and subsequent activation of Rac1/HIF-1alpha signaling. Biol. Impacts 24, 55 (2019).
29. De Francesco, E. M. & Maggiolini, M. Crosstalk between Notch, HIF-1alpha and GPER in breast cancer EMT. Int. J. Mol. Sci. 19, pii: E2011, (2018).
30. Ren, S. et al. Knockdown of circDENND4C inhibits glycolysis, migration and invasion by up-regulating miR-200b/c in breast cancer under hypoxia. J. Exp. Clin. Cancer Res. 38, 388 (2019).