Long Non-Coding RNA BC040587 is Required in Cell Invasion of Renal Cancer Induced by Acidosis

Yang Zhou

Affiliated Hospital of Jiangsu University

Di Dong

Affiliated Hospital of Jiangsu University

Qin Yao

Affiliated Hospital of Jiangsu University

binghai chen (✉ chenbhny@163.com)

Affiliated Hospital of Jiangsu University <https://orcid.org/0000-0002-0913-860X>

Primary research

Keywords: BC040587, Linc00901, MDM2, Snail, LncRNA, cell invasion, renal cell carcinoma, AKT, ERK, CRISPR/Cpf1

Posted Date: September 2nd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-70355/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Abstract

Background: It is well known that aggressive growth and metastasis of tumors are strengthened in acidic environments of cancer. Whether Long non-coding RNA (LncRNA) is involved in this process is still unknown and has never been reported in renal cell cancer(RCC).

Methods: We determined the invasion ability of 786-O and 769P cells upon acidosis. Then LncRNA profiling was used to figure out the potential acidosis-related LncRNAs. We then knocked out the LncRNA (BC040587) to investigate its role in acidosis-induced cell invasion. AKT/mTOR ,MEK/ERK pathway as well as pMDM2 were analyzed to uncover the cell signaling induced by BC040587 upon acidosis. Clinical features were also carefully analyzed.

Results: Acidosis elevated the level of Snail and induced cell invasions in RCC. BC040587 was required in this process as the increased cell invasion could be abolished by knockout of BC040587. Acidosis activated AKT/mTOR and MEK/ERK pathway in a BC040587-dependent manner. MDM2 could be the downstream of BC040587, which implicated poorer prognosis in RCC based on the clinical features.

Conclusions: BC040587 is required in cell invasion of renal cancer induced by acidosis. The BC040587-AKT/ERK-pMDM2-Snail axis is of vital importance in the development of RCC and is likely to be potential therapeutic target in RCC.

1 Background

Increasing evidence has suggested accumulation of lactic acid due to the dysfunction of aerobic oxidation in cancer cells[1]. A large amount of carbonic acid is induced in cancer cells via over-expression of carbonic anhydrase. Thus, the acid in tumor cells makes the intracellular environment acidic[2, 3]. In order to maintain a relatively stable intracellular acidic environment, tumor cells transfer intracellular acidic substances to extracellular by means of sodium-hydrogen transporter, sodium-potassium atpase and monocarboxylic acid transporter, which results in an acidic extracellular micro-environment for the cancer cells[4]. Studies have shown that aggressive growth and metastasis of tumors are strengthened in acidic environments. For example, acidosis induced cell invasion and metastasis via ASIC(acid sensing ion-channel)[5]. Moreover, It can also induce activation of ROS/ERK through ASIC1 in our previous research[6], However, whether Long non-coding RNA(LncRNA) is involved in it remains to be elaborated.

LncRNAs account for the majority of non-coding RNAs, but the mechanism of numerous LncRNAs in cell biological processes has not been clearly understood[7]. LncRNA is abnormally expressed in renal carcinoma, and it is closely related to tumor stage, grade, local invasion, and metastasis of other organs[8]. It has been indicated in other research that LncRNA can regulate cell proliferation, trigger cell apoptosis, as well as reduce the invasion of cancer cells^[9]. BC040587 (also known as Linc00901) is one of the most important LncRNAs which have not been featured. BC040587, which locates in chromosome 3q13, harbors various copy number alterations, therefore it suggests BC040587 could be of vital

importance in oncogenesis[10, 11]. Moreover, dysregulation of BC040587 expression could be a critical step in sarcoma, suggesting an example in tumorigenesis[12]

MDM2/P53 negative feedback pathway is notable in cancer research, and it is mainly related to apoptosis as well as proliferation[13]. MDM2 is considered as a notable inhibitor of P53, and its combination with P53 can degrade P53 protein, leading to inactivity of P53[14]. MDM2 is frequently altered in solid tumors of stomach[15], lung[16] as well as breast[17]. Ubiquitination of MDM2 protein inhibits the expression of downstream key factors and therefore increases both cancer cell invasion and metastasis [18].

In the present study, we show that BC040587, a bio-marker of higher stage and poor prognosis in renal cell cancer (RCC), serves as a novel factor in acid-induced phosphorylation of MDM2, resulting in invasion of cancer.

2 Results

Acidosis induces cell invasions in renal cancer cells

We recently reported that acidosis induced elevated level of Snail ,leading to invasion of prostate cancer via ASIC1 in vitro[6]. Therefore, we supposed acidosis also promoted cell invasion in renal cancer cells. We then determine the invasion ability of 786-O and 769P cell lines upon acidosis. In one hour exposed to acid medium (pH 6.6) the average invasive 769P cells number was increased three times as much as that in cells of pH7.4(Fig. 1A). In addition, similar results were found in 786-O cells(Fig. 1B). Moreover, acidosis-induced increased level of Snail also provided extra evidence(Fig. 1C and D), which has been implicated in cell invasion. The level of Snail is significantly elevated in half an hour and got a peak at 1 hour. Of interest was 769P with over-expressed Snail at as early as 30 min.

BC040587 is required in acidosis-induced cell invasion

Long non-coding RNAs(LncRNAs) is of vital importance in cancer development including invasion and metastasis[8]. Thus, we focused on which LncRNAs could be implicated in cell invasion of renal cancer response to acidosis. As shown in supplemental table, Small scale LncRNA profiling which includes 90 crucial LncRNAs uncovered potential related LncRNAs to acidosis. For example, we found 40 down-regulated LncRNAs and 44 up-regulated LncRNAs (2 LncRNAs unavailable), among which BC040587 was increased significantly (Fig. 2A). To validate the up-regulated level of BC040587 upon acidosis in renal cancer cells, we then exposure the cells to acidosis. It is indicated that acidosis induced increased BC040587. In consistent with invasion assay, BC040587 got a peak at 1 hour in both 769P and 786-O cells. (Fig. 2B)

To figure out more about the role of BC040587 in cell invasion upon acidosis, we knocked out BC040587 by CRISPR/Cpf1 system (Fig. 2C).We then use the knockout clone (#17) for further characterization. We then asked whether BC040587 was required for cell invasion upon acidosis. Acidosis cannot induce

increased invasion ability after BC040587 had been knockout in 769P cells(Fig. 2D), while cell invasion was inductive in original 769P cells. Moreover, as expected in Fig. 2E, Snail over-expression was almost abolished 1 hour after exposure to acidosis, demonstrating that BC040587 is required in acidosis-induced cell invasion.

Acidosis induces the activation of AKT/mTOR and MEK/ERK pathway in a BC040587-dependent manner

It was acknowledged that acidosis induced phosphorylation of AKT in breast cancers[5]. We previously found that ERK activates upon acidosis when there was elevated AKT background[6]. Hence, we asked whether acidosis induce activation of AKT and MEK in renal cancer cells. As Snail level and cell count in invasion assay reached a peak at 60 min, we exposed the cells at the same duration and determined some critical pathways. As shown in Fig. 3A, acidosis phosphorylated P90RSK and MEK1/2. The same trend was also found as for the AKT(Fig. 3A and B). Moreover, phosphorylation of AKT 1 hour after exposure to acidosis was abolished after knockout of BC040587. Activated MEK directly connected to ERKs through its N-terminal region, catalyzing phosphorylation of ERK and activating ERK. Therefore, we determined the activation of ERK in 769P and 769P^{BC040587(-)}. As indicated in Fig. 3C, we found ERK activation in 769P cells in response to acidosis. Of interest is the 769P^{BC040587(-)} cells, in which ERK inactivation upon acidosis were confirmed. This data suggests that BC040587 is of vital importance in activation of MEK/ERK pathway. On the other hand, acidosis can also phosphorylate the crucial factor mTOR in the AKT pathway. The role of BC040587 in activation of AKT/mTOR pathway is as some as that in MEK/ERK pathway (Fig. 3D), which suggests the BC040587-dependent manner in the both pathways in response to acidosis. However, the activation of AKT is earlier than that of ERK, which might explain the potential crosstalk between the two pathways.

MDM2 is involved in the BC040587-required cell invasion upon acidosis

Given BC040587 is essential in cell invasion upon acidosis; we asked what was the mechanism and what might be regulated by BC040587? We took advantage of the database cBioPortal[19] to search for further upstream or downstream signaling molecules. Data of 538 RCC cases from cBioPortal indicated that BC040587 was closely related to those genes including CDKN2A, MDM2, CDK4, TP53, RB1, and CCNE1(Fig. 4A). Among them, MDM2 was the most attractive one. MDM2 forms a complex with oligomeric p53, HERC2, and NEURL4[14]. Activation of either AKT or ERK can induce both phosphorylation of MDM2 and enhancement of the fundamental interaction between MDM2 and p53[20]. Of interest, MDM2 phosphorylation was induced by acidosis and was abolished by BC040587 knockout(Fig. 4B). To study the feature of MDM2 in cell invasion upon acidosis, we then knocked down MDM2 with siRNA(Fig. 4C). Acidosis-induced over-expression of MDM2 was abolished by siRNA-MDM2(Fig. 4C). Additionally, average invasive 769P cells number were not increased as long as we knocked down MDM2(Fig. 4D). The same trend was also found as for expression of Snail, which is considered as a bio-marker of cell invasion (Fig. 4E).

BC040587 indicated poorer prognosis in RCC

As BC040587 has been shown to be required in the cell invasion of RCC, we then studied the role of BC040587 in clinical cases. In consistent with findings in vitro, as expected in Fig. 5A, the level of BC040587 was elevated in stage III/IV compared with that in stage I/II (Fig. 5A). This data indicated that over-expressed BC040587 led to invasion and metastasis, which was very common in higher stage. In addition, patients were divided into two categories based on the level of BC040587. As shown in Fig. 5B, significant differences between the two groups were found. Patients with over-expressed BC040587 had poorer prognosis when overall survival was compared, which suggests BC040587 might be novel bio-marker of prognosis in RCC.

3 Discussion

The acidic environment of the tumor can induce increased cell survival and invasion. For example, ASIC1-ROS-AKT-NF- κ B pathway was shown to be crucial in invasion as well as metastasis of breast cancer[5]. We previously found that ERK was alternatively activated under high AKT background upon acidosis[6]. Whether LncRNA is involved in this process is still unknown. In this study, we offered novel insights of acidosis as well as BC040587, which is a downstream factor of acidosis and the first LncRNA reported in RCC.

In comparison with adjacent normal breast tissue, BC040587 expression significantly decreased in breast cancer [11]. What's more, Lower level of BC040587 was believed to be an bio-marker in aspect of prognosis [11]. However, we found that BC040587 is required in acidosis-induced cell invasion(Fig. 2). Acidosis can no longer induce cell invasion after BC040587 had been knockout(Fig. 1A and 2D). Moreover, higher BC040587 suggested poorer prognosis in RCC when referring to cancer stage and overall survival(OS).

It is very common that the function of genes varies from tumors to tumors. For example, miRNA serve as either an oncogene or a suppressor gene in cancer. miR-223, a suppressor in human osteosarcoma cells, inhibited the level of HSP90B1, contributing to cell apoptosis and cell survival inhibition[21]. On the contrary, miR-223 can also be an oncogene, increasing invasiveness via MEF2C[22]. Similarly, it is reasonable that the role of BC040587 in RCC differs from that in other tumors. The reasons for this phenomenon might be as follows, 1) an important mechanism of LncRNA is well known as ceRNA, in which LncRNA serve to combine miRNA; and miRNA targets various genes, which might be oncogenic or tumor suppressive. 2) BC040587 is involved in different pathway in various cancers. In the current study, acidosis induced the activation of AKT/mTOR and MEK/ERK pathway in BC040587-dependent manner. 3) In consistent of miRNA, some LncRNAs might be an oncogene or a tumor suppressor themselves. 4) The diversity might be based on different genetic and epigenetic background as well as expression signatures.

Of interest, what we have found provide more evidence that acidosis induces AKT/mTOR and MEK/ERK pathway. These pathways are of vital importance in cell invasion. For instance, AKT/mTOR signaling activation causes cell survival of glioma and metastasis [23]. Fisetin suppresses the metastasis of RCC

via MEK-ERK pathway[24]. Positive and negative cross talk was found between AKT/mTOR and MEK/ERK. Activation of Akt inhibited ER-mediated ERK phosphorylation[25]. In the present study, the cross talk between the two pathways is very likely to occur in acidosis-induced cell invasion in RCC. In spite of this, the potential mechanism needs further investigation and full elaboration.

ERK phosphorylated Mdm2 at Ser166 in liver cancer[26], while activation of the PI3K-AKT pathway phosphorylates of MDM2[27]. Likely, Activation of ERK and AKT leads to phosphorylation of MDM2 in RCC(Fig. 4B). In the present study, acidosis also induces activation of MDM2(Fig. 4C), what's more, MDM2 was not phosphorylated after activation of AKT and ERK were blocked by inhibition of BC040587, suggesting additional evidence of ERK/pMDM2 and AKT/pMDM2 axis. Moreover, activation of MDM2 elevated the level of Snail, which is critical in cell invasion. Activation of MDM2 was reported to increase Snail level[28]; in consistent with that, inhibition of MDM2 by siRNA significantly down-regulated Snail, resulting in decrease of cell invasion. Thus, these data suggests that BC040587/MDM2/Snail is crucial in the cell invasion in response to acidosis.

The clinical data further uncovered the significant role of BC040584 in RCC. Elevated level of BC040587 indicates advanced RCC and decreased OS. In consistent with the clinical results, over-expression of BC040587 could be the main reason of invasion and metastasis, accelerating the development of RCC. Thus, BC040587 might be an important target in therapy of RCC. However, the underlying mechanism of how BC040587 regulates ERK/MDM2 and AKT/MDM2 remains to be further investigated. Also, the clinical value of BC040587 could be more convincing by including more cases of RCC in various centers.

4 Conclusions

We believe that BC040587 is a novel LncRNA involved in acidosis-mediated cell invasion (Fig. 6). Acidic tumor micro-environment can induce over-expression of BC040587 which activates AKT-mTOR and MEK-ERK pathway, indicating that BC040587 sits ahead of the pathways (Fig. 6). Thus, the activation of both AKT and ERK can boost MDM2 phosphorylation in various ways, leading to increased Snail. As a cell invasion promoter, Snail can result in cell invasion and metastasis. The BC040587-AKT/ERK-pMDM2-Snail axis facilitates understanding of development and is also likely to be potential therapeutic target in RCC.

5 Materials And Methods

5.1 Reagents

Primary antibodies including pERK(Thr202/Tyr204), pAKT(Ser473), p-P90RSK(Ser380), p-MEK1/2(Ser221), mTOR, p-MDM2(Ser166), GAPDH, alpha-Tubulin, were from Cell Signaling (Danvers, MA, USA). Real time PCR primers were from Sangon (Shanghai, China). siRNAs were from GenePharma (Shanghai, China). Renal cancer cell lines 786-O and 769P, and HEK293T were obtained from ATCC (Manassas, VA, USA).

The medium was adjusted as PH6.6 as before[6].

5.2 BC040587 Knockout

Knockout plasmids were constructed based on CRISPR/Cpf1[29], targeting BC040587. Vectors without specific gRNAs were used as control. Lenti-Cpf1/BC040587 was infected before the cells were seeded. After screening with puromycin, the cell clones were picked and the level of BC040587 was validated by qPCR.

5.3 Invasion assay

Cells were cultured with acid medium for 0 h, 0.5 h and 1 h before starved for 2 hours. 2×10^4 RCC cells in serum-free medium were added to the up chambers, before being put into complete medium. After the cells in the upper chamber were wiped away, the other RCC cells were stained. Five random fields were photographed and the cells were counted.

5.4 Real time quantitative PCR

The concentration of extracted RNA was detected by spectrophotometer. Then RNA was reverse transcribed into cDNA, which was used for quantitative PCR on a Roche LightCycler® 480 Instrument II (Roche Applied Science, Mannheim, Germany). The Primers were in supplemental table.1. GAPDH was used as an internal control. Delta delta Ct method was used as previously[6].

5.5 Western blot

The whole cellular protein of harvested cells were extracted. The protein was transferred with PVDF membrane after SDS-PAGE. Western blot analysis was done as before[6].

5.6 Database analysis

BC040587 was input into cBioPortal (<http://www.cbioportal.org>)[19]. We used the dataset of kidney renal clear cell carcinoma (TCGA, Firehose Legacy), as it has the most cases among all datasets. MRNA expression z-scores relative to all samples was investigated in genomic profiles. Z-scores threshold was set as ± 2.0 .

5.7 Clinical feature characterization

59 cases of RCC cancer patients were included in this study. Data including patient cancer type, stage at diagnosis, and cancer therapy, were collected. Clinical outcomes including recurrence and overall survival were calculated.

5.8 Statistical analysis

T-test or one way analysis of variance with hoc Tukey test was used to make a comparison of differences between groups. The Welch's correction of t-test was used regarding to unequal variances. Two side of $P < 0.05$ was considered as statistically significant.

Abbreviations

RCC renal cell carcinoma

OS overall survival

MDM2 Murine Double Minute 2

Declarations

Ethics approval and consent to participate

This study was approved by the Afliated Hospital of Jiangsu University Ethics Committee.

Consent for publication

Consent for publications is all agreed on by all the patients included in this study (consent form available).

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

FUNDING:

This study was funded by the National Natural Science Foundation of China (Grant No. 81402100).

Authors' contributions

Yang Zhou and Binghai Chen designed the online analysis and experiments in vitro. Yang Zhou, Di Dong and Qin Yao finished the experiments and manuscript writing. Binghai Chen reviewed the manuscript and made modifications. All authors reviewed the manuscript before submission.

Acknowledgments

We would like to appreciate Prof. Mo from UMMC providing support of small scale LncRNA profiling.

References

1. Hong SM, Lee YK, Park I, Kwon SM, Min S, Yoon G. Lactic acidosis caused by repressed lactate dehydrogenase subunit B expression down-regulates mitochondrial oxidative phosphorylation via the pyruvate dehydrogenase (PDH)-PDH kinase axis. *J Biol Chem.* 2019;294:7810–20.
2. Lu J, Tan M, Cai Q. The Warburg effect in tumor progression: mitochondrial oxidative metabolism as an anti-metastasis mechanism. *Cancer Lett.* 2015;356:156–64.
3. McDonald PC, Winum JY, Supuran CT, Dedhar S. Recent developments in targeting carbonic anhydrase IX for cancer therapeutics. *Oncotarget.* 2012;3:84–97.
4. Kato Y, Ozawa S, Miyamoto C, Maehata Y, Suzuki A, Maeda T, Baba Y. Acidic extracellular microenvironment and cancer. *Cancer Cell Int.* 2013;13:89.
5. Gupta SC, Singh R, Asters M, Liu J, Zhang X, Pabbidi MR, Watabe K, Mo YY. Regulation of breast tumorigenesis through acid sensors. *Oncogene.* 2016;35:4102–11.
6. Chen B, Liu J, Ho TT, Ding X, Mo YY. ERK-mediated NF-kappaB activation through ASIC1 in response to acidosis. *Oncogenesis.* 2016;5:e279.
7. Rajagopal T, Talluri S, Akshaya RL, Dunna NR. HOTAIR LncRNA: A novel oncogenic propellant in human cancer. *Clin Chim Acta.* 2020;503:1–18.
8. Chen B, Wang C, Zhang J, Zhou Y, Hu W, Guo T. New insights into long noncoding RNAs and pseudogenes in prognosis of renal cell carcinoma. *Cancer Cell Int.* 2018;18:157.
9. Peng F, Shi X, Meng Y, Dong B, Xu G, Hou T, Shi Y, Liu T. Long non-coding RNA HOTTIP is upregulated in renal cell carcinoma and regulates cell growth and apoptosis by epigenetically silencing of LATS2. *Biomed Pharmacother.* 2018;105:1133–40.
10. Xie J, Lin D, Lee DH, Akunowicz J, Hansen M, Miller C, Sanada M, Kato M, Akagi T, Kawamata N, Ogawa S, Koeffler HP. Copy number analysis identifies tumor suppressive lncRNAs in human osteosarcoma. *Int J Oncol.* 2017;50:863–72.
11. Chi Y, Huang S, Yuan L, Liu M, Huang N, Zhou S, Zhou B, Wu J. Role of BC040587 as a predictor of poor outcome in breast cancer. *Cancer Cell Int.* 2014;14:123.
12. Pasic I, Shlien A, Durbin AD, Stavropoulos DJ, Baskin B, Ray PN, Novokmet A, Malkin D. Recurrent focal copy-number changes and loss of heterozygosity implicate two noncoding RNAs and one tumor suppressor gene at chromosome 3q13.31 in osteosarcoma. *Cancer Res.* 2010;70:160–71.
13. Li H, Liu Q, Wang Z, Fang R, Shen Y, Cai X, Gao Y, Li Y, Zhang X, Ye L. The oncoprotein HBXIP modulates the feedback loop of MDM2/p53 to enhance the growth of breast cancer. *J Biol Chem.* 2015;290:22649–61.
14. Garcia-Cano J, Sanchez-Tena S, Sala-Gaston J, Figueras A, Vinals F, Bartrons R, Ventura F, Rosa JL. Regulation of the MDM2-p53 pathway by the ubiquitin ligase HERC2. *Mol Oncol.* 2020;14:69–86.
15. Aboushousha T, Helal N, Hammam O, Ibrahim M, Khaled S, Mostafa A, Anas A. Overview of MDM2 and B-RAF Expression in Gastric Lesions. *Open Access Maced J Med Sci.* 2018;6:1795–802.
16. Wang B, Liu X, Liu H, Guo J, Zhang T, Zhou N, Ma Y, Yu H, Chen L, Ren Z, Fan K, Tian X. Differential expressions of MDM2 and TAP73 in cancer and cancer-adjacent tissues in patients with non-small-

cell lung carcinoma. *Pulmonology*. 2018.

17. Loo LWM, Gao C, Shvetsov YB, Okoro DR, Hernandez BY, Bargonetti J. MDM2, MDM2-C, and mutant p53 expression influence breast cancer survival in a multiethnic population. *Breast Cancer Res Treat*. 2019;174:257–69.
18. Ning Y, Hui N, Qing B, Zhuo Y, Sun W, Du Y, Liu S, Liu K, Zhou J. ZCCHC10 suppresses lung cancer progression and cisplatin resistance by attenuating MDM2-mediated p53 ubiquitination and degradation. *Cell Death Dis*. 2019;10:414.
19. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2:401–4.
20. Li H, Wang Z, Jiang M, Fang RP, Shi H, Shen Y, Cai XL, Liu Q, Ye K, Fan SJ, Zhang WY, Ye LH. The oncoprotein HBXIP promotes human breast cancer growth through down-regulating p53 via miR-18b/MDM2 and pAKT/MDM2 pathways. *Acta Pharmacol Sin*. 2018;39:1787–96.
21. Li G, Cai M, Fu D, Chen K, Sun M, Cai Z, Cheng B. Heat shock protein 90B1 plays an oncogenic role and is a target of microRNA-223 in human osteosarcoma. *Cell Physiol Biochem*. 2012;30:1481–90.
22. Yang M, Chen J, Su F, Yu B, Su F, Lin L, Liu Y, Huang JD, Song E. Microvesicles secreted by macrophages shuttle invasion-potentiating microRNAs into breast cancer cells. *Mol Cancer*. 2011;10:117.
23. Zhang T, Ji D, Wang P, Liang D, Jin L, Shi H, Liu X, Meng Q, Yu R, Gao S. The atypical protein kinase R1OK3 contributes to glioma cell proliferation/survival, migration/invasion and the AKT/mTOR signaling pathway. *Cancer Lett*. 2018;415:151–63.
24. Hsieh MH, Tsai JP, Yang SF, Chiou HL, Lin CL, Hsieh YH, Chang HR. Fisetin Suppresses the Proliferation and Metastasis of Renal Cell Carcinoma through Upregulation of MEK/ERK-Targeting CTSS and ADAM9. *Cells*. 2019; 8.
25. Dai R, Chen R, Li H. Cross-talk between PI3K/Akt and MEK/ERK pathways mediates endoplasmic reticulum stress-induced cell cycle progression and cell death in human hepatocellular carcinoma cells. *Int J Oncol*. 2009;34:1749–57.
26. Malmlof M, Roudier E, Hogberg J, Stenius U. MEK-ERK-mediated phosphorylation of Mdm2 at Ser-166 in hepatocytes. Mdm2 is activated in response to inhibited Akt signaling. *J Biol Chem*. 2007;282:2288–96.
27. Zou Y, Lei W, Su S, Bu J, Zhu S, Huang Q, Li Z. Chlamydia trachomatis plasmid-encoded protein Pgp3 inhibits apoptosis via the PI3K-AKT-mediated MDM2-p53 axis. *Mol Cell Biochem*. 2019;452:167–76.
28. Lu X, Yan C, Huang Y, Shi D, Fu Z, Qiu J, Yin Y. Mouse double minute 2 (MDM2) upregulates Snail expression and induces epithelial-to-mesenchymal transition in breast cancer cells in vitro and in vivo. *Oncotarget*. 2016;7:37177–91.
29. Zetsche B, Heidenreich M, Mohanraju P, Fedorova I, Kneppers J, DeGennaro EM, Winblad N, Choudhury SR, Abudayyeh OO, Gootenberg JS, Wu WY, Scott DA, Severinov K, et al. Multiplex gene editing by CRISPR-Cpf1 using a single crRNA array. *Nat Biotechnol*. 2017;35:31–4.

Figures

Figure 1

Acidosis induces invasion of renal cancer cells. The number of invasive 769P cells(Fig.1 A) and 786-O cells(Fig.1 B) was increased significantly after exposed to acid medium at 0.5h and 1h. Acidosis induced increased level of Snail in 769P cells(Fig.1 C) and 786-O cells(Fig.1 D).

Figure 2

BC040587 is required in acidosis-induced cell invasion. A) Small scale LncRNA profiling uncovered potential related LncRNAs to acidosis. B) BC040587 increased and got a peak at 1 hour in both 769P and 786-O cells. C) BC040587 was knocked out by CRISPR/Cpf1 system. D) Acidosis cannot induce increased invasion ability after BC040587 had been knockout in 769P cells. E) Snail over-expression 1 hour after exposure to acidosis was abolished after knockout of BC040587.

Figure 3

Acidosis induces the activation of PI3K/AKT and MEK/ERK pathway in a BC040587-dependent manner. A) acidosis phosphorylated P90RSK MEK1/2 and pAKT. B) Phosphorylation of AKT 1 hour after exposure to acidosis was abolished after knockout of BC040587. C) ERK activates in 769P cells in response to acidosis and knockout of BC040587 leads to inactivation of ERK. D) The role of BC040587 in activation of AKT/mTOR pathway is as some as that in MEK/ERK pathway.

Figure 4

MDM2 is involved in the BC040587-required cell invasion upon acidosis. A) BC040587 was closely related to some genes including CDKN2A, MDM2, CDK4, TP53, RB1, and CCNE1. B) MDM2 phosphorylation was induced by acidosis and was abolished by BC040587 knockout. C) Acidosis-induced over-expression of MDM2 was abolished by siRNA-MDM2. D) Cell invasion was not increased after knockout of MDM2 compared with that before knockout. E) Snail over-expression in response to acidosis was abolished after knockdown of MDM2.

Figure 5

BC040587 indicated poorer prognosis in RCC. A) The level of BC040587 was elevated in stage III/IV compared with that in stage I/II. B) Patients with over-expressed BC040587 had poorer prognosis when overall survival was compared.

Figure 6

BC040587 is a novel LncRNA involved in acidosis-mediated cell invasion. Acidic tumor micro-environment can induce over-expression of BC040587 which activates AKT-mTOR and MEK-ERK pathway, indicating that BC040587 sits ahead of the pathways.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplementaltablesmallscaleLncRNAprofiling.xlsx](#)