ASIC1a Stimulates the Resistance of Human Hepatocellular Carcinoma by Promoting Epithelial-mesenchymal Transition via the AKT/GSK3 β /Snail Signaling Pathway

Yinci Zhang

Anhui University of Science and Technology

Niandie Cao

Anhui University of Science and Technology

Jiafeng Gao

Anhui University of Science and Technology

Jiaojiao Liang

Anhui University of Science and Technology

Yong Liang

Anhui University of Science and Technology

Yinghai Xie

Anhui University of Science and Technology

Shuping Zhou

Anhui University of Science and Technology

Xiaolong Tang (✉ xltang2006@aust.edu)

Anhui University of Science and Technology <https://orcid.org/0000-0002-7973-5354>

Primary research

Keywords: HCC, ASIC1a, EMT, AKT/GSK-3 β /Snail, migration, invasion, chemosensitivity

Posted Date: August 24th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-776349/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: The main obstacle to the cure of hepatocellular carcinoma (HCC) is multidrug resistance. Acid sensing ion channel 1a (ASIC1a) acts as a critical roles in all stages of cancer progression, especially invasion and metastasis as well as in resistance to therapy. Epithelial to mesenchymal transition (EMT) is a phenomenon in which epithelial cells transform into mesenchymal cells after being stimulated by extracellular factors and is closely related to tumor infiltration and resistance.

Methods: Western blotting assay, Immunofluorescence (IF) staining, Immunohistochemistry (IHC) staining, MTT and colony formation assay and scratch healing assay were used to detect the level of ASIC1a and the cell proliferation, migration and invasion capabilities in this research.

Results: In this research, we found that the protein of ASIC1a is overexpressed in HCC cancer tissues. In addition, we identified that the levels of ASIC1a are highly expressed in resistant HCC cells. Compared with the parental cells, EMT occurred more frequently in drug-resistant cells. Functional studies demonstrated that inactivation of ASIC1a restrained cell migration and invasion and enhanced the chemosensitivity of cells through EMT. In HCC cells, the overexpression of ASIC1a stimulates the up-regulation of EMT characterization molecular level and proliferation, migration and invasion capabilities and further induces drug resistance, while knocking down ASIC1a with short hairpin RNA (shRNA) has the opposite effect. Further investigations found that ASIC1a increased cell migration and invasion through EMT by regulating α and β -catenin, vimentin and fibronectin expression via AKT/GSK-3 β /Snail pathway.

Conclusions: Our study demonstrated that ASIC1a acts an important assignment in drug resistance of HCC through EMT via AKT/GSK-3 β /Snail pathway, thereby lending a latent therapeutic objective and new ideas regarding to HCC.

Intruductoin

Hepatocellular carcinoma (HCC) is the sixth extremely widespread cancer,^{1,2} and a fatal malignant tumor with a high recurrence rate and chemoresistance.³ Chemotherapy is one of the primary treatment method for HCC, but the effectiveness of its treatment is severely reduced due to drug resistance.⁴ At present, although the many molecular mechanisms underlying the drug resistance of HCC have been proposed, the problem of drug resistance of chemotherapeutic treatments still exists. Therefore, continuing to explore the regulation mechanism of chemotherapy resistance is still the key to improving the effect of the treatment of HCC.

The induction and maintenance of abnormal extracellular acidic microenvironment is a key link in the formation and progression of malignant tumors.⁵ The acidic microenvironment of malignant tumor tissues is mainly caused by glycolysis and hypoxia of tumor cells. Active glycolysis produces a large amount of acidic products, forming an acidic microenvironment that is more toxic to adjacent normal tissue cells. The hypoxia caused by the high metabolic growth of tumor cells can activate hypoxia-

inducible factor 1 (HIF-1) to make tumor cells adapt to the acid environment outside the tumor, which in turn facilitates tumor tissues invading adjacent normal tissues.^{6,7} Therefore, the external acidic environment of tumor tissue accelerates tumor metastasis and malignant development, while tumor metastasis is the initiating factor in tumor resistance.⁸ Other studies have shown that the extracellular acid environment can reduce the apoptotic potential, change the genetic properties, and increase the activity of multidrug transporters to make tumor cells resistant to drug resistance, thereby evading the damage of chemotherapy drugs.^{9,10} In addition, studies have shown that the extracellular acid environment can increase the expression of interleukin 8 (IL-8), vascular endothelial growth factor (VEGF), cathepsin B (CTSB), matrix metalloproteinase 2 (MMP2) and matrix metalloproteinase 9 (MMP9).^{11,12} In short, the extracellular low-acid environment contributes to chemotherapy resistance, but the molecular mechanism is still unclear.

Acid-sensitive ion channels (ASICs) are widely present in various neurons and non-neuronal tissues. At present, it has been identified that ASICs have six subunits 1a, 1b, 2a, 2b, 3 and 4, and can mediate the influx of Ca^{2+} .^{13,14} As proton-gated channels, they are related to many pathophysiological conditions regulated by pH, which indicates that ASICs have important physiological and pathophysiological significance.^{15,16} Due to high glucose metabolism and poor perfusion in tumor patients, the tumor microenvironment is low acidity.¹⁷ Therefore, ASIC1a, as the most concerned acid-sensitive ion channel molecule, is considered to be related to cancer and has been proved to be touched upon to the proliferation and migration of a variety of cells.^{18,19} In addition, study have found that ASIC1a stimulates the drug resistance of HCC via the Ca^{2+} /PI3K/AKT pathway.²⁰ However, the way in which ASIC1a participates in HCC resistance and the downstream signaling pathway of AKT have not been studied in depth.

Epithelial-mesenchymal transition (EMT) is induced by the tumor microenvironment. It is one of the most important mechanisms for initiating and promoting tumor cell metastasis, and plays an important role in chemotherapy resistance. Recent evidence shows that the acidic microenvironment can promote the epithelial-mesenchymal transition of lung cancer and melanoma cells to promote tumor progression and metastasis.^{21,22} In this study, we explore whether the acidic microenvironment can promote the activation of EMT and further participate in the occurrence of drug resistance in HCC.

It is well known that the AKT pathway can mediate tumor cells to escape apoptosis, thereby inducing drug resistance.²³ AKT/NF-KB/snail pathway can induce EMT progression of tumor cells,^{24,25} of which snail can promote the degradation of basement membrane by activating the expression of matrix metalloproteinase (MMP), accordingly pronouncedly improving the invasion ability of tumor cells.^{26,27} In HCC cells, whether EMT involved in drug resistance via AKT/NF-KB/snail pathway is still unclear.

In this research, we inspected the degree of activation of ASIC1a in the extracellular low-acid microenvironment and its role in tumor resistance. We proved that ASIC1a mediate drug resistance of HCC by promoting EMT via AKT/NF-KB/Snail pathway, revealing a new mechanism of tumor drug

resistance, and providing a theoretical and research basis for developing more effective chemotherapy drugs for HCC.

Materials And Methods

Patient tissue collection

This study was approved by the Human Research Ethics Committee of the First Affiliated Hospital of Anhui University of Science and Technology (China) and have been conducted in accordance with Declaration of Helsinki, and the informed consent was obtained from each subject.

Cell source and culture

The HepG2 (H) and Bel7402 (B) cell lines were obtained from Mingjin Biotechnology Co., Ltd. (Shanghai, China). The oxaliplatin (OXA)-resistant HepG2 cell line (H^R) that can stably grow in 20 μ M oxaliplatin and the 5-fluorouracil (5FU)-resistant Bel7402 cell line (B^R) that can stably grow in 2 mM 5-fluorouracil were successfully induced by the concentration gradient method. Cells were cultured in RPMI-1640 containing 10% fetal bovine serum (Sijiqing Bioengineering Materials, Hangzhou, China), and incubated in 5% CO_2 at 37 °C. H^R and B^R cells were cultured in pH 6.5 and pH 7.4.

Reagents and chemicals

PcTx1 (a potent and selective ASIC1 α blocker Psalmotoxin 1) and GN25 (a novel inhibitor of Snail-p53 binding) were procured from ApexBio Technology (Houston, USA). MK-2206 (a specific inhibitor of AKT) and TWS119 (a specific inhibitor of GSK3 β) were obtained from Selleck Chemicals (Houston, TX, USA). C19 (a inhibitor of EMT) was got from Shanghai Dongcang Biological Technology Co., Ltd. (Shanghai, China). OXA and 5FU were obtained from Sigma-Aldrich (St. Louis, USA). These reagents and chemicals were dissolved in DMSO at -80 °C until use and then diluted to a suitable working concentration with Roswell Park Memorial Institute (RPMI) 1640 medium.

Western blotting assay

Cells were collected and lysed by strong RIPA lysate emboding protease inhibitors (1: 50 dilution, Bioss Biotechnology Company, Beijing, China). The BCA-200 was utilized to determine the concentration of protein in the lysate. The membranes were sealed with 5% skim milk for 1 h, and then incubated with anti-ASIC1 α (1: 200, Alomone Labs, Jerusalem, Israel), anti-AKT, Phospho-AKT, GSK3 β , Phospho-GSK3 β , Snail, β -actin (1: 1000, Cell Signaling Technology, Danvers, MA, USA), anti-MMP2, MMP9, α -catenin, β -catenin, E-cadherin, vimentin, fibronectin (1: 500, Abcam, Cambridge, UK) antibodies overnight at 4 °C. Then, the membranes were incubated in the TBST buffer solution containing the secondary antibody (1: 4000) at room temperature for 1 h. Finally, compare the result displayed by the gel imaging system with the percentage of the control signal to correct the difference between the imprints.

Immunofluorescence (IF) staining

The anti-ASIC1a (1: 200) antibody was applied in IF according to our previously described method.³⁷ Alexa Fluor 488-conjugated goat anti-rabbit IgG (H+L) (1: 1000, Abcam, Cambridge, UK) was used as fluorescent secondary antibodies. For nuclear staining, cells were incubated with DAPI for 10 min.

Immunohistochemistry (IHC) staining

The cells with a density of 3×10^5 /mL were inoculated on a 24-well plate containing slides. The endogenous peroxidase was inactivated with 3% H₂O₂ at 37 °C for 10 min. The cells were fixed with absolute ethanol and blocked with 5% BSA at 37 °C for 10 min. Then, anti-ASIC1a (1: 200) was added and incubated overnight at 4 °C. Subsequently, the biotin-labeled secondary antibody was dropwised and incubated at 37 °C for 30 min. secondly, the horseradish peroxidase-labeled streptomycin avidin working solution was dripped and incubated at 37 °C for 30min. Finally, the color was developed with DAB and washed thoroughly with tap water, and counterstained with hematoxylin.

MTT and colony formation assay

After drug treatment of each group for various times and concentrations, cell viability was measured with MTT and colony formation assay which performed according to our previously described method.²⁸ Cell cloning ability is expressed as a percentage of total cells forming a clonal mass.

Scratch healing assay

The cells with a density of 1.2×10^6 /mL were inoculated on a 12-well plate. A gap between cells was created by scraping the bottom of each well using a 20 µL sterile pipette tip. At the predetermined intervals (0 and 48 h), the gaps between cells in wells were quantitatively analyzed by the ImageJ Software to measuring the scratch length.

Transwell invasion assay

BD matrigel (Solarbio, Shanghai) was putted at 4 °C to become liquid and added to the serum-free medium at a ratio of 1: 5 and mixed (operate at 4 °C, preferably on an ice bath). Subsequently, 100 µL of the mixed solution was supplemented to the upper chamber and placed in the 37 °C incubator for 5 h. Then, the cells were digested, counted, and prepared for cell suspension. Secondly, proceed in accordance with our previously described method.⁴⁹

Silencing of ASIC1a

The ASIC1a-specific shRNA lentiviral particles (Genechem, China) was transfected into cells as flowing the manufacturer's protocol. The shRNAs for gene silencing are listed below.

ASIC1a shRNA1: CCGGCTATGGAAAGTGCTACACGTTC

TCGAGAACGTGTAGCACTTTCCATAGTTTTT;

ASIC1a shRNA2: CCGGCGAGGTCATTAAGCACAAGCTC

TCGAGAGCTTGTGCTTAATGACCTCGTTTTT;

ASIC1a shRNA3: CCGGGCTGTAGGCTACATCCTGATACT

CGAGTATCAGGATGTAGCCTACAGCTTTTTT.

Overexpression of ASIC1a

The ASIC1a-specific shRNA lentiviral particles (Genechem, China) was transfected into cells as flowing the manufacturer's protocol. The primer for ASIC1a overexpression is

5'-GAGGATCCCCGGGTACCGGTCGCCACCAT

GGAAGTGAAGGCCGAGGAGGAG-3' (forward)

5'-TCCTTGTAAGTCCATACCGCAGGTAAAGTCC

TCGAACGTG-3' (reverse).

Statistical analysis

All experiments were performed at least in triplicate, and measured in three independent experiments. The data were expressed as mean $\pm$ SD. The comparison between means of two groups was performed using analysis of Student's t-test, and the comparison among the three or more groups was performed using analysis of one-way ANOVA. $P < 0.05$ was considered statistically significant. GraphPad Prism 5 was used for all analyses.

Results

ASIC1a is overexpressed in HCC tissue and significantly upregulated in resistant cells

We disclosed the levels of ASIC1a in six pairs of HCC tissues and adjacent non-tumor tissues by western blotting. As displayed in **Figures 1A1** and **A2**, ASIC1a is overexpressed in four pairs of tumor tissues, and low expressed in one pair of tumor tissues, and no significant high expressed in one pair of tumor tissues. Overall, this is not significantly different from previous studies.²⁰

In this research, we also have observed that the levels of the ASIC1a were revealingly overexpressed in HCC resistant cells than in sensitive cells by western blotting (**Figures 1B1** and **B2**), which propounded that ASIC1a acts as an considerable role in tumor resistance. To further evaluate the level of ASIC1a in the drug-resistant of HCC cells H^R and B^R, the expression of ASIC1a was detected by immunohistochemistry and immunofluorescence staining in the two drug-resistant cells. The expression level of membrane ASIC1a was obviously up-regulated in H^R and B^R cells, especially at pH 6.5 (**Figures**

1C1, C2 and D). These results not only indicate that ASIC1a is overexpressed in the drug-resistant of HCC cells H^R and B^R, but also reveal that a low pH in out of cell accelerates ASIC1a transporting to the membrane.

EMT has correlation with the high expression of ASIC1a and the drug resistance of HCC

EMT is familiar to act as an crucial role in cancer progression, metastasis and drug resistance.^{29,30} When ASIC1a is transferred to the cell membrane, it can promote the progression of cancer and induce its drug resistance.^{20,31} To further determine the role of EMT and the correlation between ASIC1a and EMT in tumor resistance, we cultured H^R and B^R cells in a pH 6.5 and pH 7.4 medium, respectively. At first, we detected the cell viability of the drug-resistant and sensitive cells of HCC by MTT. The results displayed that H^R cells have stronger cell viability after treating with 2, 4, 8, 16, 32 μ M OXA for 24 h and 48 h respectively, especially at pH 6.5 (**Figures 2A1 and A2**). Similarly, B^R cells cultured in a pH 6.5, which have stronger cell viability after treating with 0.2, 0.4, 0.8, 1.6 and 3.2 mM 5FU (**Figures 2A3 and A4**). Secondly, the clonogenic ability of H^R cells that cultured in a pH 6.5 and pH 7.4 medium as well as normally cultured H cells were detect by clony formation assay before and after treatment with 8 μ M OXA. As shown in **Figures 2B1 and B2**, H^R cells have stronger clonogenic ability after being treated with 8 μ M OXA for 24 h compared with H cells, especially at pH 6.5. In addition, almost identical results were procured in B and B^R cells.

One of the main features of EMT is the decreased expression of E-cadherin, α -catenin, and β -catenin as markers of epithelial cells and the overexpression of vimentin and fibronectin as markers of mesenchymal cells.³² Metalloproteinases (MMPs) (mainly MMP2 and MMP9), play a vital function in cell regeneration, programmed death, angiogenesis and many other necessary tissue functions, and participate in normal development and pathological processes, such as regulate inhibition of NF- κ B, EMT and cellular invasion.^{33,34} To detect the EMT of the HCC drug-resistant cells at the molecular level and its correlation with the extracellular acidic environment, we detected the expression levels of EMT marker molecules by western blotting in drug-resistant cells and sensitive cells. The results displayed that compared with sensitive cell H, the levels of E-cadherin, α -catenin, β -catenin were decreased and the expression levels of vimentin, fibronectin, MMP2 and MMP9 were increased in drug-resistant cells H^R, especially at pH 6.5 (**Figures 2C1, C2 and C3**). Additionally, compared with B cells, E-cadherin, α -catenin, β -catenin were significantly decreased, while vimentin, fibronectin, MMP2 and MMP9 were significantly increased in B^R cells, especially at pH 6.5 (**Figures 2C1, C2 and C3**).

It is an important mechanism for tumor resistance that EMT enhances the levels of invasion and migration in cancer cells.^{33,34} We found that compared with sensitive cells, the count of the two drug-resistant cells which passing through Transwell pores were significantly increased (**Figures 2D1 and D2**) and had a larger scratch healing area (**Figures 2E1 and E2**) after culturing for 48 h, especially at pH 6.5.

Taken together, these data indicate that a significant EMT has occurred in H^R cells and has a positive correlation with the extracellular acid environment, which also means that it has a effective correlation with the level of ASIC1a.

Inhibiting the activity of ASIC1a suppresses the EMT and enhances the chemosensitivity of H^R and B^R cells

Due to the significant increase in ASIC1a expression (**Figure 1**) and the significant occurrence of EMT that positively correlated with ASIC1a expression (**Figure 2**) in H^R and B^R cells, we restrained the level of ASIC1a with PcTx1 (a potent and specific blocker of the ASIC1a) to elucidating the direct effect of ASIC1a on the chemosensitivity of H^R and B^R cells and its relationship with EMT.

First, we detected the chemoresistance of H^R and B^R cells. The viability of H^R cells treated with 8 μ M OXA and B^R cells treated with 0.8 mM 5FU and H^R and B^R cells treated with 10, 20 and 40 nM PcTx1 respectively were assessed by MTT analysis at pH 6.5. As displayed in **Figures 3A1, A2, A3 and A4**, compared with H^R cells treated with OXA and B^R cells treated with 5FU, respectively, H^R and B^R cells extra treated with PcTx1 had obvious chemosensitivity. Additionally, it has no obvious difference in cell viability of each group after treatment with various concentrations of PcTx1. Therefore, we obtained that PcTx1 could inhibit the level of ASIC1a, thereby strikingly reducing the chemical resistance of H^R and B^R cells in acidic medium (pH 6.5).

Then, at pH 6.5, we detected the expression levels of α -catenin, β -catenin, vimentin and fibronectin after treatment with 10, 20 and 40 nM PcTx1 by western blotting in H^R and B^R cells. As shown in **Figures 3B1, B2 and B3**, in acidic medium, compared with untreated H^R and B^R cells, the expression of α -catenin and β -catenin were strikingly upregulated in H^R and B^R cells after treating with 10, 20 and 40 nM PcTx1, whereas the levels of vimentin and fibronectin were strikingly decreased. These data suggest that inhibiting the activity of ASIC1a can inhibit EMT, and based on the results of statistical analysis (**Figures 3B2 and B3**) and the principle of low cytotoxicity, 20 nM PcTx1 was selected as the drug concentration for the follow-up study.

Finally, at pH 6.5, we detected the levels of migration and invasion in H^R and B^R cells after treatment with 10, 20 and 40 nM PcTx1 by scratch healing assay and the Transwell invasion assay, respectively. Compared with untreated H^R and B^R cells in acidic medium, the scratch area and number of invaded cells of H^R and B^R cells were significantly reduced after treating with 10, 20, 40 nM PcTx1 (**Figures 3C1, C2, D1 and D2**). These data indicate that inhibiting the activity of ASIC1a could restrain the levels of migration and invasion in H^R and B^R cells.

ASIC1a knockdown suppresses the EMT and enhances the chemosensitivity of H^R and B^R Cells

After ASIC1a shRNA was transfected, the results of MTT method evaluation showed that the cytochemical sensitivity of H^R and B^R cells was critical enhanced (**Figures 4A1 and 4A2**). In addition, as

indicated in **Figures 4B1, B2, and B3**, the level of ASIC1a, vimentin and fibronectin were crucially down-regulated whereas the level of α -catenin and β -catenin were significantly increased when ASIC1a knockdown. Then, we detected the migration and invasion abilities of H^R and B^R cells after ASIC1a shRNA transfection by scratch healing assay and the Transwell invasion assay, respectively. In acidic medium, the scratch area and number of invaded cells of H^R and B^R cells were significantly reduced after ASIC1a shRNA transfection (**Figures 4C1, C2, D1 and D2**). These data indicate that silenced the ASIC1a gene could inhibit the migration and invasion abilities of H^R and B^R cells.

Overexpression of ASIC1a gene promotes the EMT and reduces the chemosensitivity of H^R and B^R cells

After confirming the relationship among the suppression of ASIC1a and the chemosensitivity of H^R and B^R cells as well as EMT level in acidic medium, the ASIC1a overexpression in H and B cells were used to explore the chemosensitivity of H^R and B^R cells and its relationship with EMT in acidic medium. As displayed in **Figures 5A1 and A2**, the cell viability was relevantly increased in B and H transfectant cells by MTT assay. Then, we detected the expression levels of α -catenin, β -catenin, vimentin and fibronectin by western blot. The results indicated that overexpressed ASIC1a upregulated vimentin and fibronectin and downregulated α -catenin and β -catenin expression (**Figures 5B1, B2 and B3**). Next, we detected the levels of migration and invasion in H and B cells after ASIC1a expression vector transfection by scratch healing assay and the Transwell invasion assay, respectively. In acidic medium, the scratch area and number of invaded cells of H and B cells were significantly increased after ASIC1a expression vector transfection (**Figures 5C1, C2, D1 and D2**). These data indicate that overexpressed the ASIC1a gene can significantly stimulate the migration and invasion ability of H and B cells.

ASIC1a mediates EMT in H^R and B^R cells

As ASIC1a and EMT were notably increased in H^R and B^R cells and suppressed by inhibiting the activity of ASIC1a. Therefore, we explored whether the occurrence of EMT was mediated via ASIC1a. As shown in **Figures 6A1 and A2**, the acid-induced cell viability of H^R and B^R cells was significantly attenuated by PcTx1. The acid-induced cell viability of H^R and B^R cells was not decreased by C19 to block occurrence of EMT. However, the acid-induced cell viability of H^R and B^R cells was obviously decreased in the presence of PcTx1 and C19.

Then, we detected the acid-induced expression levels of α -catenin, β -catenin, vimentin and fibronectin. As displayed in **Figures 6B1, B2, B3 and B4**, the acid-induced low expression levels of α and β -catenin were significantly increased and the acid-induced high expression levels of vimentin and fibronectin were reduced by PcTx1 in H^R and B^R cells. The acid-induced low expression levels of α and β -catenin were not increased and the acid-induced high expression levels of vimentin and fibronectin were not reduced by C19 in H^R and B^R cells. However, the acid-induced expression levels of α and β -catenin, vimentin and fibronectin were changed by PcTx1 and C19 as in the presence of PcTx1 in H^R and B^R cells. Next, the acid-

induced levels of migration and invasion in H^R and B^R cells were not decreased by C19, whereas obviously decreased in the presence of PcTx1 and C19 (**Figures 6C1, C2, C3, D1, D2 and D3**).

Overall, the occurrence of EMT induced by acidic was withdrawn by PcTx1 to restrain activation of ASIC1a. These data reveal that ASIC1a has a preeminent effect for mediating EMT in H^R and B^R cells.

ASIC1a reduces the chemosensitivity of H^R and B^R cells by promoting EMT via the AKT/GSK3 β /Snail signaling pathway

Early studies have illustrated that the AKT/GSK3 β /Snail pathway is touched upon the regulation of EMT,^{35,36} but whether ASIC1a is involved in it is not yet known. First, we found that the expressed levels of Snail and the p-AKT and p-GSK3 β were appreciably upregulated in H^R(pH6.5) and B^R(pH6.5) cells compared with H and B cells by Western blot analysis (**Figures 7A1, A2 and A3**).

At pH 6.5, we detected the levels of migration and invasion in H^R and B^R cells after treatment with MK2206, TWS119 and GN25 by scratch healing assay and the Transwell invasion assay, respectively. Compared with untreated H^R and B^R cells in acidic medium, the scratch healing area and number of invaded cells of H^R and B^R cells were significantly reduced after treating with MK2206, TWS119 and GN25, respectively (**Figures 7B1, B2, C1 and C2**).

Additionally, compared with untreated H^R and B^R cells in acidic medium, the protein expression levels of Snail (**Figures 7D1 and D2**) and the phosphorylation levels of AKT(**Figures 7E1 and E2**) and GSK3 β (**Figures 7F1 and F2**) were significantly downregulated after treating with PcTx1 or ASIC1a shRNA in H^R (pH6.5) and B^R(pH6.5) cells. We also evaluated the effect of the AKT/GSK3 β /Snail pathway on EMT in H^R and B^R cells by western blotting. As displayed in **Figures 7G1, G2 and G3**, compared with untreated H^R and B^R cells in acidic medium, the levels of α -catenin and β -catenin were impressively increased whereas the levels of vimentin and fibronectin were impressively reduced after treating with MK2206, TWS119 and GN25 in H^R and B^R cells, respectively.

Discussion

Due to excessive rapid growth, the tumor cells often form hypoxic environment.³⁷ And hypoxia can induce the transformation of cell metabolism and promote the increase of acidosis, thereby producing high acid load.³⁸ At this time, the tumor cells can adapt to the acidic microenvironment by changing the metabolic to turn on the detoxification mode.³⁹ Studies have shown that extracellular acidification is an important feature of the cancer microenvironment, which can diametrically control the migration and invasion of tumor cells by influencing immune cell function, clonal cell evolution and drug tolerance.^{40–42} As an acid sensor, ASIC1a has been proven to promote the development of malignant tumors by promoting the migration and invasion of tumor cells, and enhance the drug tolerance of HCC cells via mediating Ca²⁺ influx.²⁰ EMT is a necessary transformation process for the local and long-distance

progression of many malignant tumors, including HCC.⁴³ Other studies have shown that it is an important mechanism that EMT enhances tumor cell invasion and metastasis for tumor resistance.^{33,34} However, it is not clear that the relationship and interaction between ASIC1a and EMT in tumor resistance. Therefore, in this study, the identification of ASIC1a and EMT functions and their molecular mechanisms and interactions in resistant HCC cells provide a reasonable explanation for why low pH is advantageous to the development of resistant cancer cells, and it also provides new ideas for reversing tumor drug resistance by inhibiting EMT.

Consistent with previous studies, the of ASIC1a overexpressed in HCC tissues and resistant cells was considerably higher than that of parental cells, especially at pH 6.5.²⁰ In addition, immunofluorescence and immunohistochemistry staining showed that membrane ASIC1a has a considerably higher expressed in H^R and B^R cells at pH 6.5. These data indicate that ASIC1a stimulate the development of the malignant and resistant tumor at low pH.

In the past, it was thought that EMT was only concerning to the invasion and metastasis of malignant cells, but in recent years, it has been detected that EMT which occurrence in cancer cells also could to resist drug resistance and apoptosis, which is a indispensable process of tumor resistance.^{33,34} So reversing EMT or killing cancer cells of EMT has been a potential treatment strategy for tumor. This study detected EMT through the detection of cell proliferation, clone formation, migration, invasion and molecular marker expression *in vitro*. Consistent with previous studies, it has significant EMT in HCC resistant cells compared with parental cells, especially at pH 6.5.⁴⁴ These data reveal that acidic extracellular pH promotes the EMT, and the up-regulation of ASIC1a may be promote EMT in HCC. However, it is not yet possible to fully understand the relationship between ASIC1a and EMT and their relationship with the drug resistance. Therefore, we restrained the expression of ASIC1a through PcTx1 to annotate the connotation of ASIC1a in resistant and EMT of H^R and B^R cells by the detection of cell proliferation, clone formation, migration, invasion and molecular marker expression *in vitro*. The results showed that at pH 6.5, the inactivation of ASIC1a by PcTx1, which arouses chemoresistance of H^R and B^R cells, was impressively reduced and the reversal of EMT by inhibiting the activity of ASIC1a was also significantly reduced. In addition, the ASIC1a gene knockdown heightens the chemosensitivity of H^R and B^R cells and inhibits EMT in H^R and B^R cells. From above results, we could acquire that ASIC1a may be liable for EMT and altered drug-resistant in HCC cells.

It is noteworthy that part of the EMT in tumor cells is believed to heighten their invasiveness, generate circulating cancer cells and cancer stem cells, and enhance resistance to anticancer drugs.⁴⁵ Interestingly, several transcriptional repressors including Snail (SNAI1), Slug (SNAI2) and ZEB family constitute the key role of EMT in cancer and development.^{26,27} Regulating Akt/GSK3 β /Snail signal transduction axis can inhibit tumor cell EMT and chemoresistance.^{24,25} Extracellular acidosis leads to drug tolerance, including decreased apoptotic latent, stimulation of autophagy, suppression of immunity and genetic changes.⁴⁶ Studies have declared that ASIC1a mediates tumor tolerance via inducing calcium influx and prevents apoptosis through its induced autophagy.^{20,47} In addition, extracellular acidification activates ASIC1a to

facilitate cancer cell migration and adhesion.⁴⁸ Based on the upon findings, we inspected whether ASIC1a-mediated EMT induces tumor tolerance through the AKT/GSK3 β /Snail pathway. We proved that the AKT/GSK3 β /Snail activity of drug-resistant cell lines H^R and B^R was higher than that of sensitive cells. Inhibition of AKT/GSK3 β /Snail pathway with MK2206 or TWS119 or GN25 can reverse the EMT of H^R and B^R cells (Fig. 7). These results recommended that the drug resistance of HCC cells regulated by ASIC1a depended at least in part on AKT/GSK3 β /Snail.

In conclusion, activation of ASIC1a by extracellular acidification through the AKT/GSK3 β /Snail stimulates the EMT to make HCC resistant (Fig. 8), which indicates that ASIC1a may reverse EMT, prevent HCC cells from developing drug resistance and controlling the progression of HCC. But this study has not yet explored the relationship between calcium influx and EMT. Therefore, other potential mechanisms that ASIC1a and EMT participate in the regulation of drug resistance in HCC cells remain to be further studied.

Declarations

Ethics approval and consent to participate

This study was approved by the Human Research Ethics Committee of the First Affiliated Hospital of Anhui University of Science and Technology (China) and have been conducted in accordance with Declaration of Helsinki, and the informed consent was obtained from each subject.

Consent for publication

All authors are in agreement with the content of the manuscript.

Availability of data and materials

The data set used and/or analyzed in the current study can be obtained from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

The National Natural Science Fund of China (NO. 82071862, 81872017, 81572431), Anhui Provincial Science and Technology program (NO. 1604a0802094, 202004j07020053), University Natural Science Research Project of Anhui Province (NO. KJ2018ZD011, KJ2018A0097, KJ2019A0093, KJ2020A0340) and Research Foundation of the Institute of Environment-friendly Materials and Occupational Health (Wuhu), Anhui University of Science and Technology (ALW2020YF11), Graduate Innovation Fund Project of Anhui University of Science and Technology funded this research.

Authors' contributions

YCZ performed the experiments, analyzed the data and was the main contributor to writing the manuscript. NDC analyzed the data. JFG was responsible for the English revision work and performed some of the experiments. YL, YHX and SPZ performed the experiments. XLT was the project leader and was responsible for the design of the project, the revision of the manuscript and performed some of the experiments. YCZ and XLT confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Kim BH. Park JW. Epidemiology of liver cancer in South Korea. *Clin Mol Hepatol*. 2018;24:1–9.
2. Sia D. Villanueva A, Friedman SL. Llovet JM. Liver Cancer Cell of Origin, Molecular Class, and Effects on Patient Prognosis. *Gastroenterology*. 2017;152:745–61.
3. Nio K. Yamashita T, Kaneko S. The evolving concept of liver cancer stem cells. *Mol Cancer*. 2017;16:4.
4. Nyongesa CO. Park S. Chemotherapeutic resistance: a nano-mechanical point of view. *Biol Chem*. 2018;399:1433–46.
5. *Shima Tavakol*. Acidic pH derived from cancer cells may induce failed reprogramming of normal differentiated cells adjacent tumor cells and turn them into cancer cells. *Med Hypotheses*. 2014;83:668–72.
6. Bång-Rudenstam A. Cerezo-Magaña M, Belting M. Pro-metastatic functions of lipoproteins and extracellular vesicles in the acidic tumor microenvironment. *Cancer Metastasis Rev*. 2019;38:79–92.
7. Chen C. Bai L, Cao F. Wang S, He H. Song M, *et al*. Targeting LIN28B reprograms tumor glucose metabolism and acidic microenvironment to suppress cancer stemness and metastasis. *Oncogene*. 2019;38: 4527–39.
8. Norouzi S. Gorgi Valokala M, Mosaffa F. Zirak MR, Zamani P. Behravan J. Crosstalk in cancer resistance and metastasis. *Crit Rev Oncol Hematol*. 2018;132:145–53.
9. Bohlooli M. Atashi A, Soleimani M. Kaviani S, Anbarlou A. Investigating Effects of Acidic pH on Proliferation, Invasion and Drug-Induced Apoptosis in Lymphoblastic Leukemia. *Cancer Microenviron*. 2016;9:119–26.
10. Yi M. Ban Y, Tan Y. Xiong W, Li G. Xiang B. 6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 and 4: A pair of valves for fine-tuning of glucose metabolism in human cancer. *Mol Metab*. 2019;20:1–13.
11. Tian XP. Wang CY, Jin XH. Li M, Wang FW. Huang WJ, *et al*. Acidic Microenvironment Up-Regulates Exosomal miR-21 and miR-10b in Early-Stage Hepatocellular Carcinoma to Promote Cancer Cell Proliferation and Metastasis. *Theranostics*. 2019;9:1965–79.

12. Vaupel P, Multhoff G. Accomplices of the Hypoxic Tumor Microenvironment Compromising Antitumor Immunity: Adenosine, Lactate, Acidosis, Vascular Endothelial Growth Factor, Potassium Ions, and Phosphatidylserine. *Front Immunol.* 2017;8:1887.
13. Vullo S, Kellenberger S. A molecular view of the function and pharmacology of acid-sensing ion channels. *Pharmacol Res.* 2020;154:104166.
14. Gupta SC, Singh R, Asters M, Liu J, Zhang X, Pabbidi MR, *et al.* Regulation of breast tumorigenesis through acid sensors. *Oncogene* 2016;35:4102-11.
15. Huber V, Camisaschi C, Berzi A, Ferro S, Lugini L, Triulzi T, *et al.* Cancer acidity: An ultimate frontier of tumor immune escape and a novel target of immunomodulation. *Semin Cancer Biol.* 2017;43:74–89.
16. Li W, Sun X. Recent Advances In Developing Novel Anti-Cancer Drugs Targeting Tumor Hypoxic and Acidic Microenvironments. *Recent Pat Anticancer Drug Discov.* 2018;13:455–68.
17. Ye X, Nie S, Wang J. Smart Superstructures with Ultrahigh pH-Sensitivity for Targeting Acidic Tumor Microenvironment: Instantaneous Size Switching and Improved Tumor Penetration. *ACS Nano.* 2016;10:6753-61.
18. Jin C, Ye QH, Yuan FL, Gu YL, Li JP, Shi YH, *et al.* Involvement of acid-sensing ion channel 1alpha in hepatic carcinoma cell migration and invasion. *Tumour Biol.* 2015;36:4309–17.
19. Chen X, Sun X, Wang Z, Zhou X, Xu L, Li F, *et al.* Involvement of acid-sensing ion channel 1a in gastric carcinoma cell migration and invasion. *Acta Biochim Biophys Sin (Shanghai).* 2018;50:440–6.
20. Zhang Y, Zhang T, Wu C, Xia Q, Xu D. ASIC1a mediates the drug resistance of human hepatocellular carcinoma via the Ca²⁺/PI3-kinase/AKT signaling pathway. *Lab Invest.* 2017;97:53–69.
21. Corbet C, Bastien E, Santiago de Jesus JP, Dierge E, Martherus R, Vander Linden C, *et al.* TGFβ₂-induced formation of lipid droplets supports acidosis-driven EMT and the metastatic spreading of cancer cells. *Nat Commun.* 2020;11:454.
22. Yu C, Liu Y, Qin Z. Metadherin contributes to epithelial-mesenchymal transition and paclitaxel resistance induced by acidic extracellular pH in nasopharyngeal carcinoma. *Oncol Lett.* 2018;15:3858–63.
23. Jacobsen K, Bertran-Alamillo J, Molina MA, Teixidó C, Karachaliou N, Pedersen MH, *et al.* Convergent Akt activation drives acquired EGFR inhibitor resistance in lung cancer. *Nat Commun.* 2017;8:410.
24. Wu W-S, Ren-In You, Cheng C-C, Ming-Che Lee, Lin T-Y, Chi-Tan Hu. Snail Collaborates With EGR-1 and SP-1 to Directly Activate Transcription of MMP 9 and ZEB1. *Sci Rep.* 2017;7:17753.
25. Hongshun Xing S, Wang Q, Li Y, Ma P, Sun. Long Noncoding RNA LINC00460 Targets miR-539/MMP-9 to Promote Meningioma Progression and Metastasis. *Biomed Pharmacother.* 2018;105:677–82.
26. Jena MK, Janjanam J. *Role of extracellular matrix in breast cancer development: a brief update.* *F1000Res.* 2018 Mar 5;7:274.
27. Skrzypek K, Majka M. Interplay among SNAIL Transcription Factor, MicroRNAs, Long Non-Coding RNAs, and Circular RNAs in the Regulation of Tumor Growth and Metastasis. *Cancers (Basel).*

2020;12:209.

28. Zhang Y. Xie C, Li A. Liu X, Xing Y. Shen J, *et al.* PKI-587 enhances chemosensitivity of oxaliplatin in hepatocellular carcinoma through suppressing DNA damage repair pathway (NHEJ and HR) and PI3K/AKT/mTOR pathway. *Am J Transl Res.* 2019;11:5134–49.
29. Du B. Shim JS. Targeting Epithelial-Mesenchymal Transition (EMT) to Overcome Drug Resistance in Cancer. *Molecules.* 2016;21:965.
30. Lu W. Kang Y. Epithelial-Mesenchymal Plasticity in Cancer Progression and Metastasis. *Dev Cell.* 2019;49:361–74.
31. Li X. Ye JX, Xu MH. Zhao MD, Yuan FL. Evidence that activation of ASIC1a by acidosis increases osteoclast migration and adhesion by modulating integrin/Pyk2/Src signaling pathway. *Osteoporos Int.* 2017;28:2221–31.
32. Aiello NM. Kang Y. Context-dependent EMT programs in cancer metastasis. *J Exp Med.* 2019;216:1016–26.
33. Scheau C. Badarau IA, Costache R. Caruntu C, Mihai GL. Didilescu AC, *et al.* The Role of Matrix Metalloproteinases in the Epithelial-Mesenchymal Transition of Hepatocellular Carcinoma. *Anal Cell Pathol (Amst).* 2019;2019:9423907.
34. Kim DH. Kim EJ, Kim DH. Park SW. Dact2 is involved in the regulation of epithelial-mesenchymal transition. *Biochem Biophys Res Commun.* 2020;524:190–7.
35. Meng Q. Shi S, Liang C. Liang D, Hua J. Zhang B, *et al.* Abrogation of glutathione peroxidase-1 drives EMT and chemoresistance in pancreatic cancer by activating ROS-mediated Akt/GSK3 β /Snail signaling. *Oncogene.* 2018;37:5843–57.
36. Peng B. Ortega J, Gu L. Chang Z, Li GM. Phosphorylation of proliferating cell nuclear antigen promotes cancer progression by activating the ATM/Akt/GSK3 β /Snail signaling pathway. *J Biol Chem.* 2019;294:7037–45.
37. Tolstun DA. Knyazer A, Tushynska TV. Dubiley TA, Bezrukov VV. Fraifeld VE, *et al.* Metabolic remodelling of mice by hypoxic-hypercapnic environment: imitating the naked mole-rat. *Biogerontology.* 2020;21:143–53.
38. Vaupel P. Schmidberger H, Mayer A. The Warburg effect: essential part of metabolic reprogramming and central contributor to cancer progression. *Int J Radiat Biol.* 2019;95:912–9.
39. Boedtker E. Pedersen SF. The Acidic Tumor Microenvironment as a Driver of Cancer. *Annu Rev Physiol.* 2020;82:103–26.
40. Teixeira J. Basit F, Swarts HG. Forkink M, Oliveira PJ. Willems PHGM, *et al.* Extracellular acidification induces ROS- and mPTP-mediated death in HEK293 cells. *Redox Biol.* 2018;15:394–404.
41. Kondo A. Yamamoto S, Nakaki R. Shimamura T, Hamakubo T. Sakai J, *et al.* Extracellular Acidic pH Activates the Sterol Regulatory Element-Binding Protein 2 to Promote Tumor Progression. *Cell Rep.* 2017;18:2228–42.
42. Zeeshan R. Mutahir Z. Cancer metastasis-tricks of the trade. *Bosn J Basic Med Sci.* 2017;17:172–82.

43. Saitoh M. Involvement of partial EMT in cancer progression. *J Biochem.* 2018;164:257–64.
44. Giannelli G. Koudelkova P, Dituri F. Mikulits W. Role of epithelial to mesenchymal transition in hepatocellular carcinoma. *J Hepatol.* 2016;65:798–808.
45. Pastushenko I. Blanpain C. EMT Transition States during Tumor Progression and Metastasis. *Trends Cell Biol.* 2019;29:212–26.
46. Corbet C. Feron O. Tumour acidosis: from the passenger to the driver's seat. *Nat Rev Cancer.* 2017;17:577–93.
47. Dai B. Zhu F, Chen Y. Zhou R, Wang Z. Xie Y, *et al.* ASIC1a Promotes Acid-Induced Autophagy in Rat Articular Chondrocytes through the AMPK/FoxO3a Pathway. *Int J Mol Sci.* 2017;18:2125.
48. Li X. Ye JX, Xu MH. Zhao MD, Yuan FL. Evidence that activation of ASIC1a by acidosis increases osteoclast migration and adhesion by modulating integrin/Pyk2/Src signaling pathway. *Osteoporos Int.* 2017;28:2221–31.
49. Zhang YC. Wu CG, Li AM. Liang Y, Ma D. Tang XL. Oxaliplatin and Gedatolisib (PKI-587) Co-Loaded Hollow Polydopamine Nano-Shells with Simultaneous Upstream and Downstream Action to Re-Sensitize Drugs-Resistant Hepatocellular Carcinoma to Chemotherapy. *J Biomed Nanotechnol.* 2021;17:18–36.

Figures

Figure 1

ASIC1a is highly expressed in HCC tissue and significantly upregulated in resistant HCC cells. (A1 and A2) Western blotting analysis of ASIC1a protein expression levels in HCC tissue and adjacent non-tumor tissue. Data were expressed as the mean $\pm$ SD, **P < 0.01 and ***P < 0.001 all versus Control group. C: Control (adjacent non-tumor tissue), T: Tumor (HCC tissue). (B1 and B2) Western blotting analysis of ASIC1a protein expression levels in HCC cells. Data were expressed as the mean $\pm$ SD, **P < 0.01 all versus H group. (C1 and C2): Expression levels and the cell distribution of ASIC1a in HCC cells were

measured by Immunofluorescence (IF) staining. Representative images were taken at $\times 400$ magnification. (D): Expression levels and the cell distribution of ASIC1a in HCC cells were measured by Immunohistochemistry (IHC) staining. Representative images were taken at $\times 400$ magnification.

Figure 2

EMT plays an important role in the drug resistance of HCC and has correlation with the high expression of ASIC1a. (A1, A2, A3 and A4) MTT analysis of the cell viability. Data were expressed as the mean $\pm$ SD.

(B1 and B2) Representative images of the colony formation assay in HCC cells. The percentage of colony formation was calculated. Data are presented as mean $\pm$ SD, n=3. **P < 0.01 and ***P < 0.001 all versus Control group. (C1, C2 and C3) Western blotting analysis of EMT marker molecule protein levels in HCC cells. Data were expressed as the mean $\pm$ SD, *P < 0.05, **P < 0.01 and ***P < 0.001 all versus H group. (D1 and D2) Invasion abilities of different group cells were determined by transwell invasion assays. Representative images were taken at $\times$ 200 magnification. Data were expressed as the mean $\pm$ SD, *P < 0.05 and **P < 0.01 all versus H group. (E1 and E2) Migration abilities of different group cells were examined by wound healing assays. Representative images were taken at $\times$ 100 magnification. Data were expressed as the mean $\pm$ SD, *P < 0.05, **P < 0.01 and ***P < 0.01 all versus 0h group.

Figure 3

Inhibiting the activity of ASIC1a suppresses the EMT and enhances the chemosensitivity of HR and BR cells. (A1, A2, A3 and A4) MTT analysis of the cell viability. Data were expressed as the mean $\pm$ SD. n.s.: no significance, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (B1, B2 and B3) Western blotting analysis. Data were expressed as the mean $\pm$ SD, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ all versus HR(pH6.5) or BR(pH6.5) group. (C1 and C2) Migration abilities of different group cells were examined by wound healing assays.

Representative images were taken at $\times 100$ magnification. Data were expressed as the mean $\pm$ SD, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.01$ all versus 0h group. (D1 and D2) Invasion abilities of different group cells were determined by transwell invasion assays. Representative images were taken at $\times 200$ magnification. Data were expressed as the mean $\pm$ SD, ** $P < 0.01$ and *** $P < 0.001$ all versus HR(pH6.5) or BR(pH6.5) group.

Figure 4

Silencing of the ASIC1a gene suppresses the EMT and enhances the chemosensitivity of HR and BR Cells. (A1 and A2) MTT analysis of the cell viability. Data were expressed as the mean $\pm$ SD. *P < 0.05 all versus HR(pH6.5)-control shRNA or BR(pH6.5)-control shRNA group. (B1, B2 and B3) Western blotting analysis. Data were expressed as the mean $\pm$ SD, **P < 0.01 and ***P < 0.001 all versus Control shRNA group. (C1 and C2) Migration abilities of different group cells were examined by wound healing assays. Representative images were taken at $\times$ 100 magnification. Data were expressed as the mean $\pm$ SD, n.s: no significance, **P < 0.01, ***P < 0.001. (D1 and D2) Invasion abilities of different group cells were determined by transwell invasion assays. Representative images were taken at $\times$ 200 magnification. Data were expressed as the mean $\pm$ SD, ***P < 0.001 all versus HR(pH6.5)-control shRNA or BR(pH6.5)-control shRNA group.

Figure 5

Overexpression of ASIC1a gene promotes the EMT and reduces the chemosensitivity of HR and BR Cells. (A1 and A2) MTT analysis of the cell viability. Data were expressed as the mean $\pm$ SD. * $P < 0.05$ versus HR(pH6.5)-mock or BR(pH6.5)-mock group. (B1, B2 and B3) Western blotting analysis. Data were expressed as the mean $\pm$ SD, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ all versus Mock group. (C1 and C2) Migration abilities of different group cells were examined by wound healing assays. Representative

images were taken at $\times 100$ magnification. Data were expressed as the mean $\pm$ SD, n.s: no significance, ***P < 0.001. (D1 and D2) Invasion abilities of different group cells were determined by transwell invasion assays. Representative images were taken at $\times 200$ magnification. Data were expressed as the mean $\pm$ SD, ***P < 0.001 versus HR(pH6.5)-mock or BR(pH6.5)-mock group.

Figure 6

ASIC1a mediates EMT in HR and BR cells. (A1 and A2) MTT analysis of the cell viability. Data were expressed as the mean $\pm$ SD. n.s: no significance, *P < 0.05, **P < 0.01, ***P < 0.001. (B1, B2, B3 and B4) Western blotting analysis. Data were expressed as the mean $\pm$ SD, n.s: no significance, *P < 0.05, **P < 0.01, ***P < 0.001. (C1 and C2) Migration abilities of different group cells were examined by wound healing assays. Representative images were taken at $\times$ 100 magnification. Data were expressed as the mean $\pm$ SD, n.s: no significance, *P < 0.05, **P < 0.01. (D1, D2 and D3) Invasion abilities of different group cells were determined by transwell invasion assays. Representative images were taken at $\times$ 200 magnification. Data were expressed as the mean $\pm$ SD, n.s: no significance, **P < 0.01, ***P < 0.001.

Figure 7

ASIC1a reduces the chemosensitivity of HR and BR cells by promoting EMT via the AKT/GSK3β/Snail signaling pathway. (A1, A2 and A3) Western blotting analysis. Data were expressed as the mean ± SD, **P < 0.01 and ***P < 0.001 versus H or B group. (B1 and B2) Migration abilities of different group cells were examined by wound healing assays. Representative images were taken at × 100 magnification. Data were expressed as the mean ± SD, n.s: no significance, **P < 0.01, ***P < 0.001. (C1 and C2) Invasion

abilities of different group cells were determined by transwell invasion assays. Representative images were taken at $\times 200$ magnification. Data were expressed as the mean $\pm$ SD, $**P < 0.01$ versus HR(pH6.5) or BR(pH6.5) group. (D1, D2, E1, E2, F1, F2, G1, G2 and G3) Western blotting analysis. Data were expressed as the mean $\pm$ SD, $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ versus HR(pH6.5) or BR(pH6.5) group.

Figure 8

Schematic diagram illustrates that ASIC1a mediates the drug resistance of human hepatocellular carcinoma (HCC) by promoting epithelial-mesenchymal transition (EMT) via the AKT/GSK3 β /Snail signaling pathway.