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Acidosis promotes tumorigenesis by activating AKT/NF-κB signaling

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Abstract
The microenvironment of solid tumors is often acidic due to poor vascular perfusion, regional hypoxia, and increased glycolytic activity of tumor cells. Although acidosis is harmful to most types of cells, tumor cells seem well adapted to such harsh conditions. Moreover, overwhelming evidence indicates that tumor cells are more invasive and more aggressive in acidic conditions by a cascade of cell signaling and upregulation of oncogenic gene expression. Therefore, how extracellular acidic signals are transduced to the cytoplasm and then into the nucleus is an interesting topic to many cancer researchers. In this review, we update on the recent advances in acidosis-induced tumorigenesis through the acid-sensing ion channels (ASICs) and activation of cell signaling.

Keywords Acidosis · Acidosis-induced tumorigenesis · AKT/NF-κB signaling

1 Introduction

Almost every type of cell is able to sense extracellular environment. Acidity is one of the important features of tumor microenvironment which are often favorable to tumor cells through adaptation and evolution. Evidence suggests that acid sensors in the cytoplasmic membrane may play a critical role in how the cells respond to acidic conditions. There are two major types of acid sensors, proton-sensing G protein-coupled receptors (GPCRs) [1] and acid-sensing ion channels (ASICs) [2]. Like other members of GPCRs, proton-sensing GPCRs are also transmembrane receptors which, however, sense acidic pH, i.e., these receptors are activated when extracellular pH falls into the range of 6.4–6.8.

For example, ovarian cancer G protein-coupled receptor 1 (OGR1) and GPR4 (G protein-coupled receptor 4) are the first two members that are shown to proton-sensing receptors involved in pH homeostasis [3]. Other members include GPR132 (G2A) and GPR65 (TDAG8) [4]. They are expressed in various tissues such as the testis, spleen, bone, lung, brain, and placenta, and may play a role in bone metabolism. Of interest, at least, some of these receptors play a role in cancer. In this regard, OGR1 has been implicated as a metastasis suppressor gene in prostate cancer, although exogenous OGR1 overexpression had no effect on primary prostate tumor growth *in vivo* [5].

On the other hand, ASICs are neuronal voltage-insensitive cationic channels activated by extracellular protons and they are primarily expressed in neuronal cells. However, increasing evidence suggests that expression of ASICs is not limited to neuronal cells. Furthermore, upregulation of ASICs has been reported in several types of cancer. They may also contribute to tumorigenesis. A substantial progress has been made in dissecting the acidosis-induced signaling pathways in cancer, and in particular, how ASICs impact tumorigenesis. Therefore, the focus of this current review is on ASICs.

2 Acidosis is a common physical hallmark of solid tumors

The extracellular pH (pHe) of solid tumors is more acidic than that of normal tissue primarily due to high glycolysis and poor perfusion within tumors; pHe values could reach 6.2~6.8 [6]. In some extreme cases, interstitial pH values could even be as low as 5.8 [7]. It is known that tumor cells can metabolize glucose to lactic acid even under normal oxygen tension, a phenomenon called aerobic glycolysis or the Warburg effect. This can lead to low intracellular pH (pHi) which is often

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deleterious to the tumor cells. Thus, the tumor cells have to employ various mechanisms to remove intracellular acids in order to maintain physiological pHi. These include Na-driven proton extrusion, V-type ATPases, Na^+/H^+ exchangers (NHEs), and those facilitated by carbonic anhydrases [2]. Among these, NHE1 is a primary cellular housekeeping Na^+/H^+ antiporter present in the membranes of many types of cells. It serves to regulate intracellular pH (pHi) and cell volume and is intimately involved in changing the growth and functional states of cells, and cell metabolism. In particular, pHi may also impact how the cells respond to therapy. *In silico* analysis suggests that alkaline pHi maximizes cancer cell proliferation coupled to increased glycolysis and adaptation to hypoxia, whereas acidic pHi disables these adaptations and compromises tumor cell growth [8].

As a result, pHe becomes acidic, which is often harmful to normal cells including host immune cells. However, such low pHe is not harmful to tumor cells; instead, it may even benefit tumor cells for their migration and invasion [9]. For example, a report showed that the peritumoral pH is acidic and heterogeneous, and pHe is negatively correlated with their invasiveness [10]. On the other hand, little tumor invasion is detected in regions with normal or near-normal pH [10]. These findings suggest that tumor cells have adapted well to extracellular acidosis which, furthermore, may be used by tumor cells as a means to promote their invasion and metastasis [11].

Acidosis changes tumor metabolism and induces expression of oncogenes. An important feature of acidosis is the production of reactive oxygen species (ROS) [12] (see Sonveaux this volume). For instance, acidosis induces MAPK phosphorylation in rat prostate carcinoma cells through the formation of ROS [13] (see Thews, this volume). ROS plays a critical role in enhancing the inflammation by the activation of NF-κB and AP-1 transcription factors, and nuclear histone acetylation and deacetylation in various inflammatory diseases including cancer. These microenvironmental stresses exert important influences on metabolic phenotypes and gene expression patterns. It is well known that the process of tumorigenesis requires genetic instability and highly selective local microenvironments, and such environmental stress promotes somatic evolution.

As an example, a variety of environmental factors can alter tumor cell metabolism to serve the distinct cellular biosynthetic and bioenergetic needs during oncogenesis. In this regard, acidosis can increase both glutaminolysis and fatty acid β-oxidation. For example, acidosis activates p53, which contributes to both the enhancement of the pentose phosphate pathway and glutaminolysis [14]. In other cases, several oncogenic genes can be induced by acidosis. For instance, an acidic pHe promotes the expression of VEGF in melanoma cells through the NF-κB transcription factor [15]. Similarly, it has been shown that acidic tumor microenvironment can promote autophagy and induce autophagy-related genes [16]. In particular, chronic autophagy can serve as a survival adaptation mechanism for tumor cells in this setting [16]. In addition, acidosis can activate extracellular matrix-degrading enzymes such as cathepsin B and hyaluronidase 2 and pro-angiogenic factors [17, 18]. Together, these findings may also explain in part why tumor cells can benefit from the acidic microenvironment.

Furthermore, acidosis also affects therapeutic resistance. For example, in lymphoma cells, acidosis (pH 6.5) blocks apoptosis induced by multiple cytotoxic metabolic stresses, including deprivation of glucose or glutamine and treatment with dexamethasone. Acidic conditions cause elevation of both Bcl-2 and Bcl-xL, while also attenuating glutamine starvation-induced upregulation of PUMA and Bim [19]. Consistent with this finding, acidosis is able to strongly induce P-glycoprotein, an important drug transporter responsible for multidrug resistance [20].

Finally, acidosis has been implicated in stimulation of autophagy and immunosuppression, and immune-evasion, promoting tumorigenesis [21, 22]. In support of this, acidosis-induced cell invasion has been reported in variety of cancer including melanoma [23, 24], cervical cancer [25], and prostate cancer [26]. Moreover, acidosis can activate NF-κB in melanoma [15], osteosarcoma [27], and ovarian cancer [28] as well as pancreatic, colon, and prostate cancer [29]. Our results show that acidosis induces NF-κB activation in breast cancer cells [30]. Therefore, acidic tumor microenvironment can have multiple effects on tumor cells and may contribute to invasiveness and metastasis through various mechanisms.

3 Role of reactive oxygen species (ROS), AKT, and NF-κB in cancer

Several studies suggest that acidic conditions increase ROS generation and activation of AKT/NF-κB [12, 13, 35–37] (see also Sonveaux, this volume). Acidosis-induced formation of ROS, probably through activation of NADPH oxidase from mitochondria, may then trigger a series of cell signaling events such as MAPK phosphorylation, activation of AKT and nuclear localization of NF-κB, and gene expression, leading to tumor growth and invasion.

The role of ROS, AKT, and NF-κB in cancer is well established. For example, AKT and NF-κB control a large number of cellular pathways and cellular functions. Similarly, ROS can serve as an inducer of genes involved in DNA repair, DNA mutation, and genome integrity. Thus, a better understanding of how these factors are regulated in acidic conditions is critical to effectively targeting the pathways associated with these factors.

ROS impacts various hallmarks of cancer such as cellular proliferation and angiogenesis through the promotion of cell signaling pathways. It is well known that ROS can damage DNA, and it subsequently increases

genome instability and the mutagenic activity, leading to breast cancer progression [33]. A variety of mechanisms can cause tumor cells to produce ROS, including alterations to metabolic pathways in tumor cells, an inadequate tumor vascular network, and macrophage infiltration of the tumors. A well-known consequence of ROS production is an increased mutation rate and accelerated tumor progression and activation of growth-promoting signaling pathways such as MAPK pathways [34]. ROS can also promote secretion of angiogenic factors and matrix metalloproteinase [35]. Oxygen radicals can promote tumor cell migration, increasing the risk of invasion and metastasis through activation of the p38 MAPK [34] (see also Thews, this volume). These are not the only mechanism that ROS impacts tumorigenesis. For instance, mitochondrial dysfunction and ROS stress can promote cancer cell motility through a CXCL14-mediated pathway [36].

AKT is a key downstream target of PI3K-mediated signaling pathway and it plays an important role in regulation of diverse cellular processes. Inappropriate activation of AKT has been reported in many types of human diseases, including cancer. As a major pathway involved in cell growth and proliferation, the AKT pathway has been intensively investigated. For instance, AKT plays a key role in the cell in response to growth factors; it controls cell survival, cell cycle progression, metabolism, and angiogenesis. Several known factors control AKT activity positively or negatively; a notable negative regulator is the tumor suppressor PTEN. ROS can deactivate several phosphatases including PTEN, leading to activation of AKT [37]. Activated AKT is capable of phosphorylating IKK, which is a kinase for IκBα, a direct inhibitor of NF-κB. Thus, AKT can serve as an upstream molecule for NF-κB in acidic conditions.

NF-κB is a ubiquitously expressed pleiotropic transcription factor that can be activated in response to a number of stimuli including low pHe [15, 28, 29]. Under normal conditions, NF-κB stays in the cytoplasm as a heterotrimeric complex consisting of the subunits p50, p65, and the inhibitory subunit IκBα. In response to inducing stimuli such as acidosis, IκBα undergoes phosphorylation, ubiquitination, and proteolytic degradation. The p65 subunit then undergoes phosphorylation and moves into the nucleus where it binds to specific DNA sequence and can activate the transcription of hundreds of genes [38]. The phosphorylation of IκBα is catalyzed by IκBα kinase (IKK), which consists of three subunits, IKK-α, IKK-β, and IKK-γ (also called NEMO). Aberrant regulation of NF-κB and the signaling pathways that control its activity often leads to inflammation, drug/radiation resistance, and tumorigenic potential of cancer cells [39]. Therefore, like AKT, NF-κB may also be critical to the acidosis-induced tumor initiation, progression, and metastasis.

4 Acid-sensing ion channels

Now, a key question is how tumor cells sense the acidic microenvironment and then transduce into the cell to impact gene expression. As discussed early, there are two major types of acid sensors, ASICs and proton-sensing G protein-coupled receptors. Since the primary focus of this review is on ASICs, we will provide a brief background of ASICs.

ASICs belong to the epithelial sodium channels/degerin (ENaC/Deg) superfamily. ASICs are neuronal voltage-insensitive cationic channels activated by extracellular protons and they are largely responsible for the acid-evoked currents in neurons. Thus, ASICs are molecular acid sensors expressed in neurons throughout the body [40]. Knockout have identified ASIC1 as a key component of acid-activated currents which are important to the processes underlying synaptic plasticity, learning, and memory [41]. There is evidence supporting roles for ASICs in pain, neurological disease, and psychiatric disease [42, 43].

ASICs are widely expressed throughout the central and peripheral nervous system [44], except for ASIC5 that is primarily expressed in the small intestine [45]. The function of ASIC5 is not known. There are eight subunits of ASICs encoded by five different genes: ASIC1a, ASIC1b1, ASIC1b2, ASIC2a, ASIC2b, ASIC3, ASIC4, and ASIC5 [40, 45, 46]. ASIC1a is a primary acid sensor in the peripheral and central nervous system. ASICs can form functional homo or hetero trimmers [47, 48]. For example, ASIC1a can form a homotrimeric or heterotrimeric complex with ASIC2b. All ASICs are expressed in the peripheral nervous system while ASIC1a, 2a, 2b, and 4 are also expressed in the central nervous system. The amino acid sequences of ASIC subunits are well conserved among different species. For example, the mouse ASIC1a and the human ASIC1a share over 98% of their amino acid sequence identity.

Acidosis is a common feature of ischemia and plays a critical role in brain injury. Overactivation of ASIC1a can lead to neuron injury. For example, ASIC2b and ASIC1a have been implicated in acidosis-induced neuronal death [49]. On the other hand, ASIC3 plays a protective role in the inflammatory arthritis by limiting inflammation through enhanced synoviocyte cell death [50]. ASICs have also been implicated in production of reactive oxygen species (ROS) in cerebral ischemia/reperfusion injury [51].

Various ASIC modulators have been identified and reported. In particular, some animal toxins have been shown to specifically inhibit ASICs. Psalmotoxin-1 (PcTx1), a potent toxin isolated from the venom of tarantula spider, possesses sub-nanomolar affinity for ASIC1 [49]. Unlike amiloride which inhibits ASICs and other channels such as sodium–hydrogen exchangers (NHEs) and epithelial sodium channels (ENaCs) [52], PcTx1 is a specific inhibitor to ASIC1. The sea anemone toxin APETx2 inhibits ASIC3-containing channels

with $IC_{50} < 100$ nM [53]. On the other hand, a snake toxin (MitTx) from the Texas coral snake is a potent and selective agonist for ASICs and causes opening of the channels [54]. Recently, co-crystal structures of chicken ASIC1a with MitTx have been reported, which indicates that MitTx-induced open state of an ASIC1 can serve as a selectivity filter of voltage-independent and sodium-selective ion channels [55]. Therefore, in addition to being potential therapeutics, these reagents can also serve as important tools for characterization of ASIC-mediated physiological and disease processes.

5 Role of ASICs in cancer

Although ASICs are expressed primarily in neurons of peripheral sensory and central nervous systems, there are reports suggesting that ASIC currents can be detected in glia, smooth muscle cells, and osteoclasts [61–64]. ASICs are upregulated in glioma, which is not surprising because they are derived from the same neuronal stem cells as neuronal cells do. Particular relevant to this review is that overexpression of ASICs is able to impact the growth and migration of glioblastoma cells [60, 61]. For example, glioma cation current is mediated by mixed ASIC1 and ASIC2; inhibition of this conductance decreases glioma growth and cell migration [60]. It has also been shown that knockdown of ASIC1 or ENaC inhibits glioblastoma cell migration [62]. In contrast to ASIC1, increasing surface expression of ASIC2 suppresses the proliferation and migration of glioblastoma cells [63].

Of interest, ASICs have been also shown to be expressed in other types of cancer. For instance, we have shown that ASICs are expressed in a subset of breast tumors, and breast cancer cell lines [32]. This upregulation of ASIC1 may in part attribute to the capability of ASIC1 to promote cell invasion and metastasis. Another example is ASIC2 which is upregulated in colorectal cancer specimens and its high expression can predict poor outcomes of patients with colorectal cancer. Of interest, acidic exposure can upregulate expression of ASIC2. Furthermore, ASIC2 overexpression promotes, whereas ASIC2 knockdown inhibits, cell proliferation *in vitro* and *in vivo*. Importantly, ASIC2 promotes CRC cell invasion under acidosis *in vitro* and liver metastasis *in vivo* [64]. This may be attributed to the activation of calcineurin/NFAT1 signaling pathway under acidosis. These observations suggest that ASICs play an important role in cancer and they may serve as potential therapeutic targets.

6 ASICs as an important mediator for acidosis-induced cell signaling

Now, we turn our focus on the importance of ASICs in acidosis-mediated cell signaling, leading to tumor cell invasion and metastasis. In particular, we will discuss how acidosis induces ROS production, and activation of AKT and NF-κB in an ASIC-dependent manner. Several lines of evidence suggest that there is acidosis-induced cell signaling where ASICs play a critical role (Fig. 1).

Acidosis induces NF-κB activation and cell invasion in breast and prostate cancer cells Although several studies have shown that acidosis promotes tumor cell growth and invasion, the detailed mechanism has not been elucidated. Thus, we first determined whether NF-κB is activated in response to acidosis because NF-κB is a known key factor for inflammation, and cancer development. When MCF-7 and MDA-MB-231 cells were exposed to pH 6.6 medium from 5 min to 48 h, the levels of nuclear NF-κB increased substantially in both cell lines [30]. This high level of p65 was maintained for at least 48 h. Consistent with these results, acidosis induced degradation of IκBα, a well-known NF-κB inhibitor [65]. Moreover, immunofluorescence (IF) staining also supports that acidosis induced the nuclear localization of p65 (Fig. 2). This was not limited to breast cancer cells. We also detected acidosis-induced activation of NF-κB in several prostate cancer cell lines [31]. These results suggest that low pHe can induce NF-κB activation and cell invasion. Further studies suggest that the acidosis-induced NF-κB activation is dependent on ASICs and ROS production (see discussion below).

Another interesting observation is that acidosis induces AKT activation in a time-dependent manner. As expected, acidosis increases the phosphorylation of AKT (pAKT at Ser473) in MCF-7 and MDA-MB-231 cells while the total level of AKT is unaffected under the same conditions. Especially, this is a time-dependent increase. For example, starting from 5 min, the pAKT level was gradually increased

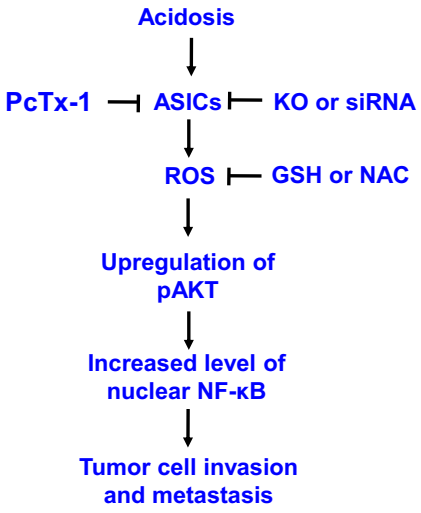

Fig. 1 ASIC is required for acidosis-induced ROS-AKT-NF-κB pathway, leading to cell invasion and metastasis. GSH, glutathione; NAC, N-acetylcysteine; KO, knock out; siRNA, small interfering RNA; PcTx1, psalmotoxin; ROS, reactive oxygen species

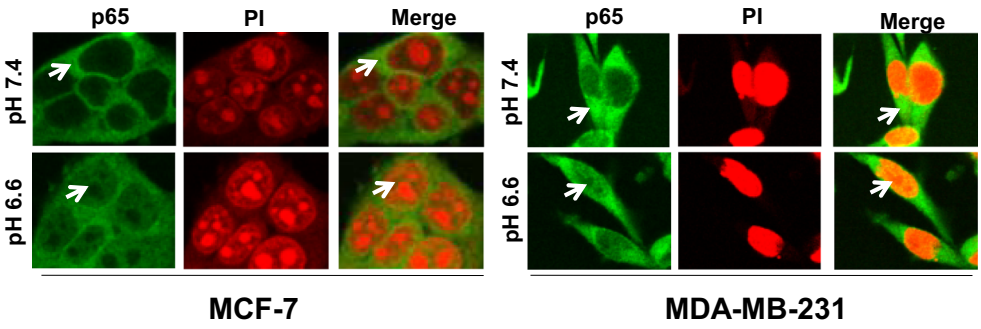

Fig. 2 Nuclear localization of NF-κB p65 in breast cancer cells under acidic environment. Cells were cultured at pH 7.4 or pH 6.6 for 1 h before fixation and immunefluorescent staining

until 2 h and then decreased to the baseline level in MCF-7 cells. From 24 h, the pAKT level started to increase again [30]. Thus, AKT activation appears to act upstream of NF-κB because AKT activation occurs earlier than NF-κB activation in response to acidosis. Moreover, AKT activation is transient whereas NF-κB activation lasts much longer.

7 Acidosis promotes ROS generation

Several studies show that acidosis induced the formation of lung metastases depends on elevated ROS production; thus, ROS plays a critical role in metastases of acidosis-primed tumor cells *in vivo* [66]. Under acidic conditions, fatty acid-derived acetyl-coA becomes oxidized, which may contribute to increased ROS production [67], highlighting the importance of fatty acid metabolism as a critical determinant of tumor cell proliferation under acidosis. This acidosis-induced ROS production may be due to mitochondrial functional impairment related to mitochondrial permeability [68].

Intensive studies have been carried out on cross-talk between ROS generation, and NF-κB and AKT activation [69], leading to invasion and metastasis. For example, acidosis significantly increases the formation of lung metastases of two rat carcinoma cell lines *in vivo* and promotes cellular motility [66].

Indeed, we showed that acid-induced ROS production in both MCF-7 and MDA-MB-231 cells as early as 5 min exposure [30]. Moreover, two well-known ROS scavengers, glutathione (GSH) and N-acetyl cysteine (NAC), both abrogated the acidosis-induced ROS production. Furthermore, both GSH and NAC suppresses the acidosis-induced NF-κB and AKT activation in a concentration-dependent manner. Finally, the acidosis-induced ROS promotes cell invasion which can be blocked by NAC.

A question is how ROS and AKT are connected. PT EN is a well-known tumor suppressor and it serves as a phosphatase to remove phosphate group attached to the 3′-hydroxyl group of the PI(3,4,5)P3 [70]. Therefore, inhibition of PTEN elevates intracellular level of PI(3,4,5)P3, leading to AKT activation. Of interest, acidosis is able to inactivate PTEN by oxidation, suggesting that PTEN is also involved in acidosis-induced AKT activation. This is consistent with the findings that catalytically active form of PTEN is able to suppress basal activation of AKT [71, 72]. Furthermore, acidosis enhances the level of pAKT in cells expressing wild-type PTEN but not in those expressing mutant PTEN [30], suggesting that acidosis-mediated ROS production induces disulfide bond formation between Cys^{71} and Cys^{124} of PTEN, leading to the loss of its phosphatase activity, and enhanced AKT phosphorylation. Thus, acidosis induces AKT activation through thiol modification of PTEN, and NADPH oxidase (NOX) may contribute to acidosis-induced ROS generation.

Acidosis-induced activation of AKT and NF-κB is specific to cancer cells Of interest, we detected acidosis-induced activation of AKT and NF-κB only in breast cancer cells, but not in non-malignant MCF-10A cells [30]. For example, we detected an obvious induction of NF-κB and AKT at 30 and 60 min exposure to pH 6.6 in MCF-7 cells; there was no such induction in MCF-10A cells under the same conditions. Moreover, there was a same trend for acidosis-induced ROS generation in these two cell lines [30]. In neutrophils, although acidosis can induce activation of AKT/ERK, it does not activate NF-κB [73], further supporting that the effect of acidosis on NF-κB signaling is cancer-specific. This may provide a basis for therapeutic targeting acidosis associated cell signaling.

ASIC1 is expressed in breast cancer cells and it promotes the acidosis-induced cell invasion To search for upstream regulators responsible for the acidosis-induced ROS generation, and activation of AKT and NF-κB, leading to increased cell invasion, we identify ASICs, in particular ASIC1, as a key player in the acidosis-induced signaling in breast cancer cells [32]. Although ASICs are primarily expressed in neuronal cells, we detected ASIC1 expression in breast tumors. For example, ASIC1 is expressed in a number of cell lines. The highest level of ASIC1 was detected in LM-4142 cell line [32], which was originally derived from MDA-MB-231 [74]. ASIC1 is a primary acid sensor and more sensitive to extracellular pH changes than ASIC2a [75]. Experiments with ASIC1 CRISPR-mediated KO and RNAi demonstrated an important

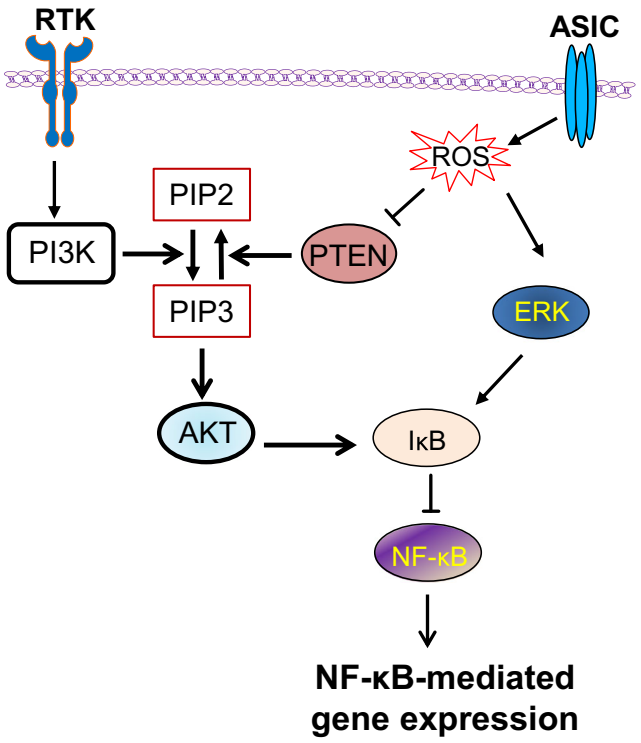

Fig. 3 Acidosis induces NF-kB through AKT-PTEN or ERK depending on the cellular context

role of ASIC1 in sensing the extracellular acidic environment and promoting breast tumor growth and invasion [32].

Of interest, patch clamp assay, a commonly used technique for measuring ion channel function [76], with ASIC1 KO cells demonstrated that while we detected current in control cells in the acidic condition, no current was detected in ASIC1 knockout cells [32]. These results suggest that ASIC1 is also functional in cancer cells.

Role of ERK pathway in ASIC-mediated NF-κB activation It is well known that tumor cells often carry deletions or mutation of PTEN [77] or mutations in the PI3K genes [78], producing highly active AKT. In this case, acidosis-induced cell signaling may take alternative pathways such as mitogen-activated protein kinase (MAPK) pathway (see Thews, this volume). Like AKT, MAPKs are a highly conserved family of serine/threonine protein kinases involved in a variety of important cellular processes such as proliferation, differentiation, motility, stress response, apoptosis, and survival. Extracellular stimuli such as mitogens, cytokines, growth factors, and environmental stress cause a cascade of MAPK signaling through phosphorylation of MAP3K, MAP2K (e.g., MEK), and MAPK (e.g., ERK). ERK serves as a substrate for MEK. Once activated by MEK, ERK can regulate a variety of downstream targets. Our study suggests that ERK plays a critical role in acidosis-induced NF-κB activation in cancer cells carrying a highly active AKT because acidosis activates ERK in a time-dependent manner and ERK inhibitors or ERK siRNAs suppress the acidosis-induced NF-κB activation. Finally, ERK inhibitors or ERK siRNAs suppress the acidosis-induced IκBα phosphorylation [31]. These findings are in line with the report that ASIC1 is also required for ERK activation in glioma cells [79]. Therefore, ERK may serve as an alternative signaling molecule in acidosis-mediated cell signaling (Fig. 3).

8 ASICs as potential therapeutic targets

Since ASICs are sensitive to inhibition by amiloride [80], we determined whether this compound can inhibit tumor growth in a xenograft nude mouse model. LM-4142 cells were injected into the mammary fat pads of female nude mice, followed by amiloride injection (i.p.). The tumor weight was suppressed by 56% in the amiloride group as compared to vehicle control [32]. Such amiloride treatment had no effect on the body weight of the mice, suggesting that mice are well tolerated at this dose. Since amiloride has multiple targets including ENaC and urokinase-type plasminogen activator [81], in addition to ASICs, it is important to determine whether amiloride-mediated suppression of tumor growth is in part through suppression of ASICs. Furthermore, intratumoral injection of PcTx1 indeed caused a reduction of tumor growth. Finally, xenograft mouse model with ASIC1 siRNA showed that ASIC1 siRNA also significantly suppressed tumor growth [32], further supporting the role of ASIC1 in tumorigenesis, and ASIC1 may serve as a therapeutic target. Given that extracellular acidosis is a common feature for solid tumors, these findings may have a broad impact. Thus, it is conceivable that ASIC1-mediated tumor cell invasion and metastasis can also occur in other types of solid tumors.

9 Conclusions and perspectives

Accumulating evidence suggests that acidic tumor microenvironment can have multiple effects on tumorigenesis through a cascade of cell signaling events such as ROS production and activation of AKT and NF-κB. Apparently, tumor cells have adapted well to extracellular acidosis, and furthermore, they use this as a means to promote their invasion and metastasis. Among many players, ASICs play a critical role in cancer development and metastasis by sensing the external acidic microenvironment. Thus, there is a critical need for a better understanding of how ASICs impact these acidosis-induced signaling events. For instance, it would be important to determine whether ASICs are key players in tumor cells that express ASICs for tumor progression and metastasis in the acidic tumor microenvironment.

In particular, how ASICs regulate ROS generation and activation of the AKT/NF-κB associated signaling pathways

will provide new insight into cancer biology in the context of the interaction between tumor cells and the acidic tumor microenvironment. Furthermore, this ASIC-mediated cell signaling may provide an opportunity for intervention in cancer therapy. For instance, ROS production is a general phenomenon in response to acidosis and ROS can cause cell death. However, co-evolution may help cancer cells to survive in the high level of ROS whereas normal or non-malignant cells may lack such a mechanism so that they cannot survive well under acidosis. Therefore, this high level of ROS in cancer cells may help them to achieve the acidosis-induced signaling cascades [82]. Evidently, this selectivity may provide a great opportunity for intervention. The availability of specific ASIC inhibitors will stimulate these studies.

One potential approach would be to neutralize the acidity in tumor environment. For example, oral administration of sodium bicarbonate is sufficient to increase peritumoral pH and inhibit tumor growth and local invasion in HCT-116 xenograft model in preclinical setting [10]. In clinical setting for large hepatocarcinoma, transarterial chemoembolization (TACE) with bicarbonate local infusion showed a significant benefit as compared to TACE without bicarbonate [83]. In addition, tumor acidosis also affects host immune system. For example, melanomas with highly acidic tumor microenvironment can regulate transcriptional repressor ICER in tumor-associated macrophages to promote their non-inflammatory phenotype, leading to tumor growth [22]. Thus, it is important to determine whether such intervention has any effect on immune cells including macrophages and infiltrated T-lymphocytes. An alternative approach would be to target acid sensors (e.g., ASICs) because they are the major players in acidosis-induced cell signaling. However, a challenge is that tumors are often very heterogeneous, and they may express different types of acid sensors even within the same tumor.

We hope that these special reviews will stimulate further discussion and attract more researchers to this field. Although much has been learned regarding how tumors maintain normal pHi under such conditions and how acidic pHe benefits tumor cells, there is still a lot to be learned, particularly how tumor cells sense the acidic signal to regulate gene expression. For instance, our unpublished results indicated that a number of long non-coding RNAs (lncRNAs) are induced in response to acidosis. Thus, it would be interesting to determine whether any of these lncRNAs are involved in acidosis-induced cell signaling. If so, it would be important to determine the underlying mechanism.

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References

1. Justus, C. R., Dong, L., & Yang, L. V. (2013). Acidic tumor microenvironment and pH-sensing G protein-coupled receptors. [review]. *Frontiers in Physiology, 4*, 354. https://doi.org/10.3389/fphys.2013.00354.
2. Damaghi, M., Wojtkowiak, J. W., & Gillies, R. J. (2013). pH sensing and regulation in cancer. [review]. *Frontiers in Physiology, 4*, 370. https://doi.org/10.3389/fphys.2013.00370.
3. Ludwig, M. G., Vanek, M., Guerini, D., Gasser, J. A., Jones, C. E., Junker, U., Hofstetter, H., Wolf, R. M., & Seuwen, K. (2003). Proton-sensing G-protein-coupled receptors. *Nature, 425*(6953), 93–98. https://doi.org/10.1038/nature01905.
4. Seuwen, K., Ludwig, M. G., & Wolf, R. M. (2006). Receptors for protons or lipid messengers or both? *Journal of Receptor and Signal Transduction Research, 26*(5–6), 599–610. https://doi.org/10.1080/10799890600932220.
5. Singh, L. S., Berk, M., Oates, R., Zhao, Z., Tan, H., Jiang, Y., Zhou, A., Kirmani, K., Steinmetz, R., Lindner, D., & Xu, Y. (2007). Ovarian cancer G protein-coupled receptor 1, a new metastasis suppressor gene in prostate cancer. *Journal of the National Cancer Institute, 99*(17), 1313–1327. https://doi.org/10.1093/jnci/djm107.
6. Hashim, A. I., Zhang, X., Wojtkowiak, J. W., Martinez, G. V., & Gillies, R. J. (2011). Imaging pH and metastasis. [Review]. *NMR in Biomedicine, 24*(6), 582–591. https://doi.org/10.1002/nbm.1644.
7. Tannock, I. F., & Rotin, D. (1989). Acid pH in tumors and its potential for therapeutic exploitation. [Research Support, Non-U.S. Gov't]. *Cancer Research, 49*(16), 4373–4384.
8. Persi, E., Duran-Frigola, M., Damaghi, M., Roush, W. R., Aloy, P., Cleveland, J. L., Gillies, R. J., & Ruppin, E. (2018). Systems analysis of intracellular pH vulnerabilities for cancer therapy. *Nature Communications, 9*(1), 2997. https://doi.org/10.1038/s41467-018-05261-x.
9. Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. [Research Support, N.I.H., Extramural Review]. *Cell, 144*(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013.
10. Estrella, V., Chen, T., Lloyd, M., Wojtkowiak, J., Cornnell, H. H., Ibrahim-Hashim, A., Bailey, K., Balagurunathan, Y., Rothberg, J. M., Sloane, B. F., Johnson, J., Gatenby, R. A., & Gillies, R. J. (2013). Acidity generated by the tumor microenvironment drives local invasion. *Cancer Research, 73*(5), 1524–1535. https://doi.org/10.1158/0008-5472.CAN-12-2796.
11. Gatenby, R. A., & Gillies, R. J. (2004). Why do cancers have high aerobic glycolysis? [review]. *Nature Reviews. Cancer, 4*(11), 891–899. https://doi.org/10.1038/nrc1478.
12. Tosco, M., Porta, C., Sironi, C., Laforenza, U., & Orsenigo, M. N. (2011). Acute and chronic acidosis influence on antioxidant equipment and transport proteins of rat jejunal enterocyte. *Cell Biology International, 35*(4), 345–353. https://doi.org/10.1042/CBI20100428.
13. Riemann, A., Schneider, B., Ihling, A., Nowak, M., Sauvant, C., Thews, O., & Gekle, M. (2011). Acidic environment leads to ROS-induced MAPK signaling in cancer cells. *PLoS One, 6*(7), e22445. https://doi.org/10.1371/journal.pone.0022445.
14. Lamonte, G., Tang, X., Chen, J. L., Wu, J., Ding, C. K., Keenan, M. M., et al. (2013). Acidosis induces reprogramming of cellular metabolism to mitigate oxidative stress. *Cancer Metab, 1*(1), 23. https://doi.org/10.1186/2049-3002-1-23.
15. Peppicelli, S., Bianchini, F., Contena, C., Tombaccini, D., & Calorini, L. (2013). Acidic pH via NF-kappaB favours VEGF-C

expression in human melanoma cells. [Research Support, Non-U.S. Gov't]. *Clinical & Experimental Metastasis, 30*(8), 957–967. https://doi.org/10.1007/s10585-013-9595-4.

16. Wojtkowiak, J. W., Rothberg, J. M., Kumar, V., Schramm, K. J., Haller, E., Proemsey, J. B., et al. (2012). Chronic autophagy is a cellular adaptation to tumor acidic pH microenvironments. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Cancer Research, 72*(16), 3938–3947. https://doi.org/10.1158/0008-5472.CAN-11-3881.
17. Bourguignon, L. Y., Singleton, P. A., Diedrich, F., Stern, R., & Gilad, E. (2004). CD44 interaction with Na+-H+ exchanger (NHE1) creates acidic microenvironments leading to hyaluronidase-2 and cathepsin B activation and breast tumor cell invasion. [Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. *The Journal of Biological Chemistry, 279*(26), 26991–27007. https://doi.org/10.1074/jbc.M311838200.
18. He, B., Zhang, M., & Zhu, R. (2010). Na+/H+ exchanger blockade inhibits the expression of vascular endothelial growth factor in SGC7901 cells. [Research Support, Non-U.S. Gov't]. *Oncology Reports, 23*(1), 79–87.
19. Ryder, C., McColl, K., Zhong, F., & Distelhorst, C. W. (2012). Acidosis promotes Bcl-2 family-mediated evasion of apoptosis: involvement of acid-sensing G protein-coupled receptor Gpr65 signaling to Mek/Erk. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *The Journal of Biological Chemistry, 287*(33), 27863–27875. https://doi.org/10.1074/jbc.M112.384685.
20. Thews, O., Dillenburg, W., Fellner, M., Buchholz, H. G., Bausbacher, N., Schreckenberger, M., & Rösch, F. (2010). Activation of P-glycoprotein (Pgp)-mediated drug efflux by extracellular acidosis: in vivo imaging with 68Ga-labelled PET tracer. [Research Support, Non-U.S. Gov't]. *European Journal of Nuclear Medicine and Molecular Imaging, 37*(10), 1935–1942. https://doi.org/10.1007/s00259-010-1504-3.
21. Corbet, C., & Feron, O. (2017). Tumour acidosis: from the passenger to the driver's seat. *Nature Reviews. Cancer, 17*(10), 577–593. https://doi.org/10.1038/nrc.2017.77.
22. Bohn, T., Rapp, S., Luther, N., Klein, M., Bruehl, T. J., Kojima, N., Aranda Lopez, P., Hahlbrock, J., Muth, S., Endo, S., Pektor, S., Brand, A., Renner, K., Popp, V., Gerlach, K., Vogel, D., Lueckel, C., Arnold-Schild, D., Pouyssegur, J., Kreutz, M., Huber, M., Koenig, J., Weigmann, B., Probst, H. C., von Stebut, E., Becker, C., Schild, H., Schmitt, E., & Bopp, T. (2018). Tumor immunoevasion via acidosis-dependent induction of regulatory tumor-associated macrophages. *Nature Immunology, 19*, 1319–1329. https://doi.org/10.1038/s41590-018-0226-8.
23. Rofstad, E. K., Mathiesen, B., Kindem, K., & Galappathi, K. (2006). Acidic extracellular pH promotes experimental metastasis of human melanoma cells in athymic nude mice. [Research Support, Non-U.S. Gov't]. *Cancer Research, 66*(13), 6699–6707. https://doi.org/10.1158/0008-5472.CAN-06-0983.
24. Moellering, R. E., Black, K. C., Krishnamurty, C., Baggett, B. K., Stafford, P., Rain, M., et al. (2008). Acid treatment of melanoma cells selects for invasive phenotypes. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Clinical & Experimental Metastasis, 25*(4), 411–425. https://doi.org/10.1007/s10585-008-9145-7.
25. Lee, W. Y., Huang, S. C., Hsu, K. F., Tzeng, C. C., & Shen, W. L. (2008). Roles for hypoxia-regulated genes during cervical carcinogenesis: somatic evolution during the hypoxia-glycolysis-acidosis sequence. [Research Support, Non-U.S. Gov't]. *Gynecologic Oncology, 108*(2), 377–384. https://doi.org/10.1016/j.ygyno.2007.10.034.
26. Castellone, R. D., Leffler, N. R., Dong, L., & Yang, L. V. (2011). Inhibition of tumor cell migration and metastasis by the proton-sensing GPR4 receptor. [Research Support, Non-U.S. Gov't]. *Cancer Letters, 312*(2), 197–208. https://doi.org/10.1016/j.canlet.2011.08.013.
27. Matsubara, T., Diresta, G. R., Kakunaga, S., Li, D., & Healey, J. H. (2013). Additive influence of extracellular pH, oxygen tension, and pressure on invasiveness and survival of human osteosarcoma cells. *Frontiers in Oncology, 3*, 199. https://doi.org/10.3389/fonc.2013.00199.
28. Xu, L., & Fidler, I. J. (2000). Acidic pH-induced elevation in interleukin 8 expression by human ovarian carcinoma cells. [Research Support, U.S. Gov't, P.H.S.]. *Cancer Research, 60*(16), 4610–4616.
29. Shi, Q., Le, X., Wang, B., Xiong, Q., Abbruzzese, J. L., & Xie, K. (2000). Regulation of interleukin-8 expression by cellular pH in human pancreatic adenocarcinoma cells. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Journal of Interferon & Cytokine Research, 20*(11), 1023–1028. https://doi.org/10.1089/10799900050198471.
30. Gupta, S. C., Singh, R., Pochampally, R., Watabe, K., & Mo, Y. Y. (2014). Acidosis promotes invasiveness of breast cancer cells through ROS-AKT-NF-kappaB pathway. *Oncotarget, 5*(23), 12070–12082. https://doi.org/10.18632/oncotarget.2514.
31. Chen, B., Liu, J., Ho, T. T., Ding, X., & Mo, Y. Y. (2016). ERK-mediated NF-kappaB activation through ASIC1 in response to acidosis. *Oncogenesis, 5*(12), e279. https://doi.org/10.1038/oncsis.2016.81.
32. Gupta, S. C., Singh, R., Asters, M., Liu, J., Zhang, X., Pabbidi, M. R., Watabe, K., & Mo, Y. Y. (2016). Regulation of breast tumorigenesis through acid sensors. *Oncogene, 35*(31), 4102–4111. https://doi.org/10.1038/onc.2015.477.
33. Brown, N. S., & Bicknell, R. (2001). Hypoxia and oxidative stress in breast cancer. Oxidative stress: its effects on the growth, metastatic potential and response to therapy of breast cancer. [review]. *Breast Cancer Research, 3*(5), 323–327.
34. Wang, X., Martindale, J. L., Liu, Y., & Holbrook, N. J. (1998). The cellular response to oxidative stress: influences of mitogen-activated protein kinase signalling pathways on cell survival. *The Biochemical Journal, 333*(Pt 2), 291–300.
35. Brown, N. S., Jones, A., Fujiyama, C., Harris, A. L., & Bicknell, R. (2000). Thymidine phosphorylase induces carcinoma cell oxidative stress and promotes secretion of angiogenic factors. [Research Support, Non-U.S. Gov't]. *Cancer Research, 60*(22), 6298–6302.
36. Pelicano, H., Lu, W., Zhou, Y., Zhang, W., Chen, Z., Hu, Y., & Huang, P. (2009). Mitochondrial dysfunction and reactive oxygen species imbalance promote breast cancer cell motility through a CXCL14-mediated mechanism. [Research Support, N.I.H., Extramural]. *Cancer Research, 69*(6), 2375–2383. https://doi.org/10.1158/0008-5472.CAN-08-3359.
37. Gough, D. R., & Cotter, T. G. (2011). Hydrogen peroxide: a Jekyll and Hyde signalling molecule. [Research Support, Non-U.S. Gov't Review]. *Cell Death & Disease, 2*, e213. https://doi.org/10.1038/cddis.2011.96.
38. Gupta, S. C., Sundaram, C., Reuter, S., & Aggarwal, B. B. (2010). Inhibiting NF-kappaB activation by small molecules as a therapeutic strategy. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. *Biochimica et Biophysica Acta, 1799*(10–12), 775–787. https://doi.org/10.1016/j.bbagrm.2010.05.004.
39. Aggarwal, B. B. (2004). Nuclear factor-kappaB: the enemy within. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S. Review]. *Cancer Cell, 6*(3), 203–208. https://doi.org/10.1016/j.ccr.2004.09.003.
40. Waldmann, R., Champigny, G., Bassilana, F., Heurteaux, C., & Lazdunski, M. (1997). A proton-gated cation channel involved in acid-sensing. [Research Support, Non-U.S. Gov't]. *Nature, 386*(6621), 173–177. https://doi.org/10.1038/386173a0.

41. Wemmie, J. A., Chen, J., Askwith, C. C., Hruska-Hageman, A. M., Price, M. P., Nolan, B. C., Yoder, P. G., Lamani, E., Hoshi, T., Freeman, J. H., Jr., & Welsh, M. J. (2002). The acid-activated ion channel ASIC contributes to synaptic plasticity, learning, and memory. [In Vitro Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. *Neuron, 34*(3), 463–477.
42. Wemmie, J. A., Taugher, R. J., & Kreple, C. J. (2013). Acid-sensing ion channels in pain and disease. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. *Nature Reviews. Neuroscience, 14*(7), 461–471. https://doi.org/10.1038/nrn3529.
43. Mazzuca, M., Heurteaux, C., Alloui, A., Diochot, S., Baron, A., Voilley, N., Blondeau, N., Escoubas, P., Gélot, A., Cupo, A., Zimmer, A., Zimmer, A. M., Eschalier, A., & Lazdunski, M. (2007). A tarantula peptide against pain via ASIC1a channels and opioid mechanisms. [Research Support, Non-U.S. Gov't]. *Nature Neuroscience, 10*(8), 943–945. https://doi.org/10.1038/nn1940.
44. Wemmie, J. A., Askwith, C. C., Lamani, E., Cassell, M. D., Freeman, J. H., Jr., & Welsh, M. J. (2003). Acid-sensing ion channel 1 is localized in brain regions with high synaptic density and contributes to fear conditioning. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. *The Journal of Neuroscience, 23*(13), 5496–5502.
45. Schaefer, L., Sakai, H., Mattei, M., Lazdunski, M., & Lingueglia, E. (2000). Molecular cloning, functional expression and chromosomal localization of an amiloride-sensitive Na(+) channel from human small intestine. [Research Support, Non-U.S. Gov't]. *FEBS Letters, 471*(2–3), 205–210.
46. Chu, X. P., & Xiong, Z. G. (2012). Physiological and pathological functions of acid-sensing ion channels in the central nervous system. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. *Current Drug Targets, 13*(2), 263–271.
47. Gonzales, E. B., Kawate, T., & Gouaux, E. (2009). Pore architecture and ion sites in acid-sensing ion channels and P2X receptors. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Nature, 460*(7255), 599–604. https://doi.org/10.1038/nature08218.
48. Jasti, J., Furukawa, H., Gonzales, E. B., & Gouaux, E. (2007). Structure of acid-sensing ion channel 1 at 1.9 A resolution and low pH. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Nature, 449*(7160), 316–323. https://doi.org/10.1038/nature06163.
49. Sherwood, T. W., Lee, K. G., Gormley, M. G., & Askwith, C. C. (2011). Heteromeric acid-sensing ion channels (ASICs) composed of ASIC2b and ASIC1a display novel channel properties and contribute to acidosis-induced neuronal death. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *The Journal of Neuroscience, 31*(26), 9723–9734. https://doi.org/10.1523/JNEUROSCI.1665-11.2011.
50. Sluka, K. A., Rasmussen, L. A., Edgar, M. M., O'Donnell, J. M., Walder, R. Y., Kolker, S. J., Boyle, D. L., & Firestein, G. S. (2013). Acid-sensing ion channel 3 deficiency increases inflammation but decreases pain behavior in murine arthritis. *Arthritis and Rheumatism, 65*(5), 1194–1202. https://doi.org/10.1002/art.37862.
51. Liu, L., Zhang, R., Liu, K., Zhou, H., Yang, X., Liu, X., Tang, M., Su, J., & Dong, Q. (2009). Tissue kallikrein protects cortical neurons against in vitro ischemia-acidosis/reperfusion-induced injury through the ERK1/2 pathway. [Research Support, Non-U.S. Gov't]. *Experimental Neurology, 219*(2), 453–465. https://doi.org/10.1016/j.expneurol.2009.06.021.
52. Matthews, H., Ranson, M., & Kelso, M. J. (2011). Anti-tumour/metastasis effects of the potassium-sparing diuretic amiloride: an orally active anti-cancer drug waiting for its call-of-duty? [review]. *International Journal of Cancer, 129*(9), 2051–2061. https://doi.org/10.1002/ijc.26156.
53. Diochot, S., Baron, A., Rash, L. D., Deval, E., Escoubas, P., Scarzello, S., Salinas, M., & Lazdunski, M. (2004). A new sea anemone peptide, APETx2, inhibits ASIC3, a major acid-sensitive channel in sensory neurons. [Research Support, non-U.S. Gov't]. *The EMBO Journal, 23*(7), 1516–1525. https://doi.org/10.1038/sj.emboj.7600177.
54. Bohlen, C. J., Chesler, A. T., Sharif-Naeini, R., Medzihradszky, K. F., Zhou, S., King, D., Sánchez, E. E., Burlingame, A. L., Basbaum, A. I., & Julius, D. (2011). A heteromeric Texas coral snake toxin targets acid-sensing ion channels to produce pain. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Nature, 479*(7373), 410–414. https://doi.org/10.1038/nature10607.
55. Baconguis, I., Bohlen, C. J., Goehring, A., Julius, D., & Gouaux, E. (2014). X-ray structure of acid-sensing ion channel 1-snake toxin complex reveals open state of a Na(+)-selective channel. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Cell, 156*(4), 717–729. https://doi.org/10.1016/j.cell.2014.01.011.
56. Chen, X., Kalbacher, H., & Grunder, S. (2005). The tarantula toxin psalmotoxin 1 inhibits acid-sensing ion channel (ASIC) 1a by increasing its apparent H+ affinity. [Research Support, Non-U.S. Gov't]. *The Journal of General Physiology, 126*(1), 71–79. https://doi.org/10.1085/jgp.200509303.
57. Chung, W. S., Farley, J. M., Swenson, A., Barnard, J. M., Hamilton, G., Chiposi, R., & Drummond, H. A. (2010). Extracellular acidosis activates ASIC-like channels in freshly isolated cerebral artery smooth muscle cells. [Research Support, N.I.H., Extramural]. *American Journal of Physiology. Cell Physiology, 298*(5), C1198–C1208. https://doi.org/10.1152/ajpcell.00511.2009.
58. Jahr, H., van Driel, M., van Osch, G. J., Weinans, H., & van Leeuwen, J. P. (2005). Identification of acid-sensing ion channels in bone. *Biochemical and Biophysical Research Communications, 337*(1), 349–354. https://doi.org/10.1016/j.bbrc.2005.09.054.
59. Lin, Y. C., Liu, Y. C., Huang, Y. Y., & Lien, C. C. (2010). High-density expression of Ca2+−permeable ASIC1a channels in NG2 glia of rat hippocampus. [Research Support, non-U.S. Gov't]. PLoS One, 5(9), doi:https://doi.org/10.1371/journal.pone.0012665.
60. Berdiev, B. K., Xia, J., McLean, L. A., Markert, J. M., Gillespie, G. Y., Mapstone, T. B., Naren, A. P., Jovov, B., Bubien, J. K., Ji, H. L., Fuller, C. M., Kirk, K. L., & Benos, D. J. (2003). Acid-sensing ion channels in malignant gliomas. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *The Journal of Biological Chemistry, 278*(17), 15023–15034. https://doi.org/10.1074/jbc.M300991200.
61. Bubien, J. K., Keeton, D. A., Fuller, C. M., Gillespie, G. Y., Reddy, A. T., Mapstone, T. B., & Benos, D. J. (1999). Malignant human gliomas express an amiloride-sensitive Na+ conductance. [Research Support, U.S. Gov't, P.H.S.]. *The American Journal of Physiology, 276*(6 Pt 1), C1405–C1410.
62. Kapoor, N., Bartoszewski, R., Qadri, Y. J., Bebok, Z., Bubien, J. K., Fuller, C. M., & Benos, D. J. (2009). Knockdown of ASIC1 and epithelial sodium channel subunits inhibits glioblastoma whole cell current and cell migration. [Research Support, N.I.H., Extramural]. *The Journal of Biological Chemistry, 284*(36), 24526–24541. https://doi.org/10.1074/jbc.M109.037390.
63. Vila-Carriles, W. H., Kovacs, G. G., Jovov, B., Zhou, Z. H., Pahwa, A. K., Colby, G., Esimai, O., Gillespie, G. Y., Mapstone, T. B., Markert, J. M., Fuller, C. M., Bubien, J. K., & Benos, D. J. (2006). Surface expression of ASIC2 inhibits the amiloride-sensitive current and migration of glioma cells. [Research Support, N.I.H., Extramural]. *The Journal of Biological Chemistry, 281*(28), 19220–19232. https://doi.org/10.1074/jbc.M603100200.
64. Zhou, Z. H., Song, J. W., Li, W., Liu, X., Cao, L., Wan, L. M., Tan, Y. X., Ji, S. P., Liang, Y. M., & Gong, F. (2017). The acid-sensing ion channel, ASIC2, promotes invasion and metastasis of colorectal cancer under acidosis by activating the calcineurin/NFAT1 axis.

Journal of Experimental & Clinical Cancer Research, 36(1), 130. https://doi.org/10.1186/s13046-017-0599-9.

65. Jacobs, M. D., & Harrison, S. C. (1998). Structure of an IkappaBalpha/NF-kappaB complex. [Research Support, Non-U.S. Gov't]. *Cell, 95*(6), 749–758.
66. Riemann, A., Schneider, B., Gundel, D., Stock, C., Gekle, M., & Thews, O. (2016). Acidosis promotes metastasis formation by enhancing tumor cell motility. *Advances in Experimental Medicine and Biology, 876*, 215–220. https://doi.org/10.1007/978-1-4939-3023-4_27.
67. Corbet, C., Pinto, A., Martherus, R., Santiago de Jesus, J. P., Polet, F., & Feron, O. (2016). Acidosis drives the reprogramming of fatty acid metabolism in cancer cells through changes in mitochondrial and histone acetylation. *Cell Metabolism, 24*(2), 311–323. https://doi.org/10.1016/j.cmet.2016.07.003.
68. Teixeira, J., Basit, F., Swarts, H. G., Forkink, M., Oliveira, P. J., Willems, P., et al. (2018). Extracellular acidification induces ROS- and mPTP-mediated death in HEK293 cells. *Redox Biology, 15*, 394–404. https://doi.org/10.1016/j.redox.2017.12.018.
69. Morgan, M. J., & Liu, Z. G. (2011). Crosstalk of reactive oxygen species and NF-kappaB signaling. [Research Support, N.I.H., Intramural Review]. *Cell Research, 21*(1), 103–115. https://doi.org/10.1038/cr.2010.178.
70. Khan, K. H., Yap, T. A., Yan, L., & Cunningham, D. (2013). Targeting the PI3K-AKT-mTOR signaling network in cancer. [Research Support, Non-U.S. Gov't Review]. *Chinese Journal of Cancer, 32*(5), 253–265. https://doi.org/10.5732/cjc.013.10057.
71. Cantley, L. C., & Neel, B. G. (1999). New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. [Review]. *Proceedings of the National Academy of Sciences of the United States of America, 96*(8), 4240–4245.
72. Papa, A., Wan, L., Bonora, M., Salmena, L., Song, M. S., Hobbs, R. M., Lunardi, A., Webster, K., Ng, C., Newton, R. H., Knoblauch, N., Guarnerio, J., Ito, K., Turka, L. A., Beck, A. H., Pinton, P., Bronson, R. T., Wei, W., & Pandolfi, P. P. (2014). Cancer-associated PTEN mutants act in a dominant-negative manner to suppress PTEN protein function. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Cell, 157*(3), 595–610. https://doi.org/10.1016/j.cell.2014.03.027.
73. Martinez, D., Vermeulen, M., Trevani, A., Ceballos, A., Sabatte, J., Gamberale, R., Alvarez, M. E., Salamone, G., Tanos, T., Coso, O. A., & Geffner, J. (2006). Extracellular acidosis induces neutrophil activation by a mechanism dependent on activation of phosphatidylinositol 3-kinase/Akt and ERK pathways. *Journal of Immunology, 176*(2), 1163–1171.
74. Minn, A. J., Kang, Y., Serganova, I., Gupta, G. P., Giri, D. D., Doubrovin, M., Ponomarev, V., Gerald, W. L., Blasberg, R., & Massagué, J. (2005). Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. *The Journal of Clinical Investigation, 115*(1), 44–55.
75. Bianchi, L., & Driscoll, M. (2002). Protons at the gate: DEG/ENaC ion channels help us feel and remember. [Review]. *Neuron, 34*(3), 337–340.
76. Sakmann, B., & Neher, E. (1984). Patch clamp techniques for studying ionic channels in excitable membranes. [Research Support, Non-U.S. Gov't Review]. *Annual Review of Physiology, 46*, 455–472. https://doi.org/10.1146/annurev.ph.46.030184.002323.
77. Rodriguez-Escudero, I., Oliver, M. D., Andres-Pons, A., Molina, M., Cid, V. J., & Pulido, R. (2011). A comprehensive functional analysis of PTEN mutations: implications in tumor- and autism-related syndromes. [Research Support, Non-U.S. Gov't]. *Human Molecular Genetics, 20*(21), 4132–4142. https://doi.org/10.1093/hmg/ddr337.
78. Yuan, T. L., & Cantley, L. C. (2008). PI3K pathway alterations in cancer: variations on a theme. [Research Support, N.I.H., Extramural Review]. *Oncogene, 27*(41), 5497–5510. https://doi.org/10.1038/onc.2008.245.
79. Rooj, A. K., McNicholas, C. M., Bartoszewski, R., Bebok, Z., Benos, D. J., & Fuller, C. M. (2012). Glioma-specific cation conductance regulates migration and cell cycle progression. [Research Support, N.I.H., Extramural]. *The Journal of Biological Chemistry, 287*(6), 4053–4065. https://doi.org/10.1074/jbc.M111.311688.
80. Benarroch, E. E. (2014). Acid-sensing cation channels: structure, function, and pathophysiologic implications. *Neurology, 82*(7), 628–635. https://doi.org/10.1212/WNL.0000000000000134.
81. Vassalli, J. D., & Belin, D. (1987). Amiloride selectively inhibits the urokinase-type plasminogen activator. [In Vitro Research Support, Non-U.S. Gov't]. *FEBS Letters, 214*(1), 187–191.
82. Trachootham, D., Alexandre, J., & Huang, P. (2009). Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? [Research Support, N.I.H., Extramural Review]. *Nature Reviews. Drug Discovery, 8*(7), 579–591. https://doi.org/10.1038/nrd2803.
83. Chao, M., Wu, H., Jin, K., Li, B., Wu, J., Zhang, G., Yang G., Hu X. (2016). A nonrandomized cohort and a randomized study of local control of large hepatocarcinoma by targeting intratumoral lactic acidosis. *Elife,* 5, doi:https://doi.org/10.7554/eLife.15691, A nonrandomized cohort and a randomized study of local control of large hepatocarcinoma by targeting intratumoral lactic acidosis, 5.