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Zn^{2+} induces apoptosis in human highly metastatic SHG-44 glioma cells, through inhibiting activity of the voltage-gated proton channel Hv1

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ABSTRACT

In contrast to the voltage-gated K^+ channels, the voltage-gated proton channel Hv1 contains a voltage-sensor domain but lacks a pore domain. Here, we showed that Hv1 is expressed in the highly metastatic glioma cell SHG-44, but lowly in the poorly metastatic glioma cell U-251. Inhibition of Hv1 activity by 140 μM zinc chloride induces apoptosis in the human highly metastatic glioma cells. Zn^{2+} ions markedly inhibit proton secretion, and reduce the gelatinase activity in the highly metastatic glioma cells. *In vivo*, the glioma tumor sizes of the implantation of the SHG-44 xenografts in nude mice that were injected zinc chloride solution, were dramatically smaller than that in the controlled groups. The results demonstrated that the inhibition of Hv1 activity via Zn^{2+} ions can effectively retard the cancer growth and suppress the cancer metastasis by the decrease of proton extrusion and the down-regulation of gelatinase activity. Our results suggest that Zn^{2+} ions may be used as a potential anti-glioma drug for glioma therapy.

1. Introduction

Voltage-gated proton (Hv) currents were recorded first in snail neurons [1], and later found in a lot of mammalian cells, such as alveolar epithelia, brain microglia, skeletal muscle, and blood cells including macrophages, neutrophils, and eosinophils [2–5]. However, the molecular identity of the voltage-gated proton channels was discovered for a long time. Until 2006, the human and mouse voltage-gated proton channels, called Hv1 and VSOP respectively, were identified using bioinformatics searches based on known cation channels (Hv1) and the voltage sensor domain of *Ciona intestinalis* VSP (VSOP) [6,7].

Hv1 was proposed to regulate intracellular pH during oxygen consumption associated with phagocytosis, called "respiratory burst" [3,8]. Hv1 is activated by depolarization and intracellular acidification, whose activity maintains intracellular pH neutral to keep reactive oxygen species (ROS) generation [9,10]. Hv1 not only regulates pH in cytoplasm, but can also provide protons in the phagosome, a closed membrane compartment for killing and digestion of a pathogen [3]. Hv1 is extremely selective for H^+, with no detectable permeability to other cations [11,12]. The voltage activation relationship of Hv1 depends strongly on both the intracellular pH (pH_i) and extracellular pH (pH_o). Increasing pH_o or lowering pH_i promotes H^+ channel opening by shifting the activation threshold to more negative potentials [3]. Furthermore, Hv1 current is inhibited by submillimolar concentrations of Zn^{2+} and Cd^{2+} and other divalent cations [13].

Hv1 contains three predicted domains: N-terminal acid and proline-rich domain, transmembrane voltage-sensor domain (VSD), and C-terminal domain. Voltage-gated K^+ channels are comprised of four subunits, each of which has a pore domain and a VSD. The four pore domains come together to form one single central pore, and four peripheral VSDs control the gate of the pore [14]. In contrast to the voltage-gated K^+ channels, the Hv1 contains a VSD but lacks the pore domain. Recent studies showed that Hv1 functions as a dimer in which the intracellular C-terminal domain is responsible for the dimeric architecture of the protein, and each subunit contains its own proton-transporting pathway [15–18]. The intracellular C-terminal domain of Hv1 forms a dimer via a parallel α-helical coiled-coil and is essential for the protein localization [17].

Tumor cells often exist in a hypoxic microenvironment, and possess high-glycolytic activity and produce acidic metabolites [19,20]. To avoid the acidosis resulting from reducing in cytosolic pH, tumor cells must extrude excessing cytosolic protons to maintain cytosolic pH, which results in acidic tumor microenvironment. The hypoxic and acidic tumor microenvironment plays a key role in cancer development, progression, and metastasis [19]. Our previous work showed that Hv1 regulates intracellular pH in breast cancer cells and plays a key role in tumor acidic microenvironment [21,22]. In the present study, we showed that Zn^{2+} ions induce apoptosis in human highly metastatic glioma, through inhibiting

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activity of the voltage-gated proton channel Hv1. Our results suggest that Zn^{2+} ions may be used as a potential anti-glioma drug for glioma tumor therapy.

2. Materials and methods

2.1. Generation of a polyclonal anti-Hv1 antibody

A polyclonal anti-Hv1 antibody was generated against the carboxyl terminal domain of Hv1. The antigen protein was purified to homogeneity after expression in *Escherichia coli* [23]. The purified antigen protein was injected into three rabbits and the polyclonal anti-Hv1 antibody was purified by an rProtein A Sepharose (GE, Healthcare) column. HRP-conjugated goat anti-rabbit IgGs were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA).

2.2. Cell culture

The human glioma tumor cell lines SHG-44 and U-251 were cultivated at 37 °C in an atmosphere of 95% air and 5% CO_2 with RPMI 1640 medium (GIBCO) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 20 mM L-glutamine.

2.3. Quantitative real-time PCR

The mRNA expression levels of Hv1 in glioma cell lines, SHG-44 and U-251, were evaluated by quantitative real-time PCR as described in our previous work [21] Relative mRNA expression levels of proteins were calculated according to the equation: $2^{-\Delta\Delta CT}$, in which $\Delta\Delta CT = (CT_{Hv1} - CT_{GAPDH}) - (CT_{Hv1} - CT_{GAPDH})_{SHG\text{-}44}$. All experiments were performed in triplicate.

2.4. Immunofluorescence cytochemistry

Immunofluorescence cytochemistry was carried out as described in our previous work [22]. Anti-Hv1 antibody (1.0 mg/ml) was diluted at 1:100, and FITC-conjugated goat anti-rabbit IgG at 1:400 in 5% fetal bovine serum and 2% BSA in PBST.

2.5. Suppressing Hv1 mRNA expression

The sequence of the siRNA targeting the Hv1 gene was 5′-CTACAAGAAATGGGAGAAT-3′, and the random sense sequence was 5′-TTCTCCGAACGTGTCACGT-3′, both of which were obtained from Ribobio (Guangzhou, China) [21]. The final concentration of siRNA was 100 nM. Silencing was examined 48 h after transfection. The efficiency of siRNA in suppressing Hv1 expression was determined by quantitative real-time PCR.

2.6. Cell survival rate assay

The survival rates of SHG-44 and U-251 cells were assayed by MTT [22]. Cells prepared at a concentration of 5×10^3 cells/100 μl were distributed in 96-well plates at 100 μl/well and cultured overnight. The medium in 96-well plates was replaced by the medium containing a concentration of 100 μM $ZnCl_2$ or a variety of $ZnCl_2$ concentrations, and the cells were cultured for 48 h.

2.7. Migration kinetics

Migration kinetics of SHG-44 and U-251 cells was assessed in wounded monolayer model [21]. To inhibit Hv1 activity, RPMI 1640 medium containing 100 μM $ZnCl_2$ was used. Cell movement was observed under phase-contrast microscopy, and were captured with a digital camera every 24 h.

2.8. Invasion and migration assays

In vitro invasion and migration assays were performed to assess the effects of Hv1 on invasive and migratory abilities of SHG-44 and U-251 cells using transwells as described in our previous work [21]. To inhibit Hv1 activity, RPMI 1640 medium containing 100 μM $ZnCl_2$ was used. The inhibitory rate of invasion or migration was calculated as [migration cell No. of test/migration cell No. of $control_{SHG\text{-}44}$] × 100% and [invasion cell No. of test/invasion cell No. of $control_{SHG\text{-}44}$] × 100%, respectively.

2.9. Measurements of intracellular pH

Intracellular pH was measured in the monolayers using the pH-sensitive fluorescent probe BCECF-AM as described in our previous work [21].

2.10. Zymography

The activities of gelatinase in the supernatants of the cultured SHG-44 and U-251 cells were measured, as described in our previous work [21]. The medium was run in 10% SDS–PAGE (containing 0.1% gelatin). The gels were stained with Coomassie brilliant blue.

2.11. Tumor xenograft

SHG-44 cells were suspended in serum-free RPMI 1640 at density of 1×10^7 cells/ml. Each mouse (4–6 weeks of age, female, BalB/c nu^+/nu^+, from Academy of Military Science, Beijing) was s.c. injected into the left upper flank region. When the tumor volume reached ~$5 \times 5 \times 5$ mm^3 (about 1 week), 100 μl with 5 mM $ZnCl_2$ in 0.85% NaCl was injected into each tumor of experiment

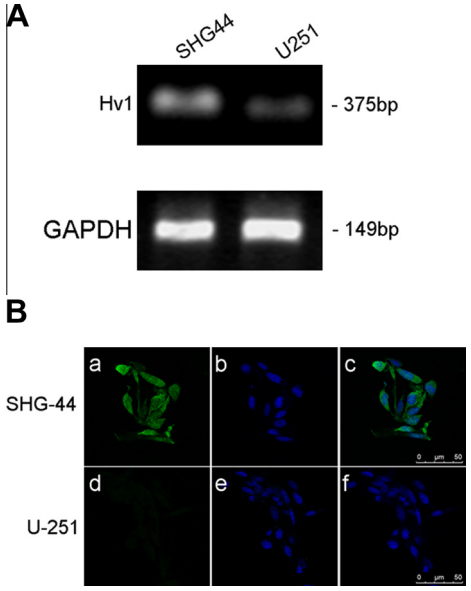

Fig. 1. Expression of Hv1 in glioma cells. (A) Expression of Hv1 mRNA in SHG-44 and U-251 cells detected by real time RT-PCR. Values are means ± SD ($n = 3$). (B) Expression of Hv1 protein in SHG-44 and U-251 cells. Observation for FITC fluorescence (a and d, green); DAPI stain to visualize the nuclei (b and e, blue); images merged the fluorescence of FITC and DAPI (c and f). A and B clearly showed that Hv1 is higher expressed in SHG-44 cells than U-251 cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

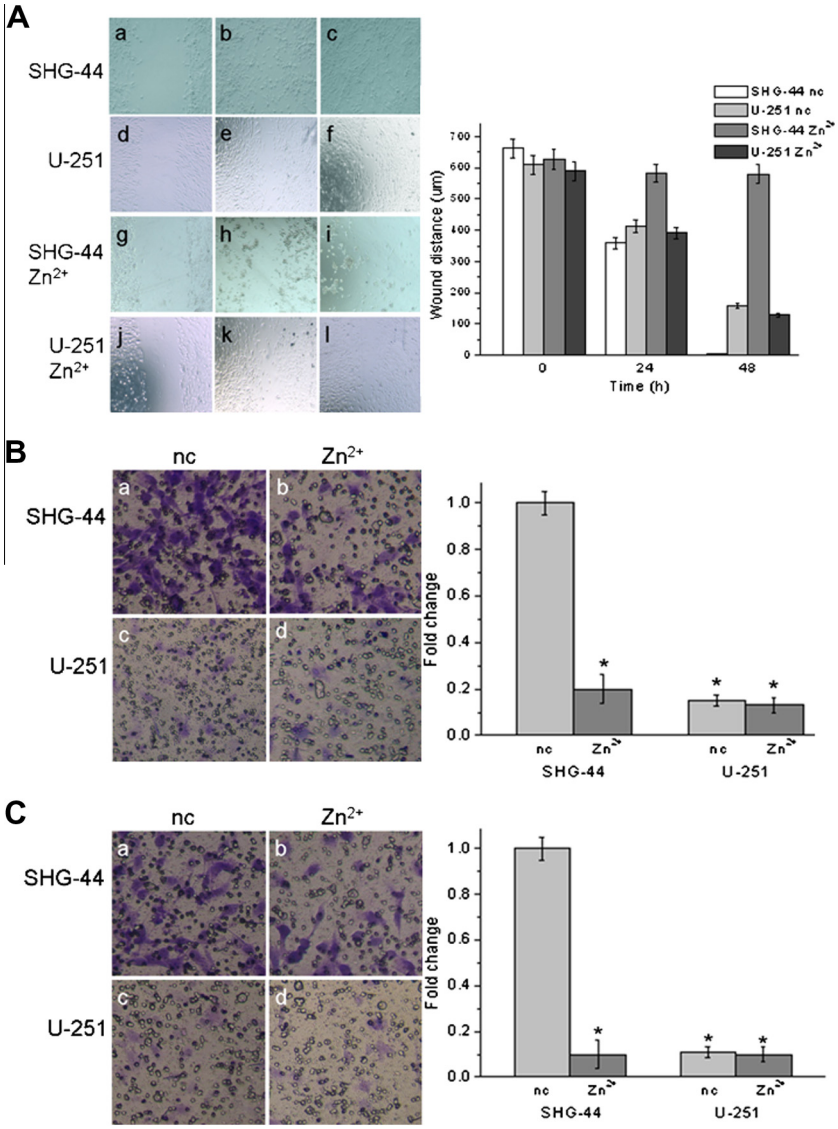

Fig. 2. Hv1 increases glioma cell migration and invasion. (A) Migration kinetics of SHG-44 and U-251 cells assayed by wounded monolayer model. The migration of SHG-44 cells (a–c, in left panel) is faster than U-251 (d–f, in left panel). Inhibition of Hv1 activity by Zn^{2+} significantly suppresses migration in SHG-44 cells (g–i, in left panel), but almost does not influence on U-251 cells (j–l, in left panel). Right panel in A shows the time-dependent wound distances of SHG-44 and U-251 cells. Values are means ± SD ($n = 5$). (B) and (C) Invasion and migration of SHG-44 and U-251 cells assayed by transwell. Migration and invasion of the highly metastatic SHG-44 cells are significantly suppressed by 100 μM $ZnCl_2$. Values are means ± SD ($n = 3$). $^*P < 0.05$, compared with SHG-44 negative control.

group everyday. After 3 weeks the mice of experiment and control groups were sacrificed.

2.12. Statistical analysis

All statistics were performed using SPSS16.0 software. Measurement data was represented as mean ± SD. Comparison of the mean between groups was performed by *t* test. *P* values <0.05 were considered significant. Survival analysis was assessed using Kaplan–Meier method and survival rate was compared by log-rank test.

3. Results

3.1. Expression of Hv1 in glioma cells

Hv1 is expressed in highly metastatic breast cancer tissues and cells, and relates with breast cancer development, progression, and metastasis [21,22]. To investigate the role of Hv1 in glioma cells, we examined whether Hv1 is expressed in SHG-44 and U-251 cells using quantitative real-time PCR and immunofluorescence with an anti-Hv1 polyclonal antibody. To examine the specificity of the antibody, 293T cells were transfected with pHv1-EGFP expression plasmid. And the expression of Hv1-EGFP was detected by immunocytochemistry and Western blotting with the antibody, and EGFP fluorescence (Supplementary Fig. S1). The results showed that the antibody specifically recognizes Hv1 and EGFP is a marker for Hv1 expression (Fig. 1A and B). As shown in Fig. 1A, real time RT-PCR clearly showed that Hv1 is expressed at a higher level in SHG-44 cells than that in U-251 cells. Expression of Hv1 in SHG-44 and U-251 cells was determined by a confocal microscopy. As shown in Fig. 1B, Hv1 was observed in SHG-44, but not in U-251 cells.

3.2. Zn^{2+} ions inhibit invasion and migration in the highly metastatic glioma SHG-44 cells

Invasion and migration are two prominent hallmarks of tumor malignancy. To evaluate the contribution of Hv1 to invasive and

migratory potential in glioma cells, we performed invasion and migration assays. First, we studied the kinetics of migratory ability of SHG-44 and U-251 cells. Fig. 2A (a–f) showed the migration kinetics of the two cell lines. A wounded monolayer of SHG-44 cells to allow for wound closure after 48 h (Fig. 2A, a–c). SHG-44 cells closed the wound faster than U-251 cells (Fig. 2A, d–f). Inhibition of Hv1 by 100 μM $ZnCl_2$ markedly decreased the migration in SHG-44 cells (Fig. 2A, g–i), but almost without affecting U-251 cells (Fig. 2A, j–l).

We then studied invasion and migration of SHG-44 and U-251 cells using transwell inserts. To inhibit Hv1 activity, RPMI 1640 medium containing 100 μM $ZnCl_2$ was used. Cells were placed into transwell inserts coated with Matrigel (invasion) or uncoated (migration) and assessed the rates of the invasion and migration of SHG-44 and U-251 cells after 24 h. The rates of the invasion and migration of SHG-44 and U-251 cells were shown in Fig. 2B and C. SHG-44 cells have obviously higher migratory and invasive abilities than U-251 cells. Inhibition of Hv1 activity remarkably decreased the invasion and migration of SHG-44 cells, but almost did not influence on U-251 cells.

3.3. Zn^{2+} induces apoptosis in the highly metastatic glioma SHG-44 cells

As shown in Fig. 2A(i), the addition of $ZnCl_2$ (a final concentration of 100 μM) into RPMI 1640 medium in SHG-44 cells for 48 h induced the apoptosis of SHG-44 cells, but not in U-251 cells. The effect of Zn^{2+} on the survival rate of SHG-44 and U-251 cells was studied *in vitro* and *in vivo*. Zn^{2+} ions induced apoptosis of SHG-44 cells with a time- and dose-dependences, whereas did not obviously affect on U-251 (Fig. 3A). Silencing Hv1 by siRNA in SHG-44 markedly inhibited the cell proliferation, but almost did not affect the U-251 cells down-regulated Hv1 by siRNA (Fig. 3A, right panel). To investigate the *in vivo* functional consequences of Hv1 expression on tumor growth, nude mice were injected s.c. with SHG-44 cells. The average size of xenografts in the experiment group that were injected with $ZnCl_2$ solution was dramatically smaller than that of the control groups (Fig. 3B). Thus, these results showed that invalidation of Hv1 brings about a dramatic decrease in tumor xenograft cell growth.

3.4. Hv1 regulates intracellular pH and gelatinase activity in SHG-44 cells

We evaluated whether inhibitions of Hv1 activity by 100 μM $ZnCl_2$ and protein expression by siRNA induces a decrease in intracellular pH in both SHG-44 and U-251 cells by BCECF fluorescence. As shown in Fig. 4B, inhibition of Hv1 activity in SHG-44 cells significantly increased acidity of intracellular pH from 7.51 to 7.32 and 7.35, which was consistent with that of silencing Hv1 by siRNA in SHG-44 cells, but not a remarkable change in U-251 cells. The finding showed that inhibition of Hv1 activity in SHG-44 cells notably suppressed proton extrusion. The above results revealed that the pH_i recovery was due to active Hv1. These results clearly showed that Hv1 regulates SHG-44 intracellular pH.

The secretion and activation of some ECM-degenerating proteases are pH-regulated. We examined the activity of MMP-2/MMP-9, which is closely related to cancer metastasis according to the previous reports [24]. Supernatants of the cultured SHG-44 and U-251 cells were collected, and the gelatinase activities were assayed. Zymography showed that the gelatinase activity in SHG-44 cells is higher than that in U-251 cells, and inhibition of Hv1 activity by 100 μM $ZnCl_2$ and expression by siRNA apparently reduced the activity of MMP-2 and MMP-9 in the supernatant of SHG-44 cell compared with U-251 cells, whereas the activity of MMP-2 and MMP-9 was almost independent of Hv1 activity, as shown in Fig. 4C, suggesting that Hv1 contributes to cellular invasiveness.

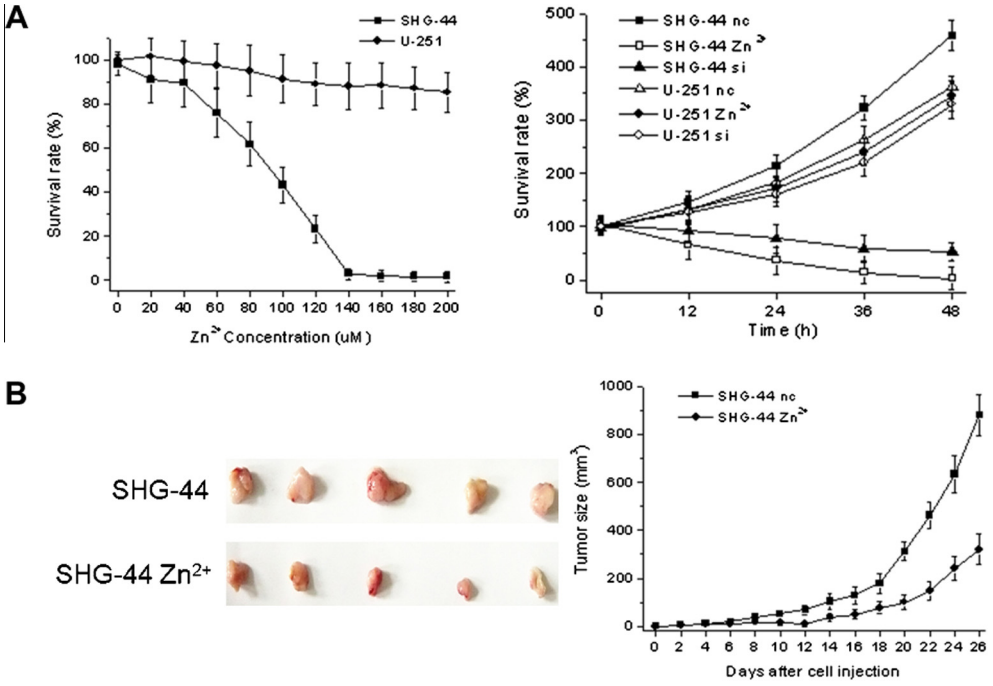

Fig. 3. Inhibition of Hv1 activity induces apoptosis in the highly metastatic glioma SHG-44 cells. (A) Zn^{2+} induces apoptosis in the highly metastatic glioma SHG-44 cells with a time (left panel, the medium containing 100 μM $ZnCl_2$ was used) and dose-dependent (right panel) manners. Down-regulated Hv1 by siRNA in SHG-44 markedly inhibited the cell proliferation (SHG-44 si), but almost did not affect the U-251 cells Hv1 silenced by siRNA (U-251 si). Values are means ± SD (n = 3). (B) Inhibition of Hv1 activity dramatically suppresses the rate of xenograft tumor growth.

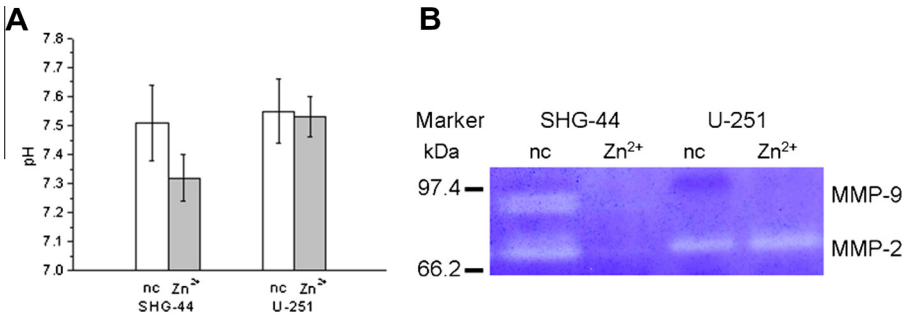

Fig. 4. (A) Expression of Hv1 in SHG-44 and U-251 cells, was detected by real time-PCR. Down-regulation of Hv1 expression was carried out by siRNA targeting Hv1 (si). (B) Suppressions of Hv1 expression by siRNA and activity by Zn^{2+} notably induces a decrease in intracellular pH in the highly metastatic SHG-44 cells. (C) The activity of gelatinase in SHG-44 cells is higher than that in U-251 cells, and inhibitions of Hv1 expression and activity apparently reduced the activity of gelatinase in SHG-44 cells, but almost does not influence on U-251 cells.

4. Discussion

The voltage-gated proton channel Hv1 extrudes intracellular protons to sustain the NADPH oxidase activity by compensating cellular loss of electrons with protons [3–6]. In present study, we showed that Hv1 is highly expressed in the highly metastatic glioma SHG-44 cells, and promotes migration and invasion of glioma cells through regulating intracellular pH and gelatinase activity. Inhibition of Hv1 activity induces apoptosis in SHG-44 cells. Our results clearly showed that SHG-44 cells have higher migratory and invasive abilities than U-251 cells. Therefore, we can come to a conclusion that SHG-44 cell line is higher metastatic cells than U-251 cell line. In our previous study we showed that Hv1 is highly expressed in the highly metastatic breast cancer cell line MDA-MB-231, but not in the poorly metastatic breast cancer cell line MCF-7 [21,22].

Maintenance of intracellular pH (pH_i) is vital for all biological processes in cells, which influences cell proliferation, cell motility, tumorigenesis, metastasis, and apoptosis [25–29]. Tumor cells often exist in a hypoxic microenvironment. The high-glycolytic metabolite in cancer cells results in an excessive production of intracellular acidity, and overly extruded acid induces extracellular acidification [30,31]. To survive the acidosis, tumor cells must exhibit a dynamic intracellular pH regulatory system. Several pH_i regulatory mechanisms in tumor cells have been proposed, such as Na^+/H^+ exchangers, bicarbonate (HCO_3^-) transporters, proton-lactate symporters, and proton pumps [19,26,31–33]. Our previous work demonstrated that high expression of Hv1 in breast tumor cells is related with tumor metastasis, development and progression [21,22]. Combined with the present study, we can come to a conclusion that the voltage-gated proton channel Hv1 is a new candidate for intracellular pH regulatory system in some cancer cells such as breast cancer and glioma cells.

The extrusion of the excessive intracellular acidity results in decreasing in extracellular pH. The low extracellular pH (pH_o) is one of hallmarks of malignant cells and optimal for cancer cells in solid tumors, on the contrary, it is toxic to normal cells [34,35]. The low pH_o increases extracellular matrix (ECM) digestion through increasing secretion and activation of proteases and remolding of ECM, induces apoptosis of adjacent normal cells, promotes angiogenesis, inhibits the host immune system, and thus contributes to cancer invasion and metastasis [30,36–38]. The activities of proteases needing a low extracellular pH to optimize their activation include cathepsin (cathepsin B, D), matrix metalloproteinase (MMP) (MMP-2, MMP-9 and MMP-3), bone morphogenetic protein-1-type metalloproteinases, tissue serine proteases, and adamalysin-related membrane proteases. Among them, the MMP family is essentially involved in degradation and remolding of ECM, due to their ability to collectively degrade all the structural components of the ECM [36,37]. The gelatinase activity in the highly metastatic SHG-44 cells is obviously higher than that in U-251 cells, and inhibition of Hv1 activity markedly decreases the gelatinase activity in SHG-44 cells, indicating that Hv1 promotes glioma invasion and metastasis through increasing gelatinase activity.

Voltage-gated proton channel Hv1 is activated by membrane voltage or pH gradients [3]. Zn^{2+} ion as an inhibitor of Hv1 is widely used to study the activity of Hv1 [3,6,7]. Inhibition of Hv1 activity by lower concentration of Zn^{2+} ions induces apoptosis in the highly metastatic SHG-44 cells, but not in the poorly metastatic U-251 cells. The mechanism is Zn^{2+} ions inhibit the extrusion of intracellular protons, and result in acidosis of glioma SHG-44 cells. Inhibition of Hv1 activity could inhibit the invasion and migration of the highly metastatic SHG-44 cells *in vitro* and *in vivo*, suggesting that Hv1 is involved in the invasion and migration of the highly metastatic glioma cells. The close relationship between Hv1 expression and glioma growth predicted that Hv1 might boost carcinogenesis and tumor progression. Zn^{2+} may be used as a novel drug for treatment of glioma.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.07.067.

References

[1] R. Thomas, R. Meech, Hydrogen ion currents and intracellular pH in depolarized voltage-clamped snail neurones, Nature 299 (1982) 826–828.
[2] T.E. DeCoursey, Hydrogen ion currents in rat alveolar epithelial cells, Biophys. J. 60 (1991) 1243–1253.
[3] T.E. Decoursey, Voltage-gated proton channels and other proton transfer pathways, Physiol. Rev. 83 (2003) 475–579.
[4] C. Eder, T.E. DeCoursey, Voltage-gated proton channels in microglia, Prog. Neurobiol. 64 (2001) 277–305.
[5] A. Kapus, R. Romanek, A.Y. Qu, O. Rotstein, S. Grinstein, A pH-sensitive and voltage-dependent proton conductance in the plasma membrane of macrophages, J. Gen. Physiol. 102 (1993) 729–760.
[6] I.S. Ramsey, M.M. Moran, J.A. Chong, D.E. Clapham, A voltage-gated proton-selective channel lacking the pore domain, Nature 440 (2006) 1213–1216.

[7] M. Sasaki, M. Takagi, Y. Okamura, A voltage sensor-domain protein is a voltage-gated proton channel, Science 312 (2006) 589–592.
[8] D. Morgan, M. Capasso, B. Musset, V.V. Cherny, E. Ríos, M.J.S. Dyer, T.E. DeCoursey, Voltage-gated proton channels maintain pH in human neutrophils during phagocytosis, Proc. Natl. Acad. Sci. USA 106 (2009) 18022–18027.
[9] R. Clark, K. Leidal, D. Pearson, W. Nauseef, NADPH oxidase of human neutrophils. Subcellular localization and characterization of an arachidonate-activatable superoxide-generating system, J. Biol. Chem. 262 (1987) 4065–4074.
[10] D. Morgan, V.V. Cherny, R. Murphy, B.Z. Katz, T.E. DeCoursey, The pH dependence of NADPH oxidase in human eosinophils, J. Physiol. 569 (2005) 419–431.
[11] V.V. Cherny, V.S. Markin, T.E. DeCoursey, The voltage-activated hydrogen ion conductance in rat alveolar epithelial cells is determined by the pH gradient, J. Gen. Physiol. 105 (1995) 861–896.
[12] T. DeCoursey, V. Cherny, Voltage-activated hydrogen ion currents, J. Membr. Biol. 141 (1994) 203–223.
[13] V.V. Cherny, T.E. DeCoursey, pH-dependent inhibition of voltage-gated H^+ currents in rat alveolar epithelial cells by Zn^{2+} and other divalent cations, J. Gen. Physiol. 114 (1999) 819–838.
[14] S.B. Long, E.B. Campbell, R. MacKinnon, Crystal structure of a mammalian voltage-dependent Shaker family K^+ channel, Science 309 (2005) 897–903.
[15] H.P. Koch, T. Kurokawa, Y. Okochi, M. Sasaki, Y. Okamura, H.P. Larsson, Multimeric nature of voltage-gated proton channels, Proc. Natl. Acad. Sci. USA 105 (2008) 9111–9116.
[16] S.-Y. Lee, J.A. Letts, R. MacKinnon, Dimeric subunit stoichiometry of the human voltage-dependent proton channel Hv1, Proc. Natl. Acad. Sci. USA 105 (2008) 7692–7695.
[17] S.J. Li, Q. Zhao, Q. Zhou, H. Unno, Y. Zhai, F. Sun, The role and structure of the carboxyl-terminal domain of the human voltage-gated proton channel Hv1, J. Biol. Chem. 285 (2010) 12047–12054.
[18] F. Tombola, M.H. Ulbrich, E.Y. Isacoff, The voltage-gated proton channel Hv1 has two pores, each controlled by one voltage sensor, Neuron 58 (2008) 546–556.
[19] S. Fais, A. De Milito, H. You, W. Qin, Targeting vacuolar H^+-ATPases as a new strategy against cancer, Cancer Res. 67 (2007) 10627–10630.
[20] E. Racker, Bioenergetics and the problem of tumor growth: an understanding of the mechanism of the generation and control of biological energy may shed light on the problem of tumor growth, Am. Sci. (1972) 56–63.
[21] Y. Wang, S.J. Li, J. Pan, Y. Che, J. Yin, Q. Zhao, Specific expression of the human voltage-gated proton channel Hv1 in highly metastatic breast cancer cells, promotes tumor progression and metastasis, Biochem. Biophys. Res. Commun. 412 (2011) 353–359.
[22] Y. Wang, S.J. Li, X. Wu, Y. Che, Q. Li, Clinicopathological and biological significance of human voltage-gated proton channel Hv1 protein overexpression in breast cancer, J. Biol. Chem. 287 (2012) 13877–13888.
[23] S.J. Li, Q. Zhao, Q. Zhou, Y. Zhai, Expression, purification, crystallization and preliminary crystallographic study of the carboxyl-terminal domain of the human voltage-gated proton channel Hv1, Acta Crystallogr. F 65 (2009) 279–281.
[24] G. Giannelli, S. Antonaci, Gelatinases and their inhibitors in tumor metastasis: from biological research to medical applications, Histol. Histopathol. 17 (2002) 339–345.
[25] J.-C. Chambard, J. Pouyssegur, Intracellular pH controls growth factor-induced ribosomal protein S6 phosphorylation and protein synthesis in the G0→ G1 transition of fibroblasts, Exp. Cell Res. 164 (1986) 282–294.
[26] R.A. Gottlieb, H.A. Giesing, J.Y. Zhu, R.L. Engler, B.M. Babior, Cell acidification in apoptosis: granulocyte colony-stimulating factor delays programmed cell death in neutrophils by up-regulating the vacuolar H^+-ATPase, Proc. Natl. Acad. Sci. USA 92 (1995) 5965–5968.
[27] R. Perona, R. Serrano, Increased pH and tumorigenicity of fibroblasts expressing a yeast proton pump, Nature 334 (1988) 438–440.
[28] A. Roos, W.F. Boron, Intracellular pH, Physiol. Rev. 61 (1981) 296–434.
[29] O. Schlappack, A. Zimmermann, R. Hill, Glucose starvation and acidosis: effect on experimental metastatic potential, DNA content and MTX resistance of murine tumour cells, Br. J. Cancer 64 (1991) 663–670.
[30] R.A. Gatenby, E.T. Gawlinski, The glycolytic phenotype in carcinogenesis and tumor invasion insights through mathematical models, Cancer Res. 63 (2003) 3847–3854.
[31] X. Lu, W. Qin, J. Li, N. Tan, D. Pan, H. Zhang, L. Xie, G. Yao, H. Shu, M. Yao, The growth and metastasis of human hepatocellular carcinoma xenografts are inhibited by small interfering RNA targeting to the subunit ATP6L of proton pump, Cancer Res. 65 (2005) 6843–6849.
[32] R. Gillies, R. Martinez-Zaguilan, Regulation of intracellular pH in BALB/c 3T3 cells. Bicarbonate raises pH via $NaHCO_3$/HCl exchange and attenuates the activation of Na^+/H^+ exchange by serum, J. Biol. Chem. 266 (1991) 1551–1556.
[33] J. Pouysségur, C. Sardet, A. Franchi, G. L'Allemain, S. Paris, A specific mutation abolishing Na^+/H^+ antiport activity in hamster fibroblasts precludes growth at neutral and acidic pH, Proc. Natl. Acad. Sci. USA 81 (1984) 4833–4837.
[34] R. Gillies, Z. Liu, Z. Bhujwalla, ^{31}P-MRS measurements of extracellular pH of tumors using 3-aminopropylphosphonate, Am. J. Physiol. Cell Physiol. 267 (1994) 195–203.
[35] J. Griffiths, Are cancer cells acidic?, Br J. Cancer 64 (1991) 425–427.
[36] G.F. Fasciglione, S. Marini, S. D'Alessio, V. Politi, M. Coletta, pH- and temperature-dependence of functional modulation in metalloproteinases. A comparison between neutrophil collagenase and gelatinases A and B, Biophys. J. 79 (2000) 2138–2149.
[37] L.L. Johnson, A.G. Pavlovsky, A.R. Johnson, J.A. Janowicz, C.-F. Man, D.F. Ortwine, C.F. Purchase II, A.D. White, D.J. Hupe, A rationalization of the acidic pH dependence for stromelysin-1 (matrix metalloproteinase-3) catalysis and inhibition, J. Biol. Chem. 275 (2000) 11026–11033.
[38] R. Martinez-Zaguilan, E.A. Seftor, R.E. Seftor, Y.-W. Chu, R.J. Gillies, M.J. Hendrix, Acidic pH enhances the invasive behavior of human melanoma cells, Clin. Exp. Metastasis 14 (1996) 176–186.