Participation of the Chaperone Hsc70 in the Trafficking and Functional Expression of ASIC2 in Glioma Cells*

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High-grade glioma cells express subunits of the ENaC/Deg superfamily, including members of ASIC subfamily. Our previous work has shown that glioma cells exhibit a basally active cation current, which is not present in low-grade tumor cells or normal astrocytes, and that can be blocked by amiloride. When ASIC2 is present within the channel complex in the plasma membrane, the channel is rendered non-functional because of inherent negative effectors that require ASIC2. We have previously shown that high-grade glioma cells functionally express this current because of the lack of ASIC2 in the plasma membrane. We now hypothesize that ASIC2 trafficking in glioma cells is regulated by a specific chaperone protein, namely Hsc70. Our results demonstrated that Hsc70 co-immunoprecipitates with ASIC2 and that it is overexpressed in glioma cells as compared with normal astrocytes. In contrast, there was no difference in the expression of calnexin, which also co-immunoprecipitates with ASIC2. In addition, glyceraldehyde and sodium 4-phenylbutyrate reduced the amount of Hsc70 expressed in glioma cells to levels found in normal astrocytes. Transfection of Hsc70 siRNA inhibited the constitutively activated amiloride-sensitive current, decreased migration, and increased ASIC2 surface expression in glioma cells. These results support an association between Hsc70 and ASIC2 that may underlie the increased retention of ASIC2 in the endoplasmic reticulum of glioma cells. The data also suggest that decreasing Hsc70 expression promotes reversion of a high-grade glioma cell to a more normal astrocytic phenotype.

Glioblastoma multiforme (GBM) are the most common type of primary tumors of the central nervous system. These tumors arise either from astrocytes or their progenitor cells (1). Gliomas have escaped most normal cell growth and cell death control mechanisms. In addition, GBMs possess an astounding ability to invade normal brain parenchyma over very long distances (2). Our laboratory has proposed that members of the epithelial Na\textsuperscript{+} channel and degenerin (ENaC/Deg) superfamily are important in the pathophysiology of high-grade, astrocyte-derived brain tumors, as we have found that cells derived from high-grade gliomas express a basally active, cation-selective current that is sensitive to amiloride. ENaC/Deg family members include the ASIC (acid-sensing ion channels) subfamily. We postulated that the glioma cation current is mediated by mixed ASIC and ENaC components, including ASIC2 (3) as ASIC1, ASIC2, and \( \gamma \)ENaC can be co-immunoprecipitated from glioma cells (3). The constitutive amiloride-sensitive cation channel activity is a specific feature of high-grade gliomas. In normal human astrocytes, no amiloride-sensitive current can be recorded, although we have shown that ASIC and ENaC subunits are expressed.

In our search for the key difference between glioma cells and normal astrocytes, we have previously shown that the increased current in gliomas is due to the loss of plasma membrane expression of ASIC2 (4). This failure in plasma membrane ASIC2 expression is manifested either as an absence in mRNA for ASIC2, because we can only detect ASIC2 mRNA in \( \sim \)50% of the glioma cell lines/tissue that we have examined or as a failure in trafficking of this subunit to its correct functional location in the plasma membrane, as ASIC2 protein can be detected in whole cell lysates, but is not found at the plasma membrane (4). Therefore, the objective of this work was to understand and define the pathways that regulate ASIC2 trafficking, and why it is dysfunctional in gliomas.

Our recent studies showed that addition of the chemical chaperone, glyceraldehyde, or the transcriptional regulator, sodium 4-phenylbutyrate (4PB), results in increased surface expression of ASIC2, a reduction in cell migration and proliferation, and loss of the amiloride-sensitive current in the glioma cell (4). These data supported our previous hypothesis that a failure of ASIC2 to traffic to the surface underlies the constitutively active cation current in glioma cells and contributes to the increased proliferation and migration characteristic of the glioma cell phenotype.

Previous studies of CFTR trafficking showed that glyceraldehyde and 4PB could rescue misfolded \( \Delta F508-CFTR \) and deliver it to the membrane (5, 6). Subsequent studies revealed that the rescue effect by 4PB was due to down-regulation of the molecular chaperone, heat shock cognate protein 70 (Hsc70) (5, 7). The data suggested that 4PB improves trafficking of \( \Delta F508-CFTR \) by allowing it to escape association with Hsc70.

Hsc70 resides in the nucleus and cytosol and is released as the proteins achieve their native conformation. Hsc70 remains attached to misfolded proteins where it can recruit the ubiquitin ligase CHIP, to facilitate proteasome-mediated degrada-
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Hsc70 overexpression causes a prolonged association with the client proteins and promotes their ubiquitination and degradation (8). Hsc70 binds directly to membrane channel proteins such as K⁺ channels and CFTR, and mediates their trafficking via multiple steps, including intracellular transport, endocytosis, and exocytosis (9–13).

In the present study we tested the hypothesis that ASIC2 trafficking is regulated by association with Hsc70 in GBMs. Using a combination of electrophysiological, cell biology, and biochemical techniques, we demonstrated that expression of Hsc70 is increased in glioma cells, and that this chaperone specifically binds ASIC2. Our results demonstrated that down-regulation of Hsc70 is associated with increased delivery of ASIC2 to the cell surface, decreased glioma cell migration, and loss of the constitutively active amiloride-sensitive cation current.

EXPERIMENTAL PROCEDURES

Cell Culture—Primary cultures of normal human astrocytes, primary cultured human glioblastoma cells (GBM), and U251-MG cells were obtained from the University of Alabama at Birmingham, Neurosurgery Brain Tissue Bank (Institutional Review Board approval X030403011). U87-MG cells were purchased from ATCC. SK-MG and D54-MG cells were gifts from Dr. Gregory Cairncross, University of Calgary, and Dr. Darell Bigner, Duke University, respectively. The cells were cultured in Dulbecco’s modified Eagle’s medium/F-12 (1:1) supplemented with 10% fetal bovine serum. All cells were maintained at 37 °C in a 95% O₂ and 5% CO₂ humidified incubator.

Transient Transfections—Glioblastoma cell lines were either transfected using Lipofectamine 2000 Transfection Reagent (Invitrogen) for 48 h following the manufacturer’s instructions or electroporated using the Electroporation Gene Pulser (Bio-Rad) and the following parameters: cuvette gap 0.4 cm, voltage 2000 V, and pulse width 1 ms. D54-MG and GBM cells were transfected with an annealed pre-designed siRNA targeting the human Hsc70 gene, HSPA8 (heat shock protein 8), with the following sequence: 5’-CCUAAUUUCGUAGCAAUU-3’, or a customized scrambled siRNA, 5’-CACUAAUUGCAUGAUAA-3’ (Ambion) at a final concentration of 100 nM. Normal human astrocytes were transfected with an annealed pre-designed siRNA targeting the human ASIC2 gene, ACCN1 (amiloride-sensitive cation channel 1), with the following sequence: 5’-GCCUCUAAUUUAUGAGACAA-3’, or a customized scrambled siRNA, 5’-AAAAUUUAAGGGCCUUCAA-3’ (Ambion) at a final concentration of 100 nM.

Antibodies, Immunoprecipitation, SDS-Polyacrylamide Gel Electrophoresis, and Western Blots—Rabbit anti-ASIC1 antibody raised against the peptide sequence DVSQHPNCPESL-RGHP or the rabbit anti-ASIC2 antibody (Alomone) were used for immunoprecipitation. The rat anti-Hsc70 monoclonal (1B5) antibody, mouse anti-actin monoclonal antibody (Abcam), rabbit anti-calnexin polyclonal antibody, rabbit anti-Grp78 polyclonal antibody, rat anti-Grp94 monoclonal antibody, mouse anti-Hsp70 monoclonal antibody, and mouse anti-Hsp90 monoclonal antibody (Stressgen) were used for Western blot detection. Briefly, for immunoprecipitation, 100-mm plates of confluent normal astrocytes, GBM, and glioblastoma cell lines were washed twice with PBS (138 mM NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 8 mM Na₂HPO₄, 1 mM MgCl₂, and 0.1 mM CaCl₂, pH 7.4). The cells were then lysed with 1% Triton X-100 in PBS supplemented with Complete Protease Inhibitors (Roche Applied Science). The lysate was placed into microcentrifuge tubes and sheared with a 23-gauge needle. 500 μg of supernatant from the lysate after centrifugation (15,800 × g for 20 min at 4 °C) was incubated with 2 μg of the corresponding antibody overnight at 4 °C on a rotator, followed by the addition of 50 μl of protein A-agarose beads. Beads were washed with PBS and pelleted five times. Samples were resuspended 1:1 (v/v) with Laemmli sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% bromphenol blue, 5% β-mercaptoethanol) and heated at 95 °C for 5 min. Specificity of the antibodies for immunoprecipitation was demonstrated by substitution of ChromoPure non-immune IgGs (Jackson ImmunoResearch Laboratories) for the primary antibodies. Standard electrophoresis and blotting protocols were followed. Briefly, proteins were run on 8% SDS-PAGE mini-gels with 4% stacking gels. Gels were transferred onto polyvinylidene difluoride membranes (Bio-Rad). Blots were blocked with 10% nonfat dry milk/Tris-buffered saline-Tween 20 overnight at 4 °C and probed with the appropriate antibody in Tris-buffered saline-Tween-20 for 1 h. After washing, blots were reacted with horseradish peroxidase-conjugated secondary antibody (Jackson ImmunoResearch Laboratories) and visualized using chemiluminescent reagents (Super Signal West Pico, Pierce) imaged onto Kodak X-Omat AR film. Gel analysis was done by obtaining the integrated density of each band using the Eagle Sight software (Stratagene).

Plasma Membrane Preparation—D54-MG cells were seeded in 100-mm culture dishes and transfected using Lipofectamine 2000 (Invitrogen) for 48 h with either Hsc70 siRNA or scrambled Hsc70 siRNA. Cells were scraped and collected by centrifugation at 600 × g for 5 min at 4 °C. A plasma membrane protein extraction kit (Bio-Rad) was used according to the manufacturer’s protocol to isolate specifically the plasma membrane from the total cellular membranes. We have previously shown that this method results in a glial membrane fraction enriched in plasma membrane markers (4). The plasma membrane fraction was dissolved in 0.5% Triton X-100 in PBS and plasma membrane protein concentration was measured using the BCA protein assay (Pierce).

Whole Cell Patch Clamp—GBM or D54-MG cells were electroporated with either Hsc70 or scrambled Hsc70 siRNA. Normal human astrocytes were electroporated with ASIC2 siRNA or scrambled ASIC2 siRNA for 48 h. Cells were placed in a chamber filled with RPMI 1640 culture medium (133 mM Na⁺, 5.3 mM K⁺, and 108.3 mM Cl⁻) and mounted on the stage of an inverted microscope. Once the cells adhered to the glass bottom of the chamber, micropipettes connected to an Axon 200A patch clamp amplifier and filled with an electrolyte solution containing (in mM) 100 K-glutamate, 30 KCl, 10 NaCl, 20 HEPES, 0.5 EGTA, <10 free Ca²⁺, and 4 ATP at pH 7.2 were abutted against the plasma membrane of individual cells. For whole cell patch clamping of normal human astrocytes, 100 mM LiCl (Eli Lilly Research Laboratories), a PKCβ inhibitor, was added to the pipette solution. Giga-ohm resistance seals
between the pipette tips and the cells were obtained by applying suction to the pipettes. Once the pipette tips were sealed to the cells, a sharp suction pulse was applied to rupture the membrane within the sealed area, leaving the seal intact. This procedure produced a low resistance electrical connection to the cellular interior. Once the whole cell configuration had been achieved, the stability of each preparation was monitored between voltage clamp protocols and during drug superfusions by clamping the membrane to positive and negative potentials continuously. The cells were held at a membrane potential of $V_{m} = -60 \text{ mV}$ and clamped sequentially for 800 ms each to membrane potentials of $V_{m} = 160$ to $40 \text{ mV}$ at intervals of 20 mV, returning to the holding potential of $-60 \text{ mV}$ for 800 ms between each test voltage. The continuous monitoring shows, in real time, the effects of inhibitors, such as amiloride, and acts as a quality control for the stability of the preparations. The currents were recorded digitally and filed in real time using the pCLAMP 6 software (Axon Instruments). Each recording under a given experimental condition was done on at least three different cells.

**FluoroBlock Migration Assay**—D54-MG cells and normal human astrocytes were seeded in 100-mm culture dishes and transfected using Lipofectamine 2000 (Invitrogen) with 1 μg of pCMV-eGFP and either Hsc70 or ASIC2 siRNA, scrambled Hsc70, or ASIC2 siRNA for 48 h prior to FluoroBlock migration assay. Transfection efficiency was determined by GFP expression before the cells were harvested. The average transfection efficiency was $\sim 30\%$ in all experiments. Filters were coated overnight with 5 μg/ml vitronectin (BIOSOURCE) in PBS 24 h before the assay. HTS FluoroBlock™ inserts (BD Falcon Labware) contain fluorescence blocking PET track-etched membranes with 8-μm pores. This migration assay allows for real-time viewing of cell migration without the need to end the

| Chaperones | % co-IP with ASIC1 ± S.D. | % co-IP with ASIC2 ± S.D. |
|------------|--------------------------|--------------------------|
| Grp94      | 40.74 ± 13.74            | 0                        |
| Hsp90      | 84.18 ± 7.65             | 0.025 ± 0.019            |
| Hsc70      | 0.084 ± 0.145            | 55.37 ± 21.98            |
| Grp78      | 68.89 ± 53.02            | 57.64 ± 46.41            |
| Calnexin   | 99.13 ± 18.44            | 67.05 ± 21.01            |
| Hsp70      | 0                        | 0                        |

**FIGURE 1. Co-immunoprecipitation of chaperones with ASIC1 and ASIC2 from D54-MG glioma cells.** A–D, gels were loaded with 50 μg of D54-MG lysate (control), 500 μg of D54-MG lysate immunoprecipitated (IP) with anti-ASIC1 (ASIC1), and 500 μg of D54-MG lysate IP with anti-ASIC2 antibody (ASIC2). Immunoblots were carried out with the following antibodies: A, rat anti-Grp94 monoclonal antibody, 98 kDa; mouse anti-Hsp90 monoclonal antibody, 90 kDa; B, rat anti-Hsc70 monoclonal antibody, 73 kDa; C, rabbit anti-Grp78 polyclonal antibody, 78 kDa; rabbit anti-calnexin polyclonal antibody, 90 kDa; D, mouse anti-Hsp70 monoclonal antibody, 70 kDa. Immunoblots of whole cell lysates show that the chaperone was expressed in the glioma cell in each case. Blots are representative of three similar experiments. The table shows the percentage of each chaperone protein that co-IP with either ASIC1 or ASIC2 ± S.D.

**FIGURE 2. Hsc70 is overexpressed in glioma cells.** A, immunoblot showing increased expression of Hsc70 in glioma cells as compared with normal astrocytes. 50 μg of cell lysate were immunobloted with rat anti-Hsc70 and mouse anti-actin antibodies. B, immunoblot showing that expression of calnexin in glioma cells is not significantly different from that in normal astrocytes. Bar graphs show the ratio of expression of each chaperone to actin, as assessed by band density, normalized to expression in normal astrocytes. The graphs represent mean values from three independent experiments ± S.D. (*, p < 0.05).
experiment and process the membranes. The FluoroBlock membrane prevents the transmission of light to cells on top of the membrane, thus only GFP-expressing cells migrating through the vitronectin-coated membrane can be viewed. Briefly, cells were lifted from their culture dishes, pelleted, and resuspended in Dulbecco’s modified Eagle’s medium/F-12 medium with 1% bovine serum albumin. 150,000 cells were added to the upper chamber. The cells were incubated at 37 °C for 24 h. LY379196 (100 nM) was added to the normal human astrocyte medium. GFP-expressing cells that had migrated through the pores to the lower side of the filter were counted in five random fields per filter using a Nikon TE200V microscope equipped for epifluorescence. Three filters equal an n of 1. This experiment was repeated three times.

RESULTS

Association between ASICs and Chaperone Proteins—We have previously shown that glioma cells exhibit a basally active cation current because trafficking of ASIC2, but not ASIC1, to the membrane is compromised (4). ASIC1 and ASIC2 are closely related proteins that exhibit an overall identity of 68% (14). Therefore, we needed to determine whether any chaperone proteins specifically interacted with ASIC2 but not ASIC1. Fig. 1 shows that chaperones exhibit varying specificities for both ASIC1 and ASIC2. Whereas Grp78 and calnexin interact with both ASIC subunits (Fig. 1C), Grp94 and Hsp90 appear to interact preferentially with ASIC1 (Fig. 1A). Importantly, only ASIC2 co-immunoprecipitated Hsc70; this chaperone does not seem to bind to ASIC1 (Fig. 1B). Somewhat surprisingly, Hsp70 did not co-precipitate with either ASIC1 or ASIC2 (Fig. 1D).

Hsc70 Is Overexpressed in Glioma Cells—Because ASIC2 specifically co-immunoprecipitated with Hsc70, we studied Hsc70 expression in glioma cells compared with nor-
normal astrocytes. Semiquantitative immunoblotting of lysates from glioma cell lines, GBM cells maintained in primary culture, and normal astrocytes using rat anti-Hsc70 and mouse anti-actin antibodies were done. The ratio of Hsc70 density to actin showed that Hsc70 is overexpressed in glioma and primary GBM cells as compared with normal astrocytes (Fig. 2A). In contrast, levels of an endoplasmic reticulum chaperone, calnexin, are similar in all cell types (Fig. 2B).

**Sodium 4-Phenylbutyrate and Glycerol Decrease Expression of Hsc70 in Gliomas**—It has been previously demonstrated that 4PB and glycerol decrease expression of Hsc70 in epithelial cells (7). If this also occurs in glioma cells, we should be able to detect this effect in immunoblots of Hsc70. Therefore, immunoblotting of normal astrocytes, primary cultures of GBM, and D54-MG cells following 48 h incubation with 5 mM 4PB and 500 mM glycerol were done. The ratio of Hsc70 density to actin showed that Hsc70 expression was markedly decreased by 4PB and glycerol. In contrast, levels of calnexin were not affected by either of these drugs (Fig. 3A). We have previously shown that 4PB and glycerol increase ASIC2 trafficking to the membrane and inhibit the constitutive current of glioma cells (4); we wanted to test the hypothesis that these compounds were affecting the interaction between Hsc70 and ASIC2. Thus, immunoprecipitation with anti-ASIC2 antibody and immunoblotting with anti-Hsc70 antibody of normal astrocytes, D54-MG cells, and primary cultures of GBM cells following 48 h incubation with 5 mM 4PB and 500 mM glycerol were done. Incubation with 4PB and glycerol reduced the amount of Hsc70 that was detected following co-immunoprecipitation with anti-ASIC2 (Fig. 3B). In both D54-MG cells and in the primary GBM cells, the extent of ASIC2/Hsc70 interaction in the presence of 4PB and glycerol was decreased to similar levels found in normal astrocytes.

**Knockdown of Hsc70 Expression in Glioma Cells with siRNA**—Although 4PB and glycerol decrease Hsc70 expression levels, these agents are relatively nonspecific. We wanted to test the hypothesis that the specific inhibition of Hsc70 expression would increase plasma membrane expression of ASIC2 and inhibit the amiloride-sensitive current as well as glioma cell migration. Thus, D54-MG and primary GBM cells were transfected with Hsc70 siRNA, and a scrambled Hsc70 siRNA as a negative control, and immunoblotted with rat anti-Hsc70 antibody and mouse anti-actin antibody. Hsc70 siRNA markedly reduced expression of Hsc70 protein, whereas the scrambled Hsc70 control siRNA had no effect (Fig. 4).

**Hsc70 siRNA Induces the Delivery of ASIC2 to the Plasma Membrane**—D54-MG cells were transfected with Hsc70 siRNA or scrambled Hsc70 siRNA. Plasma membranes were isolated as described previously (4), separated by 8% SDS-PAGE, and blotted with rabbit anti-ASIC2 antibody. Knockdown of Hsc70 was associated with the appearance of ASIC2 in the plasma membrane of D54-MG cells (Fig. 5).
Reduced Expression of Hsc70 Is Associated with Inhibition of Amiloride-sensitive Cation Current in Glioma Cells—We have shown that the constitutive current of the glioma cells results from the loss of ASIC2 expression at the plasma membrane. As knockdown of Hsc70 is associated with appearance of ASIC2 at the plasma membrane, we predicted that Hsc70 siRNA should...
also inhibit the amiloride-sensitive cation current. D54-MG and primary cultures of GBM cells were transfected for 48 h with Hsc70 siRNA or a scrambled siRNA by electroporation, and whole cell patch clamp was performed. Transfection with Hsc70 siRNA completely inhibited the amiloride-sensitive current in primary GBM (Fig. 6A) and D54-MG (Fig. 6B). These residual current traces resemble those obtained in normal human astrocytes (Fig. 8).

Decrease of ASIC2 Expression Cause the Appearance of an Amiloride-sensitive Cation Current in Normal Human Astrocytes—We also wanted to show that knocking down ASIC2 is associated with the appearance of the amiloride-sensitive current in normal human astrocytes. Therefore, normal astrocytes were transfected for 48 h with ASIC2 siRNA or a scrambled ASIC2 siRNA as a negative control. Cells were immunoprecipitated and blotted with either ASIC2 (Fig. 7A) or
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ASIC2 siRNA in normal astrocytes. Immunoprecipitation and immunoblotting of 500 µg of normal astrocytes lysate with (A) rabbit anti-ASIC2 or (B) rabbit anti-ASIC1 antibody. Cells were transfected with ASIC2 siRNA or ASIC2 scrambled siRNA by electroporation. After 48 h cells were lysed using 1% Triton X-100 in PBS. Blots show that ASIC2 siRNA decreases ASIC2 expression without affecting ASIC1.

DISCUSSION

Our studies suggest that the GBM channel may be unique among amiloride-sensitive channels in that it is likely comprised of a mixture of ASIC and ENaC subunits. Many ion channels, including ENaC and CFTR, undergo regulated trafficking as a means of controlling their plasma membrane density (15). Depending on cell type, only 1–20% of newly synthesized ENaC reaches the apical membrane in epithelia (16). The rest is ubiquinated and degraded in the proteasome (17). If, however, αβγENaC subunits are co-expressed, there is a pool of ENaC that is lysosomally targeted for degradation (18). In contrast to ENaCs, nothing is known about trafficking of ASICs to and from the plasma membrane. Thus, our major goal is to understand the mechanism of altered cellular trafficking of ASIC2 in GBMs. Ultimately, this channel and its regulatory pathways may provide unique therapeutic targets for treatment of malignant tumors.

A corollary to our hypothesis is that the amiloride-sensitive cation current, present in glioma cells but not in normal astrocytes, gives a selective advantage to the glioma cell by enhancing its ability to volume regulate. Glioma cells are characterized by extremely high rates of migration and proliferation. Migration through the tightly packed brain parenchyma requires the opposite process, regulatory volume increase, is required to reset cell volume and would be required prior to proliferation (20, 21). Regulatory volume increase usually involves the influx of Na+, Cl−, and water. It is in this latter process that we hypothesize that the glioma channel plays a major role, and it would be predicted that inhibition of the current would prevent glioma migration and proliferation.

Our previous studies suggested that in approximately half of all glioma cell lines and GBM samples we have examined, ASIC2 is trapped in the endoplasmic reticulum and thus is never inserted into the plasma membrane. In the remaining cells, ASIC2 is simply not expressed, possibly because of methylation-mediated transcriptional repression within the ASIC2 promoter (22). We hypothesize that part of the accelerated tumor cell growth and invasiveness is due to the resultant failure in cell surface expression of ASIC2. This situation is highly reminiscent of that found with CFTR, where nearly 100% of ΔF508-CFTR, and surprisingly the majority of wild-type CFTR, is subjected to degradation by the proteasome (23–25). Similarly, a failure of renal β and γENaC to be appropriately internalized and degraded is responsible for the hypertensive syndrome known as Liddle disease (17, 26).

Interaction with Hsc70 is suggested to be a key step in targeting a number of cellular proteins for degradation by the proteasome. Decreasing Hsc70 levels in the cell would decrease the rate of intracellular degradation of these proteins. Although analysis of a limited number of ASIC2 sequences from freshly resected GBM samples has not revealed any mutation that might cause misfolding, we hypothesized that ASIC2 is also a client of Hsc70. This chaperone is usually up-regulated in cancer (27).

Whereas there has been considerable progress toward characterizing ASIC/ENaC gating properties and cellular localization, virtually nothing is known about the cellular mechanisms
of ASIC trafficking. Our results provide information on the mechanisms underlying constitutive activation of these channels in glioma cells and why they are inactive in normal astrocytes. Such information is vital if we are to understand ASIC activity as it is integrated with other components of the tumor cell machinery.
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A. D54-MG

The results obtained in this study suggest that ASIC2, but importantly not ASIC1, associates with the molecular chaperone, Hsc70, and are consistent with the hypothesis that increased binding of this ASIC subunit by Hsc70 may prevent ASIC2 expression at the plasma membrane. This chaperone is up-regulated in glioma cells as compared with normal astrocytes. We believe that in the 50% of glioma cell lines that express ASIC2, the protein is degraded prior to reaching its functional domain in the plasma membrane. Therefore, an increase in Hsc70 expression also increases its association with ASIC2 and targets it for degradation. Because ASIC2 mediates inhibition of the channel complex, the ultimate result (i.e., absence of plasma membrane ASIC2), is a constitutively active cation current.

In conclusion, we suggest that the up-regulation of Hsc70 underlies the constitutively active amiloride-sensitive current found in GBMs rather than a direct problem with ASIC2, because silencing of Hsc70 permits escape of ASIC2 to the cell membrane, inhibition of the basally active current in glioma cells, and a decrease in glioma cell migration. In contrast, silencing of ASIC2 in normal astrocytes activates the amiloride-sensitive current and increases the migration of normal astrocytes. These results are consistent with our hypothesis that trafficking of ASIC2 to the plasma membrane of glioma cells inhibits the cation current and that this current is critical for maintenance of the glioma cell phenotype.

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