Lithium inhibits invasion of glioma cells; possible involvement of glycogen synthase kinase-3

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Therapies targeting glioma cells that diffusely infiltrate normal brain are highly sought after. Our aim was to identify novel approaches to this problem using glioma spheroid migration assays. Lithium, a currently approved drug for the treatment of bipolar illnesses, has not been previously examined in the context of glioma migration. We found that lithium treatment potently blocked glioma cell migration in spheroid, wound-healing, and brain slice assays. The effects observed were dose dependent and reversible, and worked using every glioma cell line tested. In addition, there was little effect on cell viability at lithium concentrations that inhibit migration, showing that this is a specific effect. Lithium treatment was associated with a marked change in cell morphology, with cells retracting the long extensions at their leading edge. Examination of known targets of lithium showed that inositol monophosphatase inhibition had no effect on glioma migration, whereas inhibition of glycogen synthase kinase-3 (GSK-3) did. This suggested that the effects of lithium on glioma cell migration could possibly be mediated through GSK-3. Specific pharmacologic GSK-3 inhibitors and siRNA knockdown of GSK-3α or GSK-3β isoforms both reduced cell motility. These data outline previously unidentified pathways and inhibitors that may be useful for the development of novel anti-invasive therapeutics for the treatment of brain tumors.
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Introduction

Invasiveness is one of the main hallmarks of primary glial brain tumors, in which malignant cells diffusely infiltrate normal brain tissue and migrate along defined structures of the brain.[1,2] Invasion prevents complete surgical tumor removal and is a major reason for the continued poor prognosis (median survival, 12 months) seen in glioblastoma multiforme, the most common primary brain tumor, with approximately 15,000 new patients diagnosed annually in the USA. Glioma invasion is associated with specific molecular alterations, including changes in extracellular matrix composition, cell adhesion, and cytoskeletal dynamics. However, potential anti-invasive therapeutics have not yet successfully translated to the clinic. Drugs developed so far, such as protease inhibitors, have not shown clinical ben-

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efits.[3] Invasion is facilitated by multiple cellular alterations. These include changes in extracellular matrix composition that allow invasion,[4] along with changes in cytoskeletal components and adhesion that increase motility[5,6] and interactions with the normal cells of the brain and the host immune system, which also contribute to the invasion process.[7]

In this study, we examined the effects of lithium on glioma cell migration and invasion. Lithium has been used clinically in the treatment of bipolar disorder for more than 50 years and has profound effects on a variety of processes, including metabolism, neuronal communication, and cell proliferation and development.[8,9] It has not been studied in the context of glioma invasion. In this report, we show that lithium potently and specifically blocks glioma cell migration in a range of assays and that additional inhibitors of the serine/threonine protein kinase glycogen synthase kinase-3 (GSK-3) have very similar effects. GSK-3 is known to be inhibited by lithium,[10,11] suggesting that GSK-3 could possibly be mediating the effects of lithium on glioma cell migration. Our data suggest that lithium and other GSK-3 inhibitors may represent a novel class of potential anti-invasive therapeutics.

Materials and Methods

Cell Lines and Chemicals

The U87 and U87ΔEGFR cell lines were provided by Drs. Huang and Cavenee (Ludwig Institute for Cancer Research, La Jolla, CA, USA). The human glioblastoma biopsies, X12 and X14 (from Dr. C. David James, Mayo Clinic, Rochester, MN, USA), were maintained as flank tumors in nude mice as described.[12] The H2BGFP fusion expression vector was obtained from Geoffrey Wahl (Salk Institute, La Jolla, CA, USA) and stably transfected into U87 cells. All cells were grown in DMEM (Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Human fetal astrocytes were purchased from Cambrex and cultured according to manufacturer's recommendations. Chemicals used were LiCl and myo-inositol (Sigma-Aldrich, St. Louis, MO, USA), AR-A014418 (Invitrogen, Carlsbad, CA, USA), SB415286 (Tocris Bioscience, Ellisville, MO, USA), and L-690,330 (Alexis Biochemicals, San Diego, CA, USA).

Cell Invasion/Migration Assays

For three-dimensional spheroid cultures, 1,000 cells were cultured as hanging drops as described.[13] The cell aggregate was transferred into 300 μl neutralized collagen I solution in an 8-well chamber slide (Nalgene, Rochester, NY, USA). Collagen (PureCol, Inamed, Fremont, CA, USA) was neutralized to pH 7.5 using 1N NaOH and supplemented with 2% FBS, penicillin-streptomycin, and 0.5× DMEM. After polymerization at 37°C, the collagen was overlaid with 300 μl of standard medium with or without inhibitors. Spheroid expansion and all other microscopy-based assays were monitored using a Zeiss LSM510 confocal microscope system (Carl Zeiss Inc., Thornwood, NY, USA) and edited/quantified using ImageJ (http://rsb.info.nih.gov/ij/) and Excel (Microsoft, Redmont, WA, USA). For detailed analysis, high-resolution images were analyzed by the method of Stein et al.[14] Wound-healing assays were performed by scratching confluent monolayers of U373 cells in 8-well chamber slides with a 20 μl pipette tip 1 h after addition of inhibitor-containing medium. For GSK-3 knockdown, 100 pmols of Smartpool GSK-3α or GSK-3β siRNA (GSK-3α, GGACAAAGGUGUUCAAAUCUU, GAACCCAGCUGCCUAACAAUU, GCGCACAGCUUCUUUGAUGUU, GCUCUAGCCUGCUGGAGUAUU; GSK-3β, GAAGAAAGAUGAGGUCUAUUU, GGACCCAAAUGUCAAACUAUU, GAUGAGGUCUAUCUUAAUCUU, GAAGUCAGCUAUACAGACAUU) or scrambled siRNA control (Dharmacon, Chicago, IL, USA) was introduced into cells with Lipofectamine 2000 (Invitrogen) in a 6-well plate 48 h prior to the wound-healing assay. The area of the gap between migrating edges of cells was used to quantitate the assay. Transwell assays were performed using 8 μm pore size inserts (ISC Bioexpress, Kaysville, UT, USA). Fifty thousand cells were allowed to migrate for 6 h prior to fixation in 1% glutaraldehyde and cell visualization by staining with 10 μg/ml DAPI (Sigma).

Brain slices were aseptically prepared from postnatal day 5–7 mice using a vibratome (Leica VT1000S). Three hundred μm slices were incubated at 36°C on 0.4 μm pore size inserts (Millicell-CM) with filtered MEM (Invitrogen) (50%), 25% FBS and 25% HBSS (Invitrogen), 1 mM L-glutamine, and 36 mM D-glucose. A glioma spheroid was carefully placed on the surface of the brain slice after 3 weeks in culture. To visualize spheroid cell migration, brain slices were fixed in 4% paraformaldehyde and stained with rabbit antihuman vimentin antibody SP20 (Neomarkers, Fremont, CA, USA).

TCF-Lef Luciferase Reporter Assay

The β-catenin reporter plasmid pSuper8XTOPflash or pSuper8XFOPflash (control) (from Dr. Randall Moon, University of Washington, Seattle, WA, USA)[15] was transfected into U87 cells, seeded at 60,000 cells/well in a 12-well plate using Lipofectamine 2000. Promoter activity was determined by measuring luciferase levels in a Fluostar Optima plate reader (BMG Labtech, Durham, NC, USA). This was carried out in triplicate and FOPflash used as a background control. The renilla luciferase expression vector pRL-tk (Promega, Madison, WI, USA) was cotransfected as a control.

Cell Viability, Cell Death, and Cell Cycle Analyses

Cell viability was measured using the WST-1 assay (Roche, Palo Alto, CA, USA) in 96 well plates, according to manufacturer's recommendations. Propidium iodide exclusion and flow cytometry–based cell cycle analysis was carried out by standard methods. Cell cycle analysis

was carried out using fluorescence-activated cell sorting (FACS) with a FACSCalibur machine (Becton Dickinson, Franklin Lakes, NJ, USA).

Immunostaining and Western Blotting

Actin staining of U87 cell spheroids in collagen I was performed with Alexa Fluor 568-phalloidin (Invitrogen) according to manufacturer's recommendations. For Western blotting, proteins (30 μg) were separated by SDS-PAGE and transferred to nitrocellulose. Antibodies used for Western blotting were mouse anti-GSK-3αβ (BD Biosciences, Rockville, MD, USA) and peroxidase conjugated secondary antibodies (Jackson Laboratories, Bar Harbor, ME, USA).

Results

Lithium Chloride Inhibits Invasion of Glioma Cells

In this study we employed glioma spheroids as a model to identify novel inhibitors of glioma invasion. These multicellular aggregates of glioma cells are embedded in a type I collagen matrix.[13] The implanted spheroid forms a cell-dense region, analogous to the core of a tumor. Over a period of days, cells migrate radially into the matrix, mimicking invading cells. This process can be monitored easily by microscopy and reproduces some key features of brain tumor invasion, such as process extension and matrix remodeling.

In the course of examining these spheroids, we serendipitously discovered that lithium, an FDA-approved drug used in the treatment of bipolar disorder, caused a near complete blockade of cell invasion in X12 glioma spheroids over a 96 h time period (Fig. 1A). In order to confirm that these effects were reproducible in another assay and with other glioma cells, we employed the wound-healing assay for cell migration. A pronounced effect on migration of U373 glioma cells across the wound gap was observed in the presence of 20 mM LiCl compared to 20 mM NaCl control (Fig. 1B).

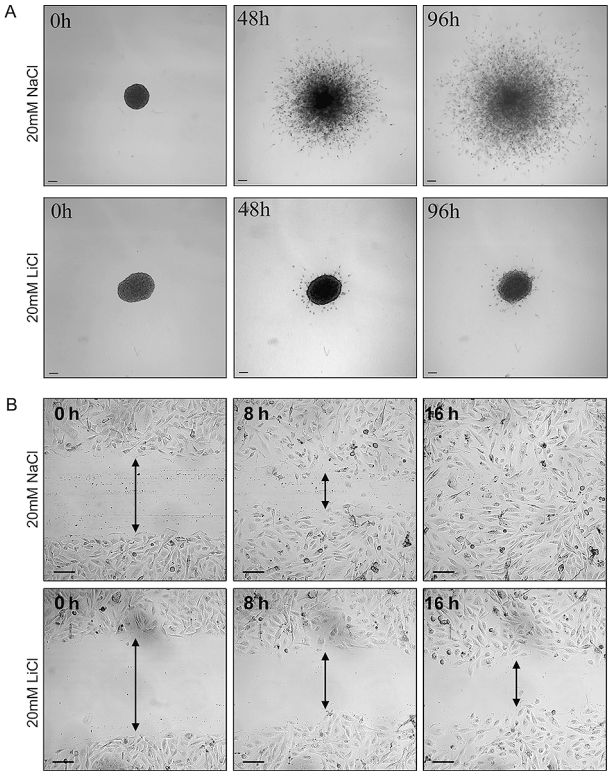

Fig. 1. Inhibition of glioma spheroid invasion by LiCl. (A) Time course of X12 glioma cell spheroid migration into a type I collagen matrix, with 20 mM NaCl (control) or 20 mM LiCl (bar = 100 μm). (B) Time course of U373 glioma cell migration in a monolayer-based wound-healing assay, in the presence of 20 mM NaCl or 20 mM LiCl (bar = 100 μm). The gap is indicated by double arrows.

Effects of Lithium on Glioma Cell Invasion Are Dose-Dependent, Reversible, and Reproducible in 6/6 Glioma Cell Lines

Further analysis of the effects of lithium on glioma cell invasion revealed that inhibition of migration was dose-dependent, with a near complete blockade at 20 mM LiCl for X12 (Fig. 2A) and U87 glioma cells (Fig. 2B). Similar effects were seen in all glioma cells examined, suggesting that the effect may be broadly applicable (Fig. 2C). As an additional control, we also examined normal human astrocytes (NHA) grown as multicellular spheroids in culture. Fig. 2C shows that lithium significantly blocked migration of NHA from the spheroid into the surrounding matrix. These findings thus show that lithium possessed inhibitory action on cell motility for both glioma cells and normal glia.

We also found that the effect of 20mM LiCl was reversible; cell migration resumed with similar kinetics to untreated spheres 24 h after LiCl removal (Fig. 2D), with an irreversible blockade observed at 40mM LiCl (data not shown).

To provide more quantitative data, confocal microscopic images of U87 cells stably expressing nuclear GFP were analyzed by counting the number of invasive cells outside the tumor core.[14] The core and rim of the spheroid can be distinguished in microsopic images as shown in Fig. 3A. The number of cells that occupied the three-dimensional volume of the matrix outside the spheroid core (the "invasive" zone) were enumerated as a function of time after treatment with several different concentrations of lithium. This showed a concentration-dependent reduction in the number of migrating cells. This result was significant even at lithium concentrations as low as 2.5 mM (Fig. 3B). In addition to measuring the absolute number of single cells found away from the tumor core, we also measured the cell density of invasion as a function of time (Fig. 3C). This showed that in addition to a decrease in cell number, lithium reduced the overall distance of migration when compared to control. Lithium therefore affects both the total distance migrated and the total numbers of cells that migrate, and these effects are detectable in vitro at lithium concentrations

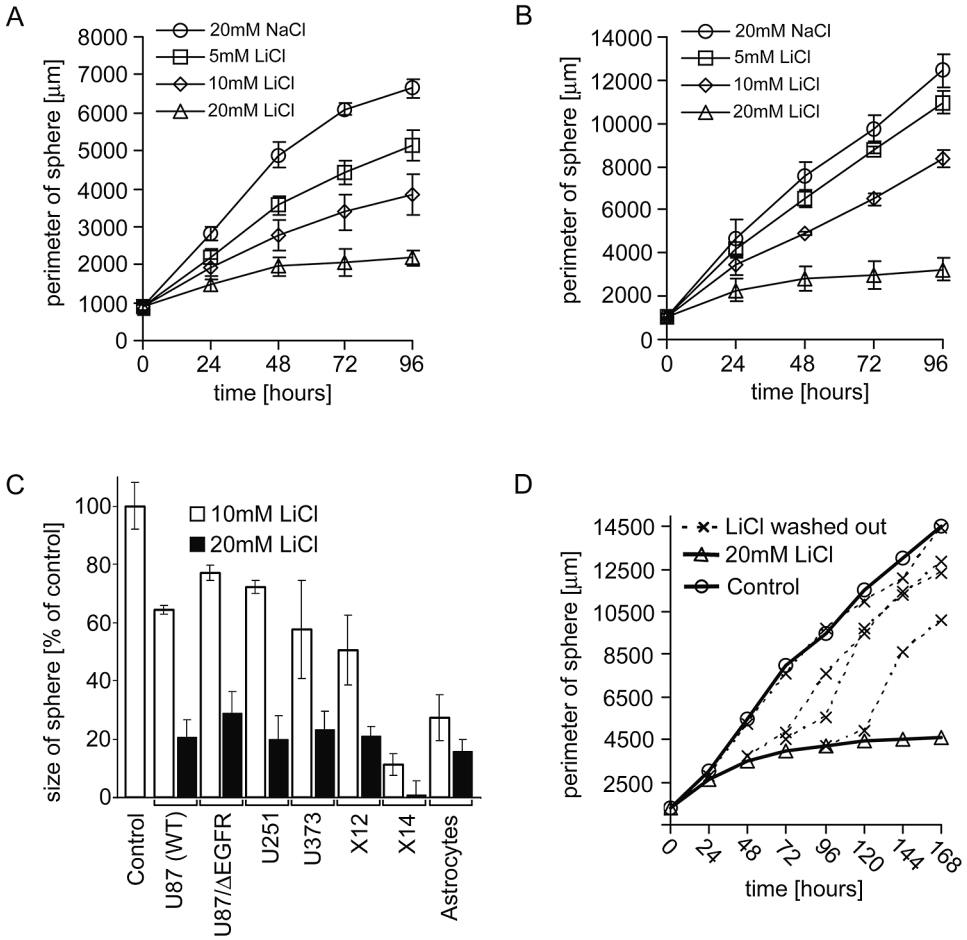

Fig. 2. Time course of X12 (A) or U87 (B) spheroid invasion, in the presence of increasing concentrations of LiCl. (C) Spheroids from 6 glioma cell lines and normal human astrocytes were grown in the presence of LiCl. Spheroid size is expressed as a percentage of control after 96 h. (D) Time course of U87 spheroid migration with 20 mM LiCl washed out at 24-h intervals, demonstrating recovery of cell migration after drug removal.

approaching those known to be therapeutically achievable in humans (<2 mM).[7]

In summary, the above results show that lithium potently and reversibly inhibits invasion of glioma cells in the spheroid model, in a time- and dose-dependent fashion.

Lithium Also Affects Cell Cycle Kinetics in Glioma Cells

We then examined the effects of lithium treatment on glioma cell viability. Compared with migration assays, the effects of lithium treatment on cell viability were quite small: a reduction in viability of about 20% was seen after 48 h of 20 mM lithium treatment in U87 cells (Fig. 4A), and at 5 mM and 10 mM lithium no significant changes in cell viability were seen, in contrast to the highly significant inhibition of migration at a similar time point ($p < 0.0001$ for 10 mM lithium; $p = 0.03$ for 5 mM lithium [Fig. 2B]).

In order to understand the reasons for this reduction in cell viability we measured cell death using propidium iodide exclusion. This showed that there was no significant increase in cell death until 48 h treatment with 20 mM LiCl, with lower concentrations having no significant effects even after 96 h treatment (Fig. 4B). Flow cytometry–based cell death assessment (subG1) revealed no increase for U87 cells and a small change from 1.3 ± 0.1% to 2.6 ± 0.3 % of the population for X12 cells after 48 h treatment with 20 mM LiCl (data not shown). FACS analysis revealed that the effect of a short-time treatment of lithium did not significantly affect cell cycle kinetics, but that 20mM lithium after 48 h treatment led to an accumulation of cells in the G2/M phase of the cell cycle (Fig. 4C). The dose- and time-dependency of this G2/M block is illustrated in Fig. 4D. This suggests that effects on the cell cycle are likely the major reason for the observed changes in cell viability when glioma cells are exposed to 20mM LiCl. Therefore, in addition to its effects on migration, lithium also slows glioma cell proliferation mainly through its effects on the cell cycle, rather than through a direct cytotoxic effect. Importantly, these effects are much less pronounced than those

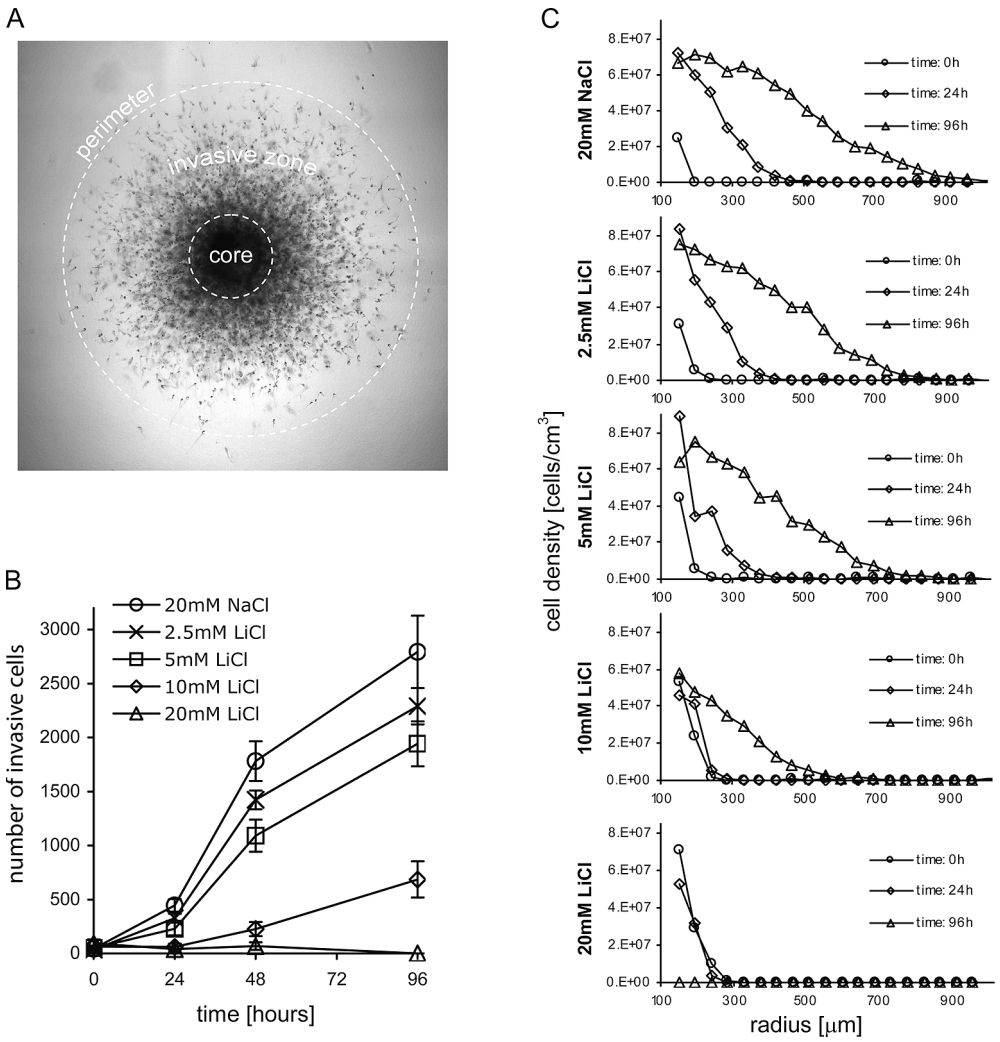

Fig. 3. Detailed examination of effects of lithium on glioma spheroid behavior. (A) Diagrammatic representation of the spheroid assay, showing core versus rim separation based on cell density. (B) Effects of varying concentrations of lithium on the number of migrating cells over time. For samples vs. control at all time points, $p < 0.01$, except 2.5 mM LiCl, where $p = 0.059$ at 24 h, $p = 0.054$ at 48 h, and $p = 0.024$ at 96 h. (C) Effects of increasing concentrations of lithium on cell density at various distances from the edge of the spheroid core.

on cell migration and are not significant at lower lithium concentrations in which reduced cell migration is readily detectable.

Lithium Treatment Inhibits Formation of Extensions at the Leading Edge of Migrating Glioma Cells

Microscopic examination revealed that the inhibition of cell migration in glioma spheroids by lithium was associated with retraction of the long protrusions at the front of the invading cells (Fig. 5A). Upon lithium removal, these morphological changes were reversible with regrowth of the extensions and resumption of migration (data not shown). X12 glioma spheroids were also cultured on mouse organotypic brain slices. Immersion of the brain slice in 20 mM LiCl for 24 h slowed glioma cell migration and caused cells to become less elongated and to round up (Fig. 5B). This effect was also observed using U87 cells (data not shown), and shows that lithium inhibits invasion in the context of a physiologically relevant matrix.

Lithium's Effects on Glioma Cell Invasion May Possibly Be Mediated by Inhibition of GSK-3

Although lithium is likely to be pleiotropic in its effects on cell processes and enzymatic activity, some molecular targets of lithium have been clearly identified.[8] The best supported of these are inositol monophosphatase (IMPase)[16] and GSK-3.[10,11] We therefore investigated the role of each of these molecules in glioma cell invasion/migration. Blockade of IMPase by lithium leads to depletion of cellular inositol levels, and IMPase-mediated effects of lithium can be rescued by addition of excess extracellular inositol.[16] We found that excess inositol did not rescue the LiCl blockade of migration in the spheroid assay, and L-690,330, an IMP inhibitor 1000-fold more potent than lithium,[17] had no significant effect on

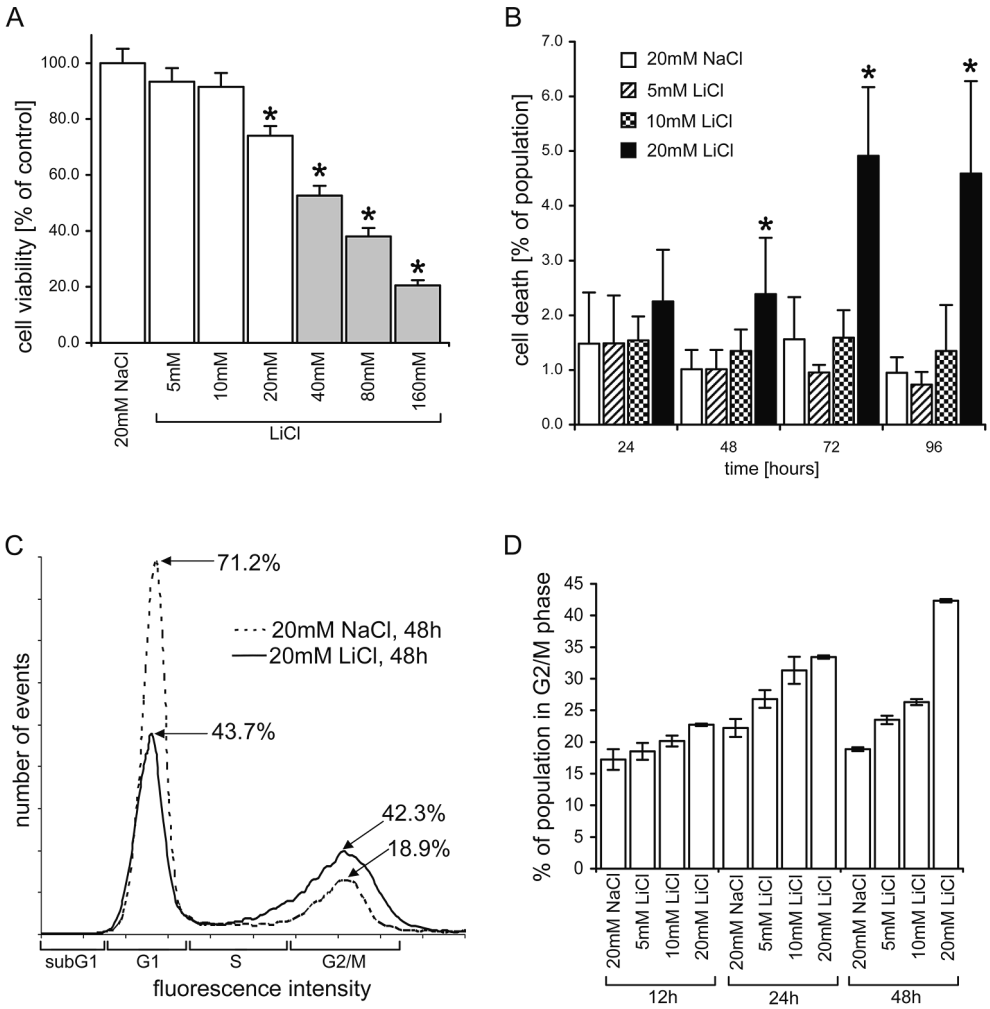

Fig. 4. Effects of lithium treatment of glioma cell viability and cell cycle. (A) U87 cell viability in the presence of increasing lithium concentrations at 48 h, as measured by WST-1 assay (*$p < 0.01$ between sample and NaCl-treated control) (B) Cell death as measured by propidium iodide exclusion in U87 cells treated with increasing lithium concentration over time (*$p = 0.01$). (C) Trace of FACS analysis of the cell cycle showing lithium-treated U87 cells 48 h after 20 mM LiCl treatment compared to 20 mM NaCl control. (D) Percentage population of U87 cells in G2/M phase after increasing lithium treatment over time (*$p < 0.01$ between sample and control).

glioma cell invasion even at concentrations as high as 400 μM (Fig. 6A). This result strongly suggests that inositol metabolism is unlikely to be an important target of LiCl in glioma cell migration. In contrast, two chemically distinct, potent, and specific pharmacological GSK-3 inhibitors, SB415286[18] and AR-A014418,[19] caused a dose-dependent blockade of invasion (Fig. 6B). The effect was observed in 6/6 glioma cells and was also reversible (data not shown). This result suggests that GSK-3 inhibition may possibly play a role in mediating the effects of lithium in glioma cell invasion.

GSK-3 inhibition is known to promote the stability and transcriptional activity of β-catenin.[15] In order to determine the level of GSK-3 inhibition in drug-treated glioma cells, we assayed the activity of a β-catenin responsive luciferase reporter plasmid in GSK-3 inhibitor treated U87 cells. A dose-dependent increase of reporter activity was seen with lithium, SB415286, and AR-A014418 (Fig. 6C). The degree of GSK-3 inhibition directly correlated with the blockade of invasion, suggesting a direct link between GSK-3 activity and the rate of glioma invasion. Wound-healing assays also showed a dose-dependent reduction in U373 glioma cell migration in the presence of GSK-3 inhibitors (Fig. 6D). Finally, siRNA was used to specifically knock down GSK-3 isoforms. Both GSK-3α– and GSK-3β–specific siRNA treatment led to a 60% reduction in protein expression. This caused reduced cell motility in wound-healing assays for U373 cells and also in transwell assays using U87 and X12 glioma cells. This indicates that both GSK-3 isoforms play a role in glioma migration.

In conclusion, lithium potently and specifically reduces glioma cell invasion, and this effect may be mediated by the inhibition of both GSK-3α and GSK-3β in lithium-treated cells.

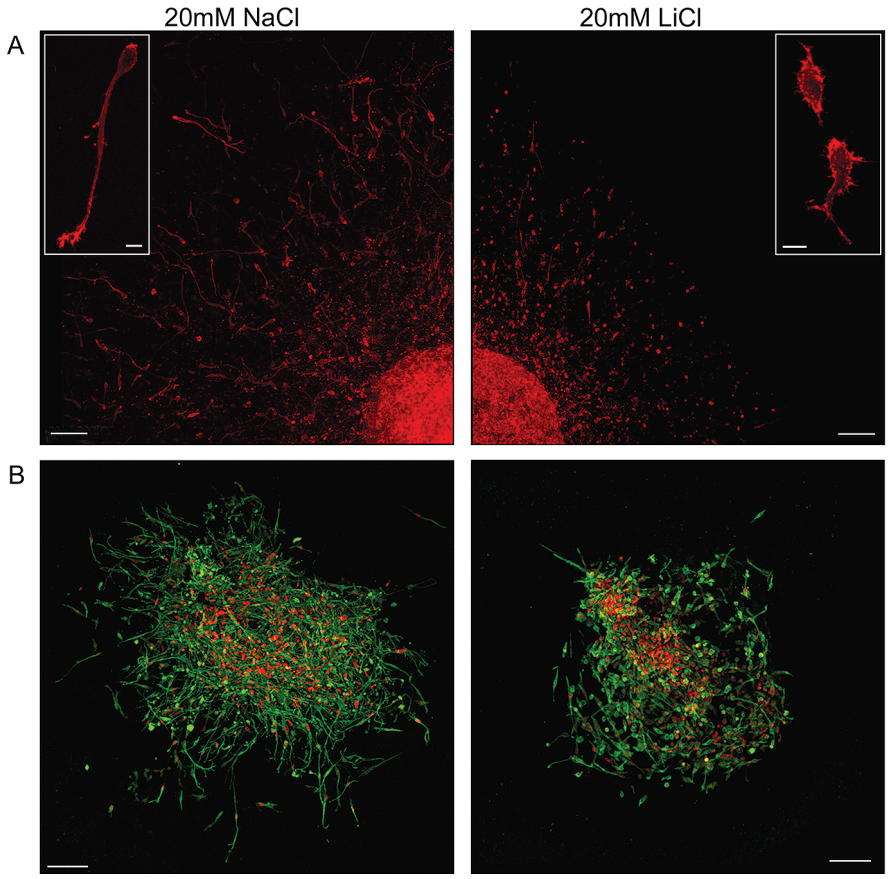

Fig. 5. LiCl affects glioma cell movement in brain slices and induces pronounced changes in glioma cell shape. (A) U87 spheroid invading collagen I matrix. A U87 spheroid was grown for 48 h and then exposed to 20 mM NaCl or LiCl for 24 h and stained with alexafluor-568 phalloidin. The image shows a quadrant of the spheroid (Bar = 100 μm). Inset shows a detailed image of a migrating cell (Bar = 10 μm). (B) X12 glioma spheroid migrating on a brain slice treated with 20 mM NaCl or with LiCl for 24 h. Tumor cells are visualized by immunohistochemical anti-vimentin (green) staining. Cell nuclei stained with Hoechst 33528 are shown in red.

Discussion

Here we report that lithium potently and reversibly blocks glioma cell migration and invasion in vitro in a range of assays and in all cell lines tested (6/6). Lithium also slows cell proliferation to a lesser degree. This effect can be distinguished from its effects on migration because: (1) the effects of lithium on migration are of much greater overall magnitude than effects on viability; (2) they occur at lower doses and earlier time points than effects on cell viability; and (3) they are reversible, even after 96 h treatment, suggesting that the blockade of migration is not due to a cytotoxic effect. Indeed, lithium treatment is accompanied by the retraction of the long protrusions at the front of the migrating cell, which is fully reversible upon lithium removal.

Lithium has profound effects on a variety of processes, including metabolism, neuronal communication, and cell proliferation and development.[8,9] These effects are cell type– and dose-dependent. For example, lithium stimulates cell proliferation in mammary tumor cells[20] and inhibits proliferation in melanoma[21] and hepatocellular carcinoma.[22] Lithium is protective against cell death in neurons,[23] but not against cellular stresses in glioma cells.[24] Several protein activities are affected by lithium, with GSK-3 and IMPase having the strongest experimental support.[10,11,25] Our data show that while IMPase is probably not important, GSK-3 may mediate the effects of lithium and may be an important driver of glioma cell motility. First, three distinct small molecule GSK-3 inhibitors (including lithium) caused a dose-dependent, reversible inhibition of glioma cell invasion in spheroid assays. Second, the degree of GSK-3 inhibition (measured by a luciferase reporter assay of β-catenin transcriptional activation) showed an inverse correlation with the degree of invasion, suggesting a direct link between GSK-3 activity and the rate of glioma invasion. This was supported by the observation that siRNA knockdown of either GSK-3α or GSK-3β slowed migration in a wound-healing assay, demonstrating that both GSK-3 isoforms play a role in glioma invasion. As with lithium, cell migration was prevented with each inhibitor, in all glioma cell types examined, and in multiple migration assays.

GSK-3 is a multifunctional serine-threonine protein kinase found in all eukaryotes that regulates diverse processes, including metabolism, cell fate specification, cell

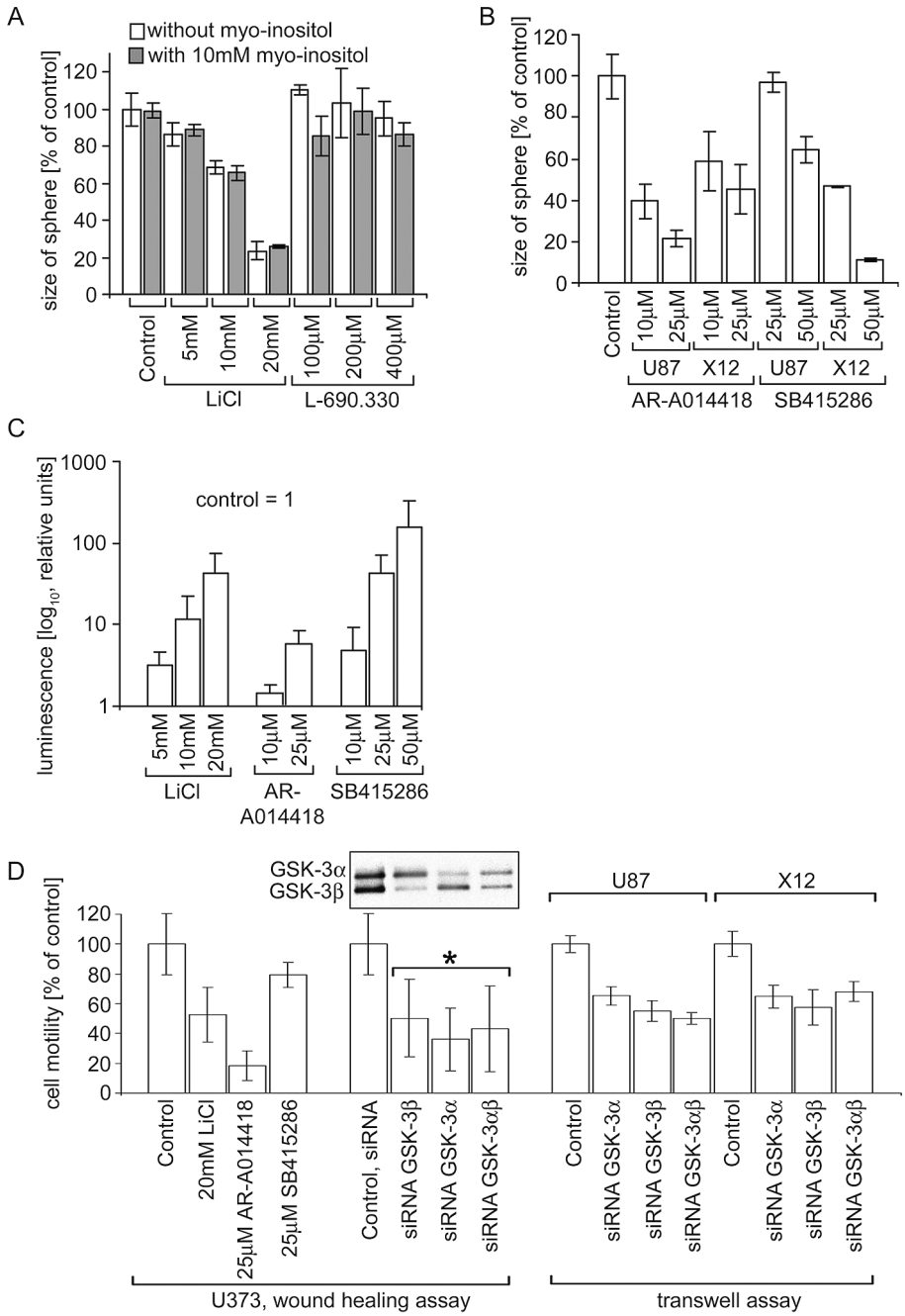

Fig. 6. GSK-3 inhibition also inhibits glioma invasion. (A) U87 spheroids were grown with LiCl in the presence or absence of 10 mM myo-inositol or with the specific IMPase inhibitor L690,330. The graph shows U87 spheroid size as a percentage of control after 96 h. (B) Effects of the specific GSK-3 inhibitors AR-A014418 and SB415286 on spheroid migration. (C) TCF/Lef-dependent activation of luciferase gene transcription is stimulated by GSK-3 inhibitors. U87 cells transfected with pSuper8XTOPflash were cultured with increasing concentrations of GSK-3 inhibitors and promoter activity determined by luciferase levels. (D) Effect of GSK-3 inhibition and siRNA treatment in wound-healing and transwell assays. The graph shows the percent inhibition of cell motility compared with controls of U373 glioma cells in a scratch assay and in transwell assays using U87 and X12 cells. The knockdown of GSK-3 isoforms is shown as a Western blot (inset). $p < 0.01$ for all samples versus control, except $^*p < 0.03$.

division, and cell death.[26,27] Two closely related isoforms, GSK-3α and GSK-3β, function in multiple pathways, including Wnt, notch, tyrosine kinase, and G-protein coupled receptor signaling. Wnt signaling inactivates GSK-3, causing β-catenin stabilization and gene transcription. Independently of Wnt signaling, GSK-3 can be inactivated by phosphorylation at an N-terminal serine (serine-9 in GSK-3β). This is a highly dynamic process, with a number of converging pathways mediated by numerous kinases, including Akt, protein kinase A, and protein kinase C, and dephosphorylated by protein phosphatase 1.[28] Interestingly, it does not appear that the

effects of lithium on migration were confined to tumor cells, since normal human astrocyte migration was also inhibited. These results suggest that the effects of lithium may be independent of the status of the PTEN/Akt pathway that is disrupted in most gliomas and regulates GSK-3 activity.[29]

The role of GSK-3 in cancer cell migration is barely studied, and current data are conflicting. For example, it has been suggested that GSK-3 inhibition may promote cancer cell invasion and metastasis by promoting the epithelial to mesenchymal transition in some cancer types,[30] and lithium was reported to promote the motility of colon cancer cells.[31] On the other hand, pharmacological GSK-3 inhibition prevents epithelial cell migration,[32] lamellipodia formation in keratinocytes,[33] filopodia formation in neurons,[34] and astrocyte cell movement.[35] Several GSK-3 substrates are known to be involved in cell migration, including paxillin,[36] FAK,[37,38] NF-κB, β-catenin, and SNAIL (reviewed in ref. 39). The observation of breakdown of long lamellipodia in lithium-treated glioma cells suggests that GSK-3 activity is important in regulating the microtubules that support this structure, as has been proposed in keratinocytes.[33] Several GSK-3 substrates play a role in microtubule regulation, including CRMPs[40] and MAPs.[41,42] The contribution of these various molecules to glioma migration is not yet known. Little is known regarding the function and status of GSK-3 in glioma cells. Akt phosphorylation and inhibition of GSK-3 may be involved in the stabilization of HIF-1α in U87 cells,[43] and the inactivation of GSK-3 by Akt also supports glycolytic energy production in hypoxic or ischemic conditions due to the activation of glycogen synthase.[44] Moreover, activation of glycolysis by GSK-3 inhibition was shown to support glioma cell migration in this study. However, GSK-3 is regulated by many other upstream signals in addition to Akt and can function independently in multiple pathways.[26] Our data, suggesting that GSK-3 inhibition can block glioma cell migration, suggest that separate pools of GSK-3 may be regulated differentially during glioma invasion. Identification of proinvasive stimuli that activate GSK-3 is a major part of our ongoing studies in this area.

Lithium has a narrow therapeutic window and is often toxic at concentrations higher than 1.5 mM in vivo, mainly due to nephrotoxicity.[7] Our data show that we could detect significant effects of lithium on glioma migration at concentrations as low as 5 mM and that at least one glioma cell (X14) was very sensitive to lithium at this dose. It is known that long-term lithium treatment in vivo does inhibit GSK-3, with a reported Ki of 1–2 mM,[10] possibly by affecting additional pathways. Interestingly, it has been reported that cancer mortality and incidence are inversely proportional to lithium dose in patients taking lithium, particularly in cancers of nonepithelial origin.[45] It is evident that detailed pharmacokinetic analysis in vivo will be required to determine if there is a dose of lithium that can significantly impact glioma cell migration. Alternative modes of administration (convection-enhanced delivery or local delivery with polymers) may be required to achieve significantly high doses of the drug in brain tumors in vivo, or alternatively, other GSK-3 inhibitors may need to be tested to find one that is applicable clinically.

On the basis of our data, we propose that lithium or other GSK-3 inhibitors may be an effective means of blocking glioma invasion as a therapeutic tool to prevent recurrence or further tumor spread. Because GSK-3 is a widely recognized therapeutic target in other diseases, such as diabetes and Alzheimer's disease, a variety of GSK-3 inhibitors have been developed, providing a rich source of candidate molecules to study as potential anti-invasive candidates.

Acknowledgments

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