Glioma Migration Can Be Blocked by Nontoxic Inhibitors of Myosin II

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ABSTRACT

Anaplastic gliomas are infiltrative tumors, and their ability to migrate through normal brain contributes to their highly malignant behavior. Invasion of brain requires cell motility, which in turn depends on the activity of the cytoskeleton. A cytoskeletal component central to this process is myosin II, the cytoplasmic analogue of smooth and skeletal muscle myosin. Myosin II activity is regulated by the enzyme myosin light chain kinase, which activates myosin II by phosphorylating it on its regulatory light chain. We have investigated the role of myosin II in glioma motility and invasiveness by examining the effects of two inhibitors of myosin light chain kinase, ML7 and KT5926. Both drugs are potent inhibitors of both glioma motility, as measured by a scrape motility assay, and an in vitro haptotaxis assay. The inhibition of in vitro haptotaxis follows the dose-response relationship expected for competitive inhibition of myosin light chain kinase by these drugs and is seen at drug concentrations that are nontoxic. These results highlight the important role that myosin II contributes to glioma invasiveness and suggest that it may serve as a target in future strategies at blocking invasion by these tumors.

INTRODUCTION

The invasive nature of anaplastic gliomas is well established. Malignant glial cells can often be found in postmortem specimens to have migrated considerable distances from the primary site, and the appearance of tumor crossing the corpus callosum or the commissures frequently heralds imminent neurological decline. The ability of malignant glial cells to invade normal brain limits the effectiveness of many of the currently available therapies and makes it clear that new targets must be identified in order to block this invasive behavior. Tissue invasion by malignant cells is a multistep process (2). Central to this is the ability of transformed cells to crawl through the extracellular matrix, a process that requires the coordination between a force generating intracellular “motor” and an attachment mechanism to the extracellular matrix. Previous studies have focused on a number of extracellular factors that contribute to or modulate invasive behavior. These include growth factors, extracellular matrix proteins, integrins, and matrix metalloproteinases. By contrast, relatively few studies have focused on the intracellular machinery that powers invasive behavior. This machinery is contained within the cytoskeleton, and includes microtubules, actin-containing microfilaments, and an array of mechanochemical enzymes collectively referred to as “molecular motors.” Both pharmacological and molecular biological methods have demonstrated the importance of myosin II in powering a variety of motile behaviors, including growth cone motility, fibroblast locomotion, and astrocyte process outgrowth and contractile responses. Thus, myosin II represents a logical target for the development of anti-invasive strategies in glioma therapy. The activity of myosin II is controlled by phosphorylation of a serine residue on its regulatory light chain by the enzyme MLCK (3). Phosphorylation of the RLC by MLCK activates myosin II. This action is opposed by dephosphorylation of the RLC, which is mediated by a specific myosin light chain phosphatase. Thus, the degree of activity of myosin II in a cell is controlled by the relative balance of activities of MLCK and myosin light chain phosphatase.

Studying the role of myosin II in tissue culture has been aided by the availability of several drugs, all derivatives of fungal metabolites, that are highly specific competitive inhibitors of ATP binding to the active site of MLCK. We have used these MLCK inhibitors to examine the contribution of myosin II to the motility and invasive behavior of malignant gliomas. We have found that these drugs are potent and specific inhibitors of motility, as measured by in vitro assays, and exert their effects at nontoxic doses. These results highlight the importance of myosin II in glioma invasion and suggest that it may serve as an important target in the future development of anti-invasion strategies for the treatment of malignant glial neoplasms.

MATERIALS AND METHODS

Chemicals. KT5926 was obtained from Kamiya Biomedical (Thousand Oaks, CA). ML7 was obtained from Sigma. Echistatin was obtained from Bachem. Transwell migration chambers with a pore size of 8 μm were obtained from Costar. Human vitronectin was obtained from Life Technologies, Inc. Buffers and crystal violet were obtained from Sigma.

Human Glioma Cell Line. U251MG glioma cells were obtained as a gift from Dr. Darell D. Bigner (Duke University, Durham, NC) and have been described previously (21). DMEM mixed 50:50 with Ham’s Nutrient Mixture F-12 (DMEM/F12; Mediatech, Inc.) supplemented to 2.6 mM l-glutamine and 7% FBS was used for culture of glioma cells at 37°C and 7.5% CO₂. FBS (Atlanta Biologicals, Atlanta, GA) was heat inactivated (50°C, 45 min). Culture media and sera were determined to have <100 pg/ml of endotoxin by the Limulus amebocyte lysate assay (Associates of Cape Cod, Inc., Woods Hole, MA). [3H] Thymidine was purchased from Amersham Corp. (Arlington Heights, IL). Tissue invasion by malignant glioma cells was assessed by the haptotaxis assay as described (22–24). Recently subcultured, subconfluent U251MG cells were harvested with 1.5 mM EDTA in PBS, counted, and suspended in DMEM + 0.1% BSA + 1.5 mM l-glutamine (migration buffer) at 400,000 cells/ml. Aliquots of 100 μl were plated in the upper chamber and incubated at 37°C in 5% CO₂ for 4 or 12 h. Migrating cells were fixed and stained with crystal violet as described (22–24) and quantified by counting 10 random 1 mm² fields in a 1-cm grid on an inverted microscope. The effects of MLCK inhibitors were examined in this assay by adding equal concentrations of drug to the upper and lower chambers at the time of plating. Each experiment was accompanied by a control consisting of a polycarbonate filter coated on the lower surface with ovalbumin, and this consistently demonstrated no migration.

Scrape Motility Assay. U251 MG cells were grown to confluence on 12-mm glass coverslips. The cell monolayer was mechanically scored with a sterile pipette tip, and coverslips were placed on the stage of a LU-CB-1 tissue chamber equipped with a NP-2 incubator (Nikon, Japan), which maintained the

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3 The abbreviations used are: MLCK, myosin light chain kinase; RLC, regulatory light chain; FBS, fetal bovine serum.
temperature at 37°C and at an atmosphere of 95% O₂ and 5% CO₂. Cells were visualized by a Nikon Diaphot inverted microscope with phase contrast optics and a ×20 objective. Images were captured on a time-lapse VHS video recorder/player and digitized off-line using an Axon Imaging Workbench 2 (Version 2.10; Axon Instruments) software. ML7 (10 μM), KT9296 (2 μM), and DMSO (0.1%; used as a vehicle for KT9296) were added directly to the growth media at the time of scraping. Afterward, media were not changed throughout the recording time.

Measurement of RLC Phosphorylation. Measurement of the effects of ML7 on RLC phosphorylation was performed on U251 MG cell lysates by using urea gel electrophoresis as described (16). Briefly, cells were collected in a 10% trichloroacetic acid solution supplemented with 10 mM DTT. Pellets were rinsed with ether and suspended in sample buffer containing 8.0 M urea, 9.0 mM DTT, 20 mM TPCK trypsin, and 55 mM Tris base. Samples were run on a 1.5% Bis-30% acrylamide, 40% glycerol gel, followed by electrophoret to a nitrocellulose membrane. The membrane was incubated overnight with a rabbit polyclonal anti-RLC antibody (kindly provided by Dr. James T. Stull, University of Texas Southwestern Medical Center Dallas, TX). RLC isoforms were then visualized by using an alkaline phosphatase secondary antibody (Vector, Burlingame, CA).

AlamarBlue Viability Assay. Gioma cells were plated at 1 × 10⁴ cells/well in 96-well plates in 0.2 ml of complete culture medium and incubated (37°C, 7.5% CO₂) for up to 72 h. Twelve h after addition of MLCK inhibitors or DMSO vehicle, AlamarBlue (40 μL; Alamar Biosciences, Sacramento, CA) was added to each well, the plates incubated for 120–180 min, and cell viability based on metabolic conversion of the deep blue dye to a pink color was assessed spectrophotometrically in situ. Absorbances of supernatants were measured at a wavelength of 562 nm, and background absorbance at 590 nm was subtracted using a semiautomatic plate reader (model EL310; Bio-Tek Instruments, Inc., Winooski, VT). Multiple experiments have confirmed that AlamarBlue does not interfere with detection of [³H]thymidine uptake (25, 26). All experiments were repeated a minimum of three times.

[³H]Thymidine Incorporation

Gioma cell proliferation was measured by [³H]thymidine incorporation. Cells were plated at 1 × 10⁴ cells/well in 96-well plates in 90 μl of complete culture medium and incubated (37°C, 7.5% CO₂) for up to 72 h. Cells were incubated with [³H]thymidine (41 Ci/mmol; 0.5 μCi/well) for an additional 15 h. Cells were detached by 30 min incubation at 37°C in cell release solution (0.1% trypsin, 2.15 mM EDTA, and 0.4 M sucrose, pH 7.5, 40 mM Hepes, 20 mM dithiothreitol, and 55 mM Tris base). Samples were run on a 1.5% Bis-30% acrylamide, 40% glycerol gel, followed by electrophoret to a nitrocellulose membrane. The membrane was incubated overnight with a rabbit polyclonal anti-RLC antibody (kindly provided by Dr. James T. Stull, University of Texas Southwestern Medical Center Dallas, TX). RLC isoforms were then visualized by using an alkaline phosphatase secondary antibody (Vector, Burlingame, CA).

Fluorescence Microscopy. For examining the effects of MLCK inhibitors on actin distribution, U251 MG cells cultured on glass coverslips were treated with KT9296 or ML7 for 1 h and were then fixed with 4.0% (w/v) PBS-buffered paraformaldehyde for 5 min and permeabilized with 0.15% Triton X-100 in PBS for 5 min. Permeabilized cells were then incubated with a 1:50 dilution of rhodamine phallolidin (Molecular Probes) in blocking buffer (2% BSA in PBS) for 2 h at room temperature in the dark. Cells were then washed three times for 10 min each with PBS and visualized with a Leica fluorescence microscope. Images were captured and digitized with ImarCap software (Imagograph Corporation, Chelmsford, MA) and with a DEI-750 CCD camera.

RESULTS

Effects of Kinase and Integrin Inhibitors on in Vitro Gioma Haptotaxis. Within 4 h of plating, U251 MG cells can be demonstrated to have migrated through a vitronectin-coated polycarbonate membrane. By contrast, when the membrane is coated with ovalbumin, no cells are seen to have migrated (data not shown). We examined the effects of the MLCK inhibitor KT9296 (2 μM) and the cGMP-dependent protein kinase inhibitor KT5823 (2 μM) on this process. In addition, echistatin, a peptide inhibitor of RGD-dependent integrin binding to vitronectin, was also examined in this assay at a concentration of 1 μM. Fig. 1 demonstrates the average number of cells migrating per mm² after 4 h of incubation in chambers in the absence of drug and in the presence of KT9296, KT5823, and echistatin. As can be seen from the figure, KT9296 reduces Transwell migration in this assay by nearly 95%, whereas KT5823 has no appreciable effect. By comparison, echistatin, which blocks integrin-mediated attachment to vitronectin (27), reduced migration by 65%.

Fig. 1. Average number of U251 MG cells migrating through a Transwell chamber as a function of inhibitor. Chambers were coated on the undersurface of the polycarbonate membrane with vitronectin prior to use, as described in "Materials and Methods." For untreated cells (Control) or cells treated with 2 μM KT9296, 2 μM KT5823, and 1 μM echistatin are expressed as means; bars, 1 SD. Although KT9296 inhibits migration by >95%, KT5823 has no effect, and echistatin (a competitive inhibitor of RGD-dependent integrin binding) reduces migration by 65%.
To further substantiate the argument that the effect of these MLCK inhibitors is in fact due to inhibition of MLCK and not some other protein kinase, the dose-response relationship for migration inhibition was measured for both KT5926 and ML7. Results of these studies are depicted in Fig. 2A for KT5926 and Fig. 2B for ML7. Data presented in these figures is for 12-h incubations. Shorter incubation periods (4 h) gave qualitatively similar results but with larger SD. Data were fit to a Michaelis-Menten dependence of cell count on drug concentration using a curve-fitting procedure (DeltaGraph Pro 3). Both KT5926 and ML7 act as competitive inhibitors of ATP binding to the active site of MLCK (28, 29). Assuming a 1:1 correspondence between MLCK inhibition and inhibition of *in vitro* haptotaxis, the relationship between drug concentration and inhibition of haptotaxis is as follows:

\[
C_i = \frac{C[ATP]}{K_m \left(1 + \frac{[I]}{K_i}\right) + [ATP]}
\]

where \(C_i\) is the average number of cells migrating in the presence of drug concentration \([I]\), \(C\) is the average number of cells migrating in the absence of drug; \([ATP]\) is the intracellular ATP concentration; \(K_m\) is the Michaelis constant for ATP binding to MLCK, which is 50 \(\mu\)M (30), and \(K_i\) is the binding constant of drug to the active site of MLCK, which was obtained from published values (28, 29, 31). Curve fitting was accomplished by setting \(K_m\) and \(K_i\) equal to the published values and allowing \([ATP]\) to be a dependent variable. This led to acceptable fitting with intracellular ATP concentrations of 1.47 mM for studies with KT5926 and 2.82 mM for studies with ML7. Given the errors inherent in the curve-fitting procedure, these values for intracellular ATP concentration are remarkably close to values measured directly in both normal and transformed cells, including gliomas (2.2–3.4 mM; Refs. 32–34). Fitting of the data by using the \(K_m\) and \(K_i\) values for other protein kinases that are inhibitable by these drugs led to values of intracellular ATP concentration that are nonphysiological (47–72 \(\mu\)M). Thus, we conclude that the inhibition of *in vitro* haptotaxis seen with these drugs is due to specific inhibition of myosin light chain kinase.

**Effect of MLCK Inhibitor ML7 on Regulatory Light Chain Phosphorylation.** As a further test of the specificity of the MLCK inhibitors, we have examined the effect of ML7 at concentrations of 20 and 40 \(\mu\)M on phosphorylation of the myosin II regulatory light chain in U251MG cells. There are two isoforms of the myosin regulatory light chain in nonmuscle cells, which can be distinguished by their different mobilities on urea gel electrophoresis due to their different net charge in 8 M urea (16, 35). Phosphorylation of these regulatory light chains enhances their mobility further, allowing a relative measure of the percentage of RLC phosphorylation. Both serine 19 and threonine 18 can be phosphorylated both *in vitro* and *in vivo* by MLCK (13). In untreated U251 MG cells, the bulk of the RLCs are phosphorylated (Fig. 3), as evidenced by the large apparent ratio of phosphorylated to unphosphorylated RLC. The limited resolution of the urea gel system prevents a precise measure of the number of phosphorylated species present. However, at least three and possibly four bands of phosphorylated RLC are evident, which correspond to mono- and diphosphorylation of the two isoforms (arrows). By contrast, treatment for 3 h with ML7 at doses that abolish haptotaxis and motility leads to a marked reduction in RLC phosphorylation. This is demonstrated by the near disappearance of the more mobile, phosphorylated species.

**Effects of MLCK Inhibitors on U251MG Motility and Attachment.** Haptotaxis in the Transwell chamber *in vitro* assay is a complex process that involves several steps. These include the ability of the cells to detect a gradient of vitronectin, to migrate toward this gradient, and to attach to a vitronectin-coated surface. To examine which of these steps is dependent on myosin II, we have examined the effects of KT5926 and ML7 on the ability of U251MG cells to move and to attach to a vitronectin-coated surface.

![Fig. 3. Urea gel electrophoresis to assess myosin II regulatory light chain phosphorylation. Cell lysates were dissolved in urea and electrophoresed on polyacrylamide gels, electrotransferred to nitrocellulose, and blotted with an anti-RLC antibody. The bulk of phosphorylated RLC. The limited resolution of the urea gel system prevents a precise measure of the number of phosphorylated species present. However, at least three and possibly four bands of phosphorylated RLC are evident, which correspond to mono- and diphosphorylation of the two isoforms (arrows). Three-h treatment with ML7 at either 20 or 40 \(\mu\)M results in a substantial decrease in the amount of RLC phosphorylation.](image-url)
Cell movement was monitored with a scrape motility assay. As demonstrated in Fig. 4A, U251MG cells can be seen to move toward and fill in a 150–200-μm-wide cell-free zone over a 24-h incubation period. When this assay is performed in the presence of 2 μM KT5926 (Fig. 4B) or 10 μM ML7 (Fig. 4C), this process is completely blocked.

In addition to its role in powering cell motility, myosin II has also been implicated in the organization of surface receptors (18). Thus, MLCK inhibitors might also alter the ability of U251MG cells to organize their integrin receptors for vitronectin and inhibit attachment to a vitronectin-coated substrate. As Fig. 5 shows, both KT5926 and ML7 partially inhibit attachment over the same range of concentrations that block in vitro haptotaxis. By contrast, KT5823 has little effect in this assay. Similar results have been reported previously in lung carcinoma cells attaching to fibronectin (29).

**Effect of MLCK Inhibitors on U251 Viability.** To insure that the effects of the MLCK inhibitors on U251 MG invasiveness and motility are not due to toxicity, we measured U251MG viability in the presence of a range of concentrations of these drugs. Viability was measured both by the uptake and reduction of a vital stain (Alamar Blue) as well as by [3H]thymidine uptake. KT5926 reduced [3H]thymidine uptake at most by 28%, at the highest concentration tested, where in vitro haptotaxis is reduced by >95%. ML7 had no appreciable effect in this assay, and neither drug appears to affect viability, as measured by AlamarBlue uptake and reduction (data not shown).

**Effects of MLCK Inhibitors on Integrin, Focal Adhesion, and Microfilament Distribution.** To determine whether inhibition of MLCK affected cytoskeletal organization in cells adherent to vitronectin, we performed immunofluorescence microscopy of U251 MG cells stained with anti-vinculin (a marker of focal adhesions) or with an antibody to the αv subunit of the αvβ3 integrin. As Figs. 6 and 7 demonstrate, untreated U251MG cells demonstrate focal adhesion localization of both proteins, which are located both centrally and peripherally, consistent with the appearance of mature focal adhesions (A, arrows).

Addition of either ML7 (panel B) or KT5926 (panel C) produces a redistribution of focal adhesions toward the cell cortex (arrows).

The spatially coordinated distribution of membrane receptors, such as integrins and focal contacts, requires an intact actin-based microfilament system (36). Because the above results indicate that myosin...
II helps regulate the distribution of focal adhesions, it is reasonable to ask what effect inhibition of myosin II function has on microfilaments. We have addressed this issue by staining U251 MG cells with rhodamine phalloidin in the presence and absence of MLCK inhibitors. As Fig. 8A shows, untreated U251 cells demonstrate both prominent stress fibers as well as F-actin in a submembranous layer and in membrane ruffles, a pattern that has been described in cultured astrocytes (37). Treatment of cells with KT5926 (Fig. 8B) or ML7 (Fig. 8C) for 3 h leads to a marked reduction in the number of stress fibers, in formation of punctate, centrally located actin staining, and with relative preservation of F-actin in membrane ruffles and along the plasma membrane. Pretreatment of cells with either drug for 24 h, followed by replacement with drug-free medium for an additional 24 h, was performed to determine reversibility of this effect. Cells were then fixed and stained with rhodamine phalloidin, and this revealed a normal complement of stress fibers (data not shown).

DISCUSSION

One of the hallmarks of astrocytic neoplasms is their marked ability to migrate and to invade normal brain (1). This invasiveness insures that small groups of malignant cells are surrounded by an intact blood-brain barrier and protects these migratory cells from water-soluble chemotherapeutic agents. It also complicates efforts at controlling local disease with surgery or focused radiation. This argues that the components that are required for invasiveness could serve as targets for new therapeutic interventions. Invasion of normal brain by malignant cells is a multi-step process requiring a coordinated effort by a number of intracellular and extracellular components. Motile forces, generated intracellularly, are transmitted through focal adhesions and through the integrin-mediated contacts with the extracellular matrix to generate movement. Although much effort has been expended to investigate the contributions of integrins and extracellular matrix to glioma motility (3–9), relatively little is known about how...
mM KT5926 (B) or 10 mM ML7 (C). Actin staining is seen in stress fibers, along the plasma membrane, and in membrane ruffles. Addition of MLCK inhibitors leads to loss of stress fibers, formation of centrally located punctate F-actin staining, and preservation of the submembranous and membrane ruffle-associated actin. Bar, 100 μm.

Fig. 8. Rhodamine-phalloidin staining of F-actin in U251 cells in the absence of MLCK inhibitors (A) or in the presence of 2 μM KT5926 (B) or 10 μM ML7 (C). Actin staining is seen in stress fibers, along the plasma membrane, and in membrane ruffles. Addition of MLCK inhibitors leads to loss of stress fibers, formation of centrally located punctate F-actin staining, and preservation of the submembranous and membrane ruffle-associated actin. Bar, 100 μm.

Inhibitors of Glioma Migration

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process include actin and myosin II. Molecular genetic, immunological, and pharmacological interventions have been used to demonstrate that myosin II plays a central role in the formation of a cleavage furrow in cytokinesis, in receptor internalization, in secretion, in wound closure, in generation of contractile forces, and in directed cell movement (12–19, 38, 39). More relevant studies of the importance of myosin II to the central nervous system have been published recently (16, 40, 41) and have demonstrated the importance of myosin II in growth cone motility and in astrocyte contractility. In this study, we have extended these observations to a tumor derived from astrocytes, glioblastoma multiforme.

Myosin II, like its smooth muscle counterpart, is regulated by calmodulin-dependent phosphorylation of the regulatory light chain (11, 20). The importance of myosin II regulation in cell function is underscored by the multiple signal transduction pathways that control RLC phosphorylation. These include mitogen-activated protein kinase, which regulates MLCK activity in a phosphorylation-dependent manner, as well as the G protein Rho, which can induce RLC phosphorylation through its effects on Rho kinase and myosin light chain phosphatase (42–44). Phosphorylation allows myosin II to interact with actin in a force-productive manner (20), and dephosphorylation of the regulatory light chain by a myosin light chain phosphatase inactivates this system. The balance of activities of MLCK and myosin light chain phosphatase determine how motile a cell is. Thus, drugs that inhibit MLCK, such as KT5926 and ML7, would be expected to lead to an eventual dephosphorylation of the myosin regulatory light chains and inactivation of the contractile apparatus. In this study, we have used KT5926 and ML7 to determine whether inhibition of myosin function indeed blocks motile-dependent behavior in U251MG, a human glioblastoma cell line. As demonstrated in Figs. 1 and 2, both drugs are potent inhibitors of glioma motility and invasiveness, as measured in vitro. Other protein kinases, including calmodulin-kinase II and protein kinase C, can be inhibited by these drugs. However, the $K_i$ for protein kinase C for KT5926 is 723 nM, which is 40-fold higher than that for MLCK. Likewise, the $K_i$ of ML7 for protein kinase C is 42 μM, which is 140-fold higher than that for MLCK. Furthermore, although calmodulin-kinase II is effectively inhibited by KT5926, it is essentially unaffected by ML7. Thus, the dose-response relationship seen with these agents together can only be explained by their specific inhibition of MLCK. This is also supported by urea gel electrophoresis of U251MG lysates (Fig. 3), which provides direct evidence that ML7 at doses that abolish haptotaxis and migration also leads to a significant reduction in RLC phosphorylation. In addition to phosphorylation at serine 19 and threonine 18 by MLCK, the RLC can be phosphorylated by protein kinase C at serine 1, and this phosphorylation reaction inhibits actomyosin contractility (45). Thus, the small amount of phosphorylation still present at high doses of ML7 (Fig. 3) may reflect the effects of residual protein kinase C activity.

Although the scrape motility assay is sensitive to the motile behavior of the glioma cells, the in vitro haptotaxis assay used in this study depends not only on cell motility but also on the ability of glioma cells to detect a gradient of vitronectin, crawl toward it, and attach to a vitronectin-coated membrane. This in turn may require gliomas to bind vitronectin through their membrane-bound integrins, internalize these complexes, and recycle the vitronectin receptors. Localization of membrane-bound receptors and their internalization are myosin II-dependent processes (18), and thus, myosin II may also be responsible for the ability of U251MG cells to sample their environment and migrate toward a chemical gradient. This may explain why KT5926 and ML7 also inhibited attachment of U251MG cells to a vitronectin-coated surface and redistributed focal adhesions to the cell cortex. The latter distribution is seen during early cytoskeletal organization, when focal adhesions are immature.

Immunofluorescence microscopy has demonstrated that stress fi-
bers terminate at focal adhesions, and both direct and indirect interactions exist between specific components of focal adhesions and F-actin (46, 47). Stress fibers contain both actin and myosin II (46, 47), and if myosin II activity were necessary for stress fiber integrity, then its inhibition with KT5926 or ML7 might be expected to disrupt not only focal adhesions but also stress fibers as well. As shown in Fig. 8, this is in fact the case, and these results are consistent with other studies that have demonstrated the necessity of myosin II for normal stress fiber localization (29).

The results of this study thus demonstrate that myosin II has several important functions in supporting the process of invasion by glioma cells. Myosin II drives cell motility, but in addition, its activity may be necessary for sampling the environment for chemoattractant cues and attachment to the extracellular matrix. However, myosin II is not a homogeneous population of molecular motors. There are two major isoforms of myosin II, referred to as IIA and IIB (41), and they differ in their intracellular localization and tissue distribution (46). Both isoforms are expressed in human glioblastoma surgical specimens at levels that are severalfold higher than in normal brain.4 Because the activity of both myosin II isoforms is controlled by regulatory light chain phosphorylation, it is not possible to deduce which isoform is responsible for glioma invasiveness on the basis of pharmacological studies with MLCK inhibitors. Such investigations will likely require more specific methods of suppressing myosin II function, such as utilization of isoform-specific antisense oligonucleotides (48). Nevertheless, the results of this study make it clear that myosin II plays a central role in glioma invasiveness and could therefore serve as a target for the development of invasion-inhibiting therapies.

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