Involvement of mTOR Signaling Pathways in Regulating Growth and Dissemination of Metastatic Brain Tumors *via* EMT

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Abstract. *Background: Metastatic dissemination to the brain may involve a process termed epithelial-mesenchymal transition (EMT), which results in a migratory, invasive and proliferative cell phenotype. Recent studies suggest that Mechanistic target of rapamycin (mTOR, that exists in two multi-protein complexes (mTORC1 and mTORC2), may regulate EMT, in addition to controlling cell growth, survival, metabolism and motility. However, the role of mTOR in brain metastases remains elusive. We hypothesize that mTOR plays a crucial role in the process of EMT in brain metastasis and therefore serves as a target of therapy. Materials and Methods: Immunohistochemical analyses were performed to determine the expression of components of mTOR pathways. Immunofluorescence and immunoblotting were executed to determine the markers of EMT after treatments with siRNA or inhibitors of mTOR pathways. Cell proliferation using MTT, S-phase entry by determining EdU-incorporation, chemotactic and scratch-wound migration assays were performed. Results: Metastatic tumor samples expressed components of mTOR pathways, namely, mTOR, Raptor and Rictor with a significant overlap. Metastatic potential was enhanced in an astrocytic environment and suppressed following mTOR inhibition. mTOR inhibition resulted in nuclear localization of the epithelial marker of EMT, E-cadherin, and enhancement in expression of the mesenchymal marker vimentin. Conclusion: Results suggest that the mTOR pathway is activated in metastatic brain tumors, and inhibition of mTOR signaling could provide therapeutic value in the management of patients with brain metastases.*

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In the United States, more than 40% of cancer patients develop metastases to the brain over the course of their illness (1). The incidence is estimated to be about 170,000/year in the United States, 10-times higher than that of primary malignant brain tumors (2), affecting approximately 10% of patients with solid tumors (3-5). Primary tumors that have a tendency to metastasize to the brain include lung (20%), breast (5%), melanoma (7%), renal (7%) and colorectal (2%), with breast cancer patients aged 20 to 39 years having the highest proportional risk of brain metastases (6). Median survival of patients with multiple brain metastases is estimated at 3 to 4 months, with a 1-year survival rate of 12% (7).

Development of brain metastases requires tumor cells to access the brain vasculature by attaching to microvessel endothelial cells, extravasate into the brain parenchyma, induce angiogenesis and proliferate in response to growth factors (8-9). Tumor cells that survive form micrometastases may develop into clinically-significant lesions after a fairly unpredictable period of latency (dormancy), time spent meeting requirements for cell division in the new microenvironment (10-11). Eighty percent of all brain metastases occur in the cerebral hemisphere, while 15% are found within the cerebellum and 5% in the brainstem (12). It is important to note that, unlike primary brain malignancies, such as gliomas, metastatic tumors develop distinctly well-defined margins separating the metastatic lesion from the surrounding brain tissue.

Although the genetic basis of tumorigenesis may vary to a great extent between different cancer types, the cellular and molecular steps required for metastatic spread are generally similar for all solid tumor cells (13-14). Formation of overt clinical metastases requires a multi-step tumor dissemination process involving cellular escape from the primary tumor by invasion of the surrounding tissue, entry and survival in the bloodstream (intravasation), arrest and/or extravasation at the secondary site and survival and proliferation at the distant location (15-17). This process requires primary tumor cells to undergo a unique, reversible cellular reprogramming

termed epithelial-mesenchymal transition (EMT). EMT is typified by the dissolution of cell-cell junctions and loss of apico-basolateral polarity resulting in the formation of migratory mesenchymal cells with invasive properties (18). Migrating cancer cells that underwent EMT undergo reversal mesenchymal-epithelial transition (MET) and revert back to their epithelial phenotype once they reach the target organ, a process that may involve the role of cancer stem cells (19). In fact, the expression of various stem cell markers has been shown in these transiting cells (20).

In recent years, efforts have been made to decipher genes associated with brain metastases (21-23). Researchers have demonstrated that the PI3K/AKT signaling pathway is activated in many tumors by either loss of tumor suppressive *PTEN* or an activating mutation of *PI3-K*. Downstream from this pathway is the mechanistic target of rapamycin (mTOR), a key regulator of cell growth, proliferation, differentiation and survival (24-25). The mTOR pathway regulates several processes including autophagy, ribosome biogenesis and metabolism by integrating signals from growth factors, nutrients, oxygen and energy status (24, 26). Furthermore, mTOR plays an essential role in the regulation of tumor cell motility, invasion and metastasis (27-29). In addition, mTOR has been linked to cancer stem cell regulation (30).

mTOR forms two multiprotein complexes, mTORC1 and mTORC2, with discrete substrate specificity to coordinate various cellular and metabolic functions. Recent studies implicate mTORC1 and mTORC2 as key regulators of EMT by virtue of their expression in metastatic tumors (31-32). This conclusion is based on the observation that the components of the mTOR pathway are expressed in metastatic colon cancer, while silencing mTORC1 and mTORC2 results in suppression of migration and reduced production of the factors that enhance the motility of cancer cells (32). Consistent with these findings, a study demonstrated that the cytokine transforming growth factor-beta (TGFβ), which is known to play a major role in promoting EMT, induces activation of mTOR signaling *via* the mTORC1 activity and phosphorylation of p70S6K and 4E-BP1, which subsequently increased protein synthesis and cell size (33). Furthermore, suppression of mTOR signaling inhibited cell migration and invasion associated with TGFβ-induced EMT *via* mTORC2 (34). However, the role of the mTOR pathway in metastatic brain tumors remains to be elucidated. In the present study, we investigated the activation of mTOR and the effects of its inhibition in metastatic brain tumors.

Materials and Methods

Cell lines and reagents. Samples of confirmed metastatic tumors were obtained from the Department of Pathology at Westchester Medical Center, Valhalla, NY after Institutional Review Board approval. The breast cancer cell line MDA-MB 231 and commercially available neurosphere forming primary glioblastoma cell lines U87, and LN18 (ATCC, Manassas, VA, USA) were used. Cells were maintained in Dulbecco's modified Eagle's medium (DMED; Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS and 1% penicillin/streptomycin/amphotericin in a humidified incubator with 5% CO_2 at 37˚C. Cells were made quiescent by serum deprivation 24 h prior to treatment. Treatment included the mTORC1 inhibitor rapamycin (RAPA, 100 nM; EMD Chemicals, Billerica, MA, USA) and mTORC1/2 inhibitor PP242 (PP242, 2.2 μm; EMD Chemicals). Cells were transfected with siRNA for *mTOR* (FRAP; NM_004958; CAGGCCTATGGTCGAGATTTA), *Raptor* (KIAA1303; NM_020671; CTGGGTCTTCAACAA GAACTA) and *Rictor* (NM_152756; ATGACCGATCTGGACC CATAA) according to manufacturer's instructions (Qiagen, Valencia, CA, USA) using HiPerFect transfection reagent (Qiagen). AllStar Hs Cell Death Control and non-specific AllStar Negative Control (Qiagen) were used as positive and negative controls, respectively.

Immunohistochemistry. The standard immunohistochemistry technique was utilized to determine the expression of mTOR (Cell Signaling, Beverly, MA, USA), Raptor and Rictor (Abcam, Cambridge, MA, USA). In brief, tumor slides were baked at 60˚C for 30 min. Specimens were deparaffinized in xylene and rehydrated in graded concentrations of ethanol. Antigen retrieval was done by using citrate buffer and incubating the slides at the highest temperature in a pressure cooker for 10 min. Tumor sections were then incubated at room temperature for 30 min with the anti-mTOR, anti-Raptor or anti-Rictor antibody. Detection of mTOR, Raptor and Rictor was done using a horseradish peroxidase-conjugated detection kit (Invitrogen, Frederick, MD, USA) and counterstained with hematoxylin.

Scratch/wound healing migration assay. The scratch wound migration technique was used to determine the motility of tumor cells following treatment. MDA-MB 231 cells were grown to confluent monolayerand, when approaching 100% cell confluence, scratching the surface as uniformly as possible with a pipette tip formed a wound. This initial wounding and migration of cells in the scratched area was photographically monitored using the Axiovert Zeiss 200 microscope (Carl Zeiss, Thornwood, NY, USA) with ×10 magnification (NA 0.25). The migration rate was expressed as a percentage of the control and calculated as the proportion of the mean distance between both borderlines caused by scratching to the distance that remained cell-free after migration. Two independent series of experiments were performed in quadruplicate.

Chemotactic migration. Directional migration was performed using a 48-well modified Boyden chamber kit (NeuroProbe, Gaithersburg, MD, USA). Quiescent cells were subjected to rapamycin or PP242. Vehicle-treated cells served as controls. Cells were aliquoted (3,000 cells/μl) in either serum-free media or their respective rapamycin or PP242 treated media. U87 was used as a chemoattractant and cells were allowed to migrate for 24 h through a polyvinyl chloride membrane (8-μm pore). The membrane was fixed in 70% ethanol, scraped along the non-migrated cell surface and stained with DiffQuick (IMEB, San Marcos, CA, USA). Migrated cells were imaged at ×10 (Axiovert 100M) and analyzed as a percentage of total microscopic field occupied by migrated cells (ImageJ; NIH, Bethesda, MD, USA).

Table I. *Primary tumors that displayed metastases to the Brain.*

Primary tumors (Metastases to the brain)	Number of samples (% of control)
Lung	53
Breast	27
Renal	7
Colon	7
Gallbladder	7

5-ethynyl-2-deoxyuridine (EdU) incorporation proliferation assay. Proliferating cells were visualized by utilizing the Click-iT EdU Imaging Kit (Invitrogen). Control, rapamycin- or LN18-treated samples were incubated for 4 h in 10 μM EdU. Cells were subsequently fixed in 4% paraformaldehyde for 15 min at room temperature and permeabilized for 15 min in 0.1% Triton X-100 in phosphate buffered saline (PBS). EdU incorporation was detected by incubation in the Click-iT reaction cocktail (as prescribed by the kit) at room temperature. The samples were then washed for 5 min in PBS three times. Frequency maps of the cell proliferation were constructed from fluorescence images using the Axiovert Zeiss 200 microscope (Carl Zeiss).

Cell proliferation assays. Cell growth was measured by MTT assay according to the manufacturer's protocol (Chemicon, Billerica, MA, USA). Cells (3,000/well) were seeded onto a 96-well plate and made quiescent for 24 h prior to treatment. After completion of treatment, fresh media containing 10 μl of MTT reagent was added to cells, plates were incubated at 37˚C for 4 hours, 100 μl of detergent reagent was added and absorbance was measured after 2 h.

Fluorescence immunohistochemistry. Cells were treated with PP242, rapamycin, mTOR siRNA, Raptor siRNA or Rictor siRNA for 24 h. After treatments, cells were fixed in 4% PFA, blocked with 10% goat serum in PBS/0.1% Triton-X100 and stained with E-cadherin (Cell Signaling) and vimentin (Abcam) according to manufacturer's instructions. FITC-Green or Rhodamine-Red secondary antibody was used (Jackson ImmunoResearch, West Grove, PA, USA) with 4',6-diamidino-2-phenylindole (DAPI) nuclear counterstaining. Cells were visualized using the imaging system of Axiovert 100M; Zeiss microscope (Carl Zeiss).

Isolation of protein. Protein extraction was performed with whole cell lysis buffer containing 1% Triton X-100, 10 mM Tris-HCl, pH 7.5, 150 mM NaCl and 5 mM EDTA containing phosphatase and protease inhibitors (Sigma-Aldrich, St. Louis, MO, USA). Protein concentrations were determined by the modified Lowry method (Bio-Rad Laboratories, Hercules, CA, USA).

Western blot analysis. Equal amounts of protein were resolved on a 10% SDSPAGE gel and then electrotransferred onto nitrocellulose membrane. Membranes were processed according to the manufacturer's instructions (Santa Cruz Biotechnology, Dallas, TX, USA; Cell Signaling Technology, Beverly, MA, USA). A routine procedure utilized primary antibodies for E-cadherin and vimentin at 1:1000 dilutions (Santa Cruz Biotechnology; Cell Signaling Technology), was followed by detection by chemiluminescence (Millipore, Billerica, MA, USA). Blots were stripped (Pierce Protein Biology Products, Rockford, IL, USA) and re-probed with actin or respective total antibodies to ensure equal loading. Densiometric analysis was performed using ImageJ (NIH). Experiments were conducted at least three times.

Statistical analysis. Values are presented as the mean±standard error of the mean (SEM). Two-tailed *t*-tests were used for single comparisons between control and treated groups. A p-value of <0.05 was considered significant.

Results

Expression of mTOR markers in metastatic brain tumors. We analyzed various primary tumor samples that have a tendency to metastasize to the brain. Our cohort indicates that lung and breast were the primary sites with the greatest propensity to metastasize (Table I). Other primary sites of origin included in our study were renal, colon and gallbladder, which metastasized with a lesser frequency (7%). To delineate the role of the mTOR pathway we examined the expression of its components, mTOR, Raptor (mTORC1) and Rictor (mTORC2) (Figure 1A). As demonstrated in the Venn diagram (Figure 1B), about 47% of samples expressed all three components, mTOR, Raptor and Rictor, and over 70% of all tumor samples exhibited at least two of these protein molecules. These observations suggest that the mTOR pathway may play a critical role in establishing metastases to the brain.

Expression of EMT markers following inhibition of mTORC1 and mTORC2. To investigate the influence of mTOR on the epithelial and mesenchymal markers of EMT in metastatic tumors, we studied the expression of E-cadherin (epithelial) and vimentin (mesenchymal) markers following treatment with inhibitors of the mTOR pathway (Figure 1D, 1E). Immunohistochemical analysis demonstrated that vehicle-treated controls showed marginal expression of E-cadherin, which was suppressed by pharmacological treatment with rapamycin (mTORC1 inhibitor), as well as PP242 (mTORC1/2 inhibitor). Upon treatment with rapamycin, E-cadherin translocated from the cytoplasm to the nucleus, an observation that was not visualized following PP242 treatment. However, following both rapamycin and PP242 treatment, vimentin expression was enhanced. In order to confirm that complete suppression of the mTOR pathway affects the markers of EMT, we treated cells with siRNA for *mTOR, Raptor* and *Rictor*. HiPerFect-treated control demonstrated expression of E-cadherin, which was suppressed following siRNA treatments of *mTOR*, *Raptor* and *Rictor*. The expression of vimentin increased in *mTOR* siRNA- and *Rictor* siRNA-treated cells. These observations demonstrated that treatment with the inhibitors of the mTOR pathway or suppression of mTOR, Raptor and Rictor expression *via* siRNA, altered the levels of EMT markers.

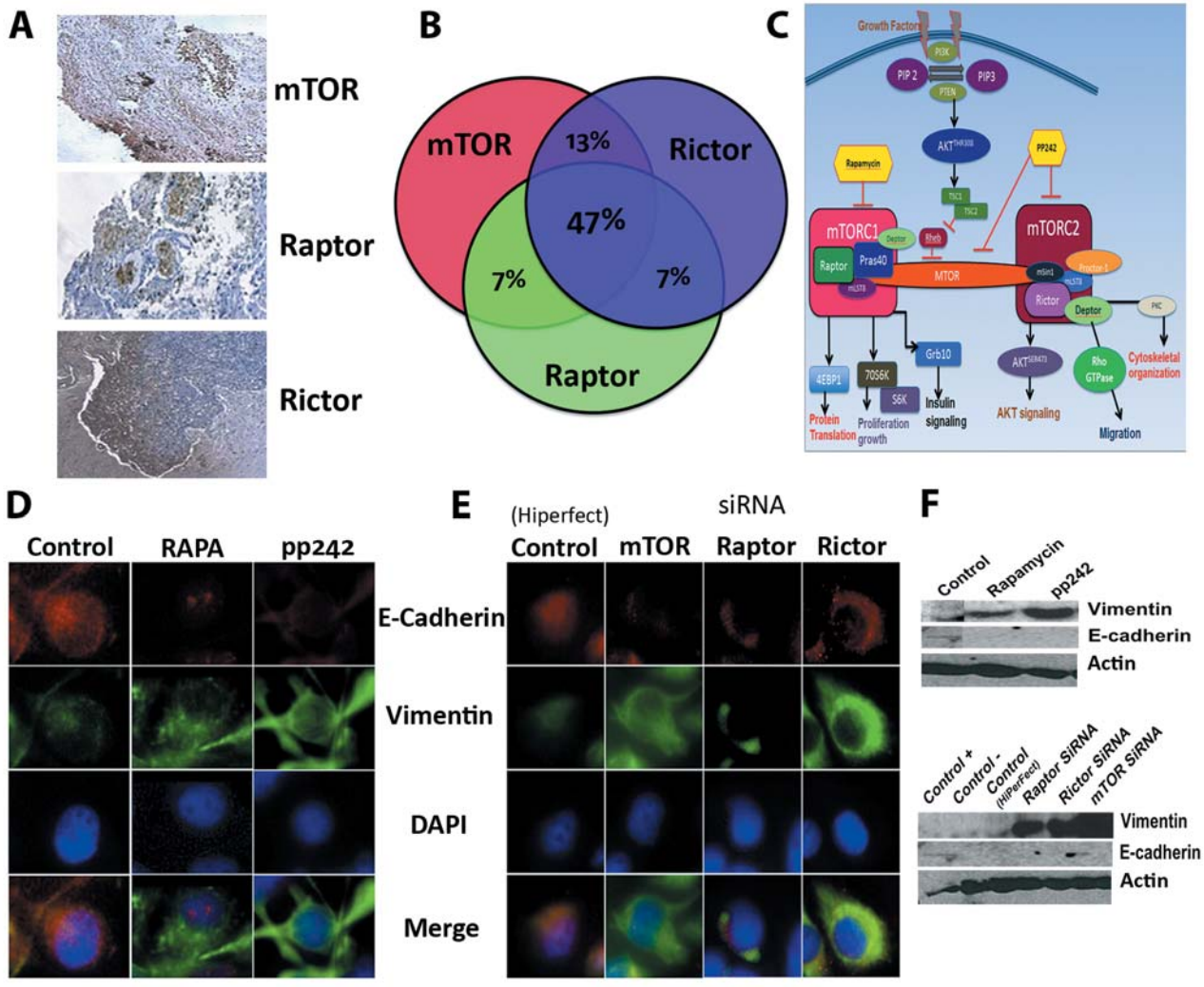

Figure 1. *Role of mTORC1/2 in metastatic brain tumors. Immunohistochemical analysis demonstrating the expression of mTOR components: mTOR, Raptor and Rictor. (A) Expression of mTOR, Raptor and Rictor in metastatic brain tumors. (B) Venn diagram with 47% of tumor samples expressing all three protein markers. (C) Cartoon diagram depicting mTOR and its components in regulating multiple cellular processes. The mTORC1 inhibitor rapamycin (RAPA) and the mTORC1/2 inhibitor PP242 are used to decipher their role in EMT. (D) Treatment with rapamycin displaces expression of E-cadherin from the cytoplasm to the nucleus. Although E-cadherin expression decreased following PP242 treatment, there was no effect on E-cadherin re-localization. The expression of vimentin was enhanced following mTOR inhibition using rapamycin or PP242. (E) siRNA treatment for mTOR, Raptor or Rictor demonstrates reduced E-cadherin expression and enhanced vimentin expression. (F) Top panel: Immunoblotting analysis demonstrates decreased E-cadherin and enhanced vimentin expression following rapamycin or PP242 treatment. Bottom panel: Immunoblotting depicts suppression of E-cadherin and enhancement of vimentin levels following treatment with siRNA for mTOR, Raptor or Rictor.*

Alterations in EMT markers following mTOR inhibition were confirmed by western blotting analysis. As shown in Figure 1F (top panel), treatment with rapamycin showed increased expression of vimentin compared to control and inhibition *via* PP242 treatment showed an even greater increase in vimentin levels as compared to control. Control cells demonstrated subtle E-cadherin expression, which dissipated completely following treatment with rapamycin or PP242. As with mTOR inhibition using rapamycin or PP242, vimentin expression was significantly increased in cells treated with *mTOR* siRNA, *Raptor* siRNA or *Rictor* siRNA as compared to controls treated with HiPerFect (Figure 1F; bottom panel).

mTORC1/2 components involved in the regulation of brain metastasis. In order to establish that the cerebral

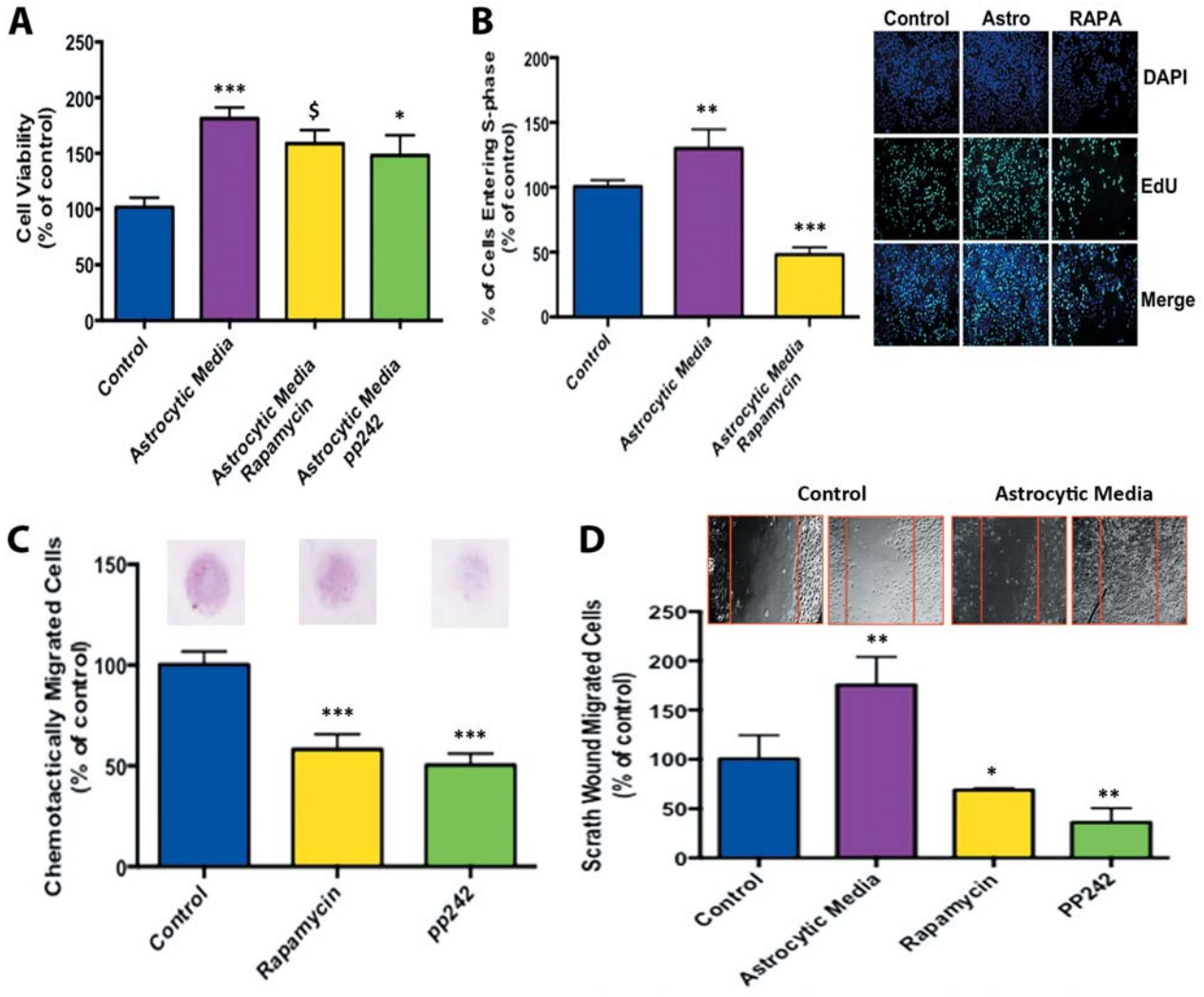

Figure 2. *Influence of mTORC1/2 in determining cell proliferation, S-phase entry and migration of metastatic tumor cells grown in astrocytic media. (A) Cell proliferation was significantly enhanced in the presence of astrocytic media ($p<0.001$) compared to control. Treatment with mTORC1 inhibitor (rapamycin) caused a trend towards significance in suppressing proliferation relative to the astrocytic media ($p=0.69$); mTORC1/2 inhibitor, PP242, significantly reduced proliferation ($p<0.05$). (B) Cell-cycle entry analysis depicting a significant number of cells entering S-phase in astrocytic media compared to control ($p<0.01$). Treatment with rapamycin significantly reduced S-phase entry ($p<0.001$ relative to astrocytic media). The fluorescent picture depicts incorporation of EdU with Alexa4 staining. (C) Cells were allowed to migrate towards the astrocytic media in the chemotactic assay. Control cells migrated extensively towards astrocytic media. Rapamycin or PP242 treatment significantly halted migration ($p<0.001$). (D) Scratch wound migration analysis demonstrates profuse migration of cells in astrocytic media relative to control ($p<0.01$). This migration was abrogated following treatment with rapamycin ($p<0.05$) or PP242 ($p<0.01$).*

microenvironment plays a significant role in tumor growth, proliferation, invasion and survival, we exposed the breast cancer cell line MDA-MB 231 to astrocytic media. Furthermore, to determine the role of the mTOR pathway in the interaction between the primary tumor cells and the surrounding brain milieu, we performed a series of functional analyses following treatment with mTOR inhibitors.

MTT analysis was performed to assess the ability of metastatic tumor cells to grow in an astrocytic environment and to examine the effect of mTOR pathway inhibition (Figure 2A). The mesenchymal breast cancer cell line MDA-MB 231, which exhibits a high affinity for the cerebral atmosphere, proliferated profusely in the astrocytic medium ($p<0.001$). PP242 significantly suppressed cell proliferation in the astrocytic media ($p<0.05$). Rapamycin also suppressed

cell growth; however, only achieved a trend towards significance (p=0.69).

Figure 2B depicts cell-cycle entry analysis using the clickit EdU technique. We demonstrated that a significantly greater percentage of tumor cells entered S-phase when exposed to astrocytic media compared to control (p<0.01). When tumor cells were exposed to astrocytic media and then treated with rapamycin, S-phase entry was dramatically reduced (p<0.001).

Migration analysis, as demonstrated by chemotactic migration or scratch wound migration, was used to ascertain the migration of breast cancer cells in or towards astrocytic media. Breast cancer cells extensively migrated towards the astrocytic media; however, the chemotactic migration capacity was abrogated significantly following treatment with rapamycin or PP242 (p<0.001) (Figure 2C). Scratch wound migration also showed extensive migration of breast cancer cells in astrocytic media (p<0.01). Treatment with rapamycin (p<0.05) or PP242 (p<0.01) suppressed migration; however, PP242 was more effective at suppressing migration (Figure 2D).

Discussion

The findings of this study demonstrate that a noticeably high proportion of metastatic brain tumor samples express components of the mTOR pathway, mTOR, Raptor and Rictor. Pharmacological inhibition of the mTOR pathway using the mTORC1 inhibitor rapamycin or mTORC1/2 inhibitor PP242 altered the expression of EMT markers. Similarly, siRNA inhibition of *mTOR, Raptor* and *Rictor* demonstrated suppression of E-cadherin and its translocation from the cytoplasm to the nucleus, while vimentin's expression was enhanced. These observations were confirmed using immunoblotting analysis. Tumor cells grew robustly in astrocytic media. Functional analyses using mTORC1 and mTORC1/2 inhibitors showed reduced cellular proliferation, cell cycle entry and migration.

In evaluating cells from the distant metastasis, we were unable to determine if mTOR was present in the primary tumor and its expression enhanced following the development of metastases or if mTOR expression originated after metastatic spread took place. However, recent observations have illustrated that key components of the mTOR pathway are expressed in pre-metastatic stages of cancer and their expression enhanced upon metastatic spread (32). Our results are consistent with these findings as we show a high proportion of metastatic brain tumor samples expressed components of the mTOR pathway (Figure 1A, 1B). These observations suggest that the mTOR pathway plays a critical role in metastatic disease to the brain.

In this study, we utilized a breast cancer cell line with a mesenchymal phenotype that has a tendency to metastasize to the brain. Following suppression of these cells using mTOR inhibitors, E-cadherin expression was reduced and predominantly localized to the nucleus, while vimentin expression was enhanced. These observations were confirmed by Western blotting analysis (Figure 1F). Similar results were obtained following siRNA inhibition. Alterations of these markers upon inhibition of the mTOR pathway suggest its involvement in the reverse process of EMT, MET. However, these observations should be evaluated with caution as this cell line expresses only subtle levels of epithelial markers. These results also imply that inhibition of the mTOR pathway is involved in maintaining the epithelial phenotype in mesenchymal cells, which provides tumor cells the ability to sustain their metastatic potential. The findings implicate mTORC1 and mTORC2 as key regulators of the process of EMT and its counterpart MET. This conclusion is based on the observation that silencing mTORC1 and mTORC2 induces a repertoire of biochemical (decreased E-cadherin and increased vimentin expression), morphologic (decreased cell-cell contact, decreased formation of lamellipodia) and functional (decreased migration and cellular division) changes characteristic of EMT/MET. These findings also provide the rationale for including mTOR kinase inhibitors, which target the ATP binding pocket inhibiting both mTORC1 and mTORC2 more completely, as part of the therapeutic regimen for treating patients.

mTOR forms two multiprotein complexes with distinct functions and substrates. The chief function of mTORC1 is to regulate cell growth, proliferation and survival *via* phosphorylation of its downstream effector molecules S6K1 and 4E-BP1. mTORC1 responds to mitogen, energy and nutrient signals through the upstream regulators tuberous sclerosis complex 1/2 (TSC1/2) and Rheb (25). As shown in our study (Figure 2A), suppression of mTORC1, using rapamycin, produced reduction of proliferation of cells grown in astrocytic media. In addition, cell cycle's S-phase entry was suppressed following rapamycin treatment (Figure 2B).

Independently from mTORC1, mTORC2 regulates the cell's actin cytoskeleton by mediating the PKC phosphorylation state (35). mTORC2 also directly phosphorylates Akt on Ser473, adding a new insight into the role of mTOR in cancer (24). Akt was known to be a key regulator of signal transduction processes that control several cellular functions, such as nutrient metabolism, cell survival and motility (36-37). We observed that scratch and chemotactic migration was suppressed by the inhibition of mTORC1 (Figure 2C, 2D). However, such suppression was more pronounced with simultaneous inhibition of both complexes. Treatment with rapamycin also suppresses the migration of tumor cells by abrogating the F-actin re-organization and inhibited the growth factor-induced phosphorylation of focal adhesion proteins, such as FAK and BAXILLIN, by disrupting the mTOR/rapamycin complexes

(38). Observed suppression of chemotactic migration (Figure 2C) may be a result of disruption in these complexes. It is important to note that mTORC2 regulates cell migration *via* Rho and its related proteins (25) and is perhaps the reason that combined mTORC1/2 inhibition using PP242 was more effective than mTORC1 inhibition alone (Figure 2C, 2D). Differences in response may be related to manipulations of cellular architecture and cytoskeletal organization. Concurrently, the haptotactic cell migration, as revealed by the scratch wound technique, demonstrated that inhibition of both mTOR complexes significantly suppressed migration (60% from the control), compared to migration following mTORC1 inhibition (30% from the control) (Figure 2D).

The major hypothesis in the development of metastasis is established on the grounds of 'seed and soil' theory (39). This theory implies that the host organ provides a suitable atmosphere for distant primary cancer cells to grow and flourish. To determine whether the cerebral atmosphere is suitable for the primary tumor cells, we treated mesenchymal breast cancer cells in astrocytic media and we observed robust growth, migration and proliferation. Consistent with this theory, we observed that primary tumor cells proliferated robustly, showed enhanced S-phase entry and demonstrated increased migratory potential in astrocytic media (Figure 2A, 2B, 2C, 2D). This observation implies that the formation of brain metastases is achieved when the host milieu is amiable to the primary metastatic tumor cells.

The findings of this study, along with results demonstrated by Gulhati *et al*. (31-32), suggest that mTOR signaling may be a critical mode in regulating cancer progression and metastasis. Utilization of specific mTORC1 and mTORC2 targeting therapies, aimed at interrupting tumor cell proliferation, migration, growth and survival, could be beneficial treatment approaches for patients with metastatic disease.

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