Secreted protein acidic, rich in cysteine (SPARC), mediates cellular survival of gliomas through AKT activation*

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Secreted protein acidic, rich in cysteine (SPARC), is an extracellular matrix protein expressed in many advanced cancers, including malignant gliomas. We and others have previously shown that human glioma cell lines engineered to overexpress SPARC adopt an invasive phenotype. We now show that SPARC expression increases cell survival under stress initiated by serum withdrawal through a decrease in apoptosis. Phosphatidylinositol 3-OH kinase/AKT is a potent pro-survival pathway that contributes to the malignancy of gliomas.

Cells expressing SPARC display increased AKT activation with decreased caspase 3/7 activity. Exogenous SPARC rapidly induces AKT phosphorylation, an effect that is blocked by a neutralizing SPARC antibody. Furthermore, AKT activation is essential for the anti-apoptotic effects of SPARC as the decreased apoptosis and caspase activity associated with SPARC expression can be blocked with dominant-negative AKT or a specific AKT inhibitor. As tumor cells face stressful microenvironments particularly during the process of invasion, these results suggest that SPARC functions, in part, to promote tumor progression by enabling tumor cells to survive under stressful conditions.

Malignant gliomas remain essentially lethal cancers despite maximal therapy because of resistance to all conventional therapies (1). Gliomas, like all cancers, share a restricted set of characteristics essential to tumor development and progression (reviewed in Ref. 2). The ability to resist apoptotic stimuli is prominent among these characteristics. Cancer cells face many noxious stimuli that may induce cell death, including nutrient restriction, hypoxia, acidosis, loss of cell attachment, and genomic instability. Gliomas commonly develop mechanisms through which they resist cell death either through disruption of apoptotic processes or activation of survival signals (3). Microenvironmental cues, including interactions between cells and the extracellular matrix, control cellular survival and resistance to apoptosis. Cancers display alterations of the normal cell-matrix interactions linked to increased proliferation, invasion, and angiogenesis (4). One component of the extracellular matrix is SPARC,1 also known as osteonectin or BM-40, which is a 43-kDa, secreted extracellular glycoprotein that plays important roles in development, tissue healing and remodeling, and angiogenesis (5, 6). SPARC was originally discovered as a component of bone (7) but is also expressed in epithelia exhibiting high rates of turnover (8, 9). Targeted disruption of SPARC expression in mice is associated with several phenotypes as follows: early cataract formation, increased wound healing and adipogenesis, and osteopenia (5). SPARC induces inhibition in endothelial cells (5, 6, 10). In addition to its normal physiological role, SPARC has been linked to cancer progression as many cancer types express increased SPARC levels upon invasion or metastasis (5, 6, 11, 12). Malignant gliomas rarely metastasize but are highly invasive leading to a failure of curative surgery (13). Gliomas express SPARC at sites of invasion and neoangiogenic blood vessels at the brain-tumor interface (11, 14). We and others have shown that malignant glioma cell lines engineered to overexpress SPARC adopt an invasive phenotype both in vitro and in vivo associated with an increased expression of specific matrix metalloproteinases (15–17). SPARC thus represents a potentially important contributor to glioma malignancy.

The mechanisms through which SPARC functions in cancer progression remain complex and depend on tumor cell type and the microenvironment. Mice with disrupted SPARC expression are less sensitive to the development of squamous cell carcinomas induced by ultraviolet radiation (18), but some tumor xenografts grow more aggressively in SPARC knockout mice with a lack of tumor capsule (19, 20). Primary endothelial cells may be growth-suppressed in response to SPARC (21), but we have previously shown that glioma cell lines expressing SPARC do not exhibit significant differences with control lines in either cellular proliferation or apoptosis in 10% serum cell culture conditions (15). However, cells grown in vivo experience much greater restriction of the growth factors found in serum as many growth factors are reversibly bound to the matrix. Glioma cells undergoing invasion may be faced with even more stressful conditions relative to adherent cells as they move

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1 The abbreviations used are: SPARC, secreted protein acidic, rich in cysteine; PI3K, phosphoinositide 3-kinase; FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagle’s medium; DPBS, Dulbecco’s phosphate-buffered saline; PBS, phosphate-buffered saline; PVDF, polyvinylidene difluoride; VEG, vector; BrdUrd, bromodeoxyuridine; EGFR, epidermal growth factor receptor; PDGFRβ, platelet-derived growth factor receptor-β; GSK3, glycogen synthase kinase 3-α/β; IGF1R, insulin-like growth factor-1 receptor.
along brain white matter tracts (13). Recently, Rempel and co-workers (22) have shown that a glioma cell line engineered to overexpress SPARC exhibits differential cell number and morphology relative to parental control cells in serum-restricted (0.1%) conditions. Although the proliferation of cells expressing SPARC varied from parental lines with different extracellular matrices, no matrix preferentially modified the effect of SPARC (22). As SPARC may directly interact with growth factors or indirectly regulate growth factor receptor pathways (5), the cellular impact of SPARC may vary with the withdrawal of growth factors and other survival factors in serum.

We have now shown that the expression of SPARC by gliomas induces cellular survival in serum-free conditions associated with AKT activation. Thus, SPARC-mediated cell survival may represent a novel mechanism by which PI3K-AKT activity may be induced by malignant gliomas. Furthermore, the ability of tumor cells to survive in stressful conditions may represent a critical aspect of glioma invasion as invading cells may face a loss of external survival signals from the microenvironment. Therefore, SPARC may represent an important therapeutic cancer target as it can modulate critical advanced tumor phenotypes, including invasion and resistance to apoptosis.

**EXPERIMENTAL PROCEDURES**

**Cells and Culture**—The genetically defined glioma cell line THR was described previously (23). Briefly, normal human astrocytes were transformed with a combination of retroviruses encoding simian virus 40 early T antigen, the human telomerase catalytic subunit hTERT, and an oncogenic Harvey-ras (23). The well characterized human malignant glioma xenograft D54MG is the Duke University subline of A-172 (24). U87MG cells were purchased from the American Type Culture Collection (Manassas, VA). THR, D54MG, and U87MG human glioma cell lines that were infected with either empty control retrovirus (VEC) or a retrovirus encoding a 1.5-kb cDNA fragment of SPARC (a generous gift from Sandra Rempel, Henry Ford Hospital) were described previously (15). Early passage polyclonal cultures selected for antibiotic resistance were used for all experiments. Glioma cultures were maintained in culture in 10-cm tissue culture dishes in DMEM (THR) or Zinc Option (D54MG and U87MG) media containing 10% fetal bovine serum (In Vitrogen) and glutamine (Invitrogen) until ready for use. All other tissue culture reagents were purchased from Invitrogen unless otherwise described.

**Reagents**—Human platelet osteonectin (SPARC) and bovine osteonectin (SPARC) were purchased from Hematech (Essex Junction, VT). Recombinant human SPARC was a kind gift of E. Helene Sage (Hope Heart Institute) (25). The neutralizing anti-SPARC antibody AON33 (aSP303) was a kind gift of Rolf Brekken (University of Texas Southwestern) (26). AKT inhibitor SH-5 and phosphoinositide 3-kinase (PI3K) inhibitors LY294002 and wortmannin were purchased from Calbiochem, dissolved in Me2SO at 10 mM concentration, and stored at −70 °C before use. All other chemicals were purchased from EMD Chemicals (Gibbstown, NJ) unless otherwise described.

**Cell Number Density Measurements**—Glioma cultures were plated in quadruplicate 6-well dishes at various densities for each cell line and allowed to attach overnight in medium with 10% fetal bovine serum (FBS). The following morning, the medium was removed; cells were washed twice with Dulbecco’s phosphate-buffered saline (DPBS), and serum-free medium was added. Cultures were visually inspected daily. Cell numbers were determined after trypan blue staining of viable tumor cells in parallel plates.

**Annexin V Assays**—Cells were plated in triplicate in 6-well plates at variable densities in medium containing 10% serum overnight. Cultures were then washed twice with DPBS and then fed with serum-free media. At various incubation times, media were collected and combined with adherent cells harvested by trypsinization. After centrifugation, cells were washed, resuspended, and stained for annexin V and pro-
Papideum iodide per the manufacturer’s instructions (Pharmingen). Samples were analyzed on a BD Biosciences flow cytometer and analyzed with manufacturer’s software.

Bromodeoxyuridine (BrdUrd) Staining—Cells were plated in individual wells on slides in media containing 10% FBS overnight. Cells were washed twice with DPBS and allowed to grow in serum-free media. Cells were pulsed with BrdUrd for 4 h, fixed with methanol, and stained with diaminobenzidine. The ratio of cells incorporating BrdUrd was calculated using the number of brown cells relative to the total number of cells. Assays were performed in triplicate.

Western Analysis—For each assay, a 100-mm plate was lysed in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% Nonidet P-40, and 50 mM NaF plus protease inhibitors and phosphatase inhibitors) and centrifuged at 14,000 rpm for 5 min at 4 °C. An equal amount of protein was run on polyacrylamide gels (SDS-PAGE), transferred to PVDF membrane (Millipore, Billerica, MA), hybridized, and detected by using an enhanced chemiluminescence system (Pierce). All antibodies were used according to the manufacturer’s instructions. Phospho-specific antibodies for AKT (Ser-473), epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor-α (PDGFRα), and insulin-like growth factor receptor-1 (IGFIR), as well as total AKT antibodies were purchased from Cell Signaling Technology (Beverly, MA). Caspase3/7, poly(ADP-ribose) polymerase antibodies were purchased from Cell Signaling Technology (Santa Cruz, CA). Osteonectin (SPARC) antibody was purchased from Hematologic Technologies. An anti-tubulin antibody was purchased from Sigma. Secondary antibodies were goat anti-rabbit from Zymed Laboratories Inc.

Flow Cytometric Analysis—Cells were plated into 100-mm plates at a density of 5 × 10⁵ cells per plate, serum-starved overnight after attachment, and then fed with media containing inhibitors for 24 h. Cells were then trypsinized, fixed in 70% ethanol, washed once in DPBS, and resuspended in RNaseA (Sigma, 100 μg/ml) and propidium iodide (Sigma, 50 μg/ml). Samples were analyzed on a FACScan (BD Biosciences) flow cytometer. Each experiment was performed in triplicate.

Transfection—Cells were maintained in medium containing 10% FBS until they reached 50–60% confluence. Plasmid DNAs were incubated with FuGENE 6 (Roche Diagnostics) for 30 min and added to cells per the manufacturer’s instructions. Transfected cells were maintained for 24 h and then split for subsequent experiments.

Caspase-3/7 Activity Assay—Apo-ONE Caspase-3/7 Activity Kits were purchased from Promega (Madison, WI). Assays were performed under the manufacturer’s instructions in a 96-well black wall clear bottom tissue culture plate. 4000 cells/well were plated in the assay. Fluorescent intensity was measured at an excitation wavelength of 485 nm and emission wavelength of 520 nm on SpectraMax Gemini plate scanner.

Nonradioactive Cell Proliferation Assay—CellTiter96 cell proliferation assay kit was purchased from Promega (Madison, WI). Assays were performed, following the manufacturer’s instructions, on a 96-well black wall clear bottom tissue culture plate. 4000 cells/well were plated in the assay. Plates were read for absorbance on VESAMax plate scanner at wavelengths 570 nm (measure) and 650 nm (background).

Nonradioactive AKT Kinase Assay—Cells were plated in 100-mm plates and subjected to serum starvation. After 24 h of incubation with either PBS or human platelet SPARC (2 μg/ml), cell lysates were prepared. Equal protein was incubated with a glycogen synthase kinase 3-α/β (GSK3) fusion protein per the manufacturer’s instructions (Cell
Signaling). The lysates were resolved by SDS-PAGE, transferred to a PVDF membrane, and hybridized. Chemiluminescence was used to detect the phosphorylated GSK3β/H9251/H9252 protein.

Statistical Analysis—Wilcoxon Rank Sum Test was used in all analyses (27).

RESULTS

SPARC Expression Is Associated with Increased Cellular Survival in Serum-free Conditions—Glioma cells that express SPARC exhibit differences relative to parental cells in cellular morphology and growth on different substrates in low serum conditions (22). We have characterized previously the impact of SPARC expression in several glioma cell lines, and we found a consistent increase in invasion measures both in immunocompromised rodents and in Matrigel invasion assays but little impact of SPARC expression on cellular proliferation or apoptosis in standard culturing conditions (15). We serendipitously found that glioma cell lines expressing SPARC survived better relative to control cell lines when left unfed for prolonged periods (data not shown). Therefore, we examined the response of glioma cultures expressing SPARC to withdrawal of serum relative to vector controls. In each cell line tested (the genetically defined human glioma cell line (THR) as well as cell lines derived from patient specimens (D54MG and U87MG)), we consistently observed greater numbers of cells in SPARC-expressing cell lines in serum-free conditions relative to vector control cell lines (Fig. 1). The increase in cell number associated with SPARC expression may be due to increased cellular proliferation or resistance to cell death. Pulsed incorporation of bromodeoxyuridine was used to determine the percentage of cells undergoing DNA replication. All cell lines had very low proliferative indices upon serum starvation, but we found no relative difference between SPARC and vector control cell lines (data not shown). In contrast, flow cytometric measurement of cellular apoptosis by annexin V levels in serum-free conditions revealed a consistent decrease in apoptosis with SPARC expression across each glioma line tested (Fig. 2). Cell cycle analysis of glioma cell lines expressing SPARC or vector control was performed using propidium iodide labeled flow cytometric analysis. We confirmed the decrease in apoptotic index with SPARC expression in each cell line as measured by the sub-G0 fraction, but the remaining cell cycle fractions displayed no relative differences between one another (data not shown). In sum, these results suggest that glioma cells expressing SPARC are resistant to apoptosis induced upon withdrawal of growth factors contained in serum.

SPARC Expression Leads to Increased AKT Phosphorylation in Serum-free Conditions—The underlying mechanisms by which serum may increase cell survival continue to be elucidated. Serum treatment induces activation of several growth factor receptors, recruitment of intracellular signaling mediators to the internal surface of the cell membrane, and initiation of pathways regulating apoptosis. Prominently, PI3K and the downstream effector protein kinase B/AKT are activated in response to serum and decrease the activity of pro-apoptotic proteins (28, 29). Like many cancers, malignant gliomas display increased AKT activation through activity of growth factor receptors or loss of the tumor suppressor gene PTEN (30–32). The THR cell line expresses a wild type PTEN, whereas
D54MG expresses a PTEN protein with deletion of exons 3–9, and U87MG does not express PTEN protein (data not shown). AKT has been linked not only to apoptotic resistance but also to increased tumor cell invasion (33, 34). Thus, AKT appeared to be an excellent potential downstream target of SPARC as both SPARC and AKT mediate tumor invasion and resistance to apoptosis. Indeed, glioma cell lines expressing SPARC displayed increased phosphorylation of a key activating residue of AKT (Ser-473) relative to vector cells in the absence of serum (Fig. 3, A and B). In contrast, the phosphorylation of the pro-proliferative extracellular signal-regulated kinases-1 and-2 (ERK1/2) did not differ between the control and SPARC cell lines (Fig. 3A), suggesting a specific relationship between SPARC and AKT. These findings provide further explanation for the selective impact of SPARC expression on resistance to apoptosis with a lack of proliferative advantage that we have demonstrated.

**SPARC Expression Associates with Decreased Executor Caspase Activity**—Caspases are cysteine proteases that play critical roles as effectors of apoptotic cell death (35). As SPARC expression is associated with a decrease in apoptosis, we expected that SPARC expression would be associated with decreased caspase activity. Indeed, glioma cell lines under serum-starved conditions that constitutively express SPARC displayed significantly lower levels of cleaved (active) caspase 3 and one of its common substrates poly(ADP-ribose) polymerase on Western analysis (Fig. 3C). As further confirmation, glioma cell lines expressing SPARC in the absence of serum also exhibit decreased caspase activity of the executor caspases 3 and 7 using a fluorescent caspase 3/7 substrate assay (Fig. 3D).

**SPARC Treatment Acutely Induces AKT Phosphorylation**—Although we have shown that glioma cell lines that constitutively express SPARC express increased AKT phosphorylation, we sought to define whether the impact of SPARC on AKT is a primary effect or is secondary to other cellular effects of long term SPARC expression. Therefore, we examined the effect of exogenous human SPARC purified from platelets on AKT phosphorylation of the parental glioma cell lines (THR, D54MG, and U87MG) from which our engineered cell lines were derived. SPARC induced a rapid phosphorylation of AKT in 2–10 min on Western analysis depending on which cell line was examined (Fig. 4A), suggesting that extracellular SPARC initiates the activity of signal transduction pathways that result in AKT activation. Corresponding to our findings with cell lines constitutively expressing SPARC, we found that exogenously administered SPARC had no impact on ERK phosphorylation (data not shown). AKT phosphorylation in response to exogenous SPARC was self-limited, returning to near base-line levels in 60–240 min (Fig. 4A and data not shown) in a manner reminiscent of that of ligand-receptor signaling. As SPARC can bind or influence several growth factor receptor pathways, we examined critical phosphorylating events in several mitogenic growth factor receptor pathways commonly active in malignant...
We found that EGFR, IGF1R, and PDGFR were not phosphorylated on Western analysis in response to exogenous SPARC treatment rapidly enough or to a sufficient degree to explain the rapid induction of AKT phosphorylation (data not shown). Recent reports suggest that SPARC may activate key intracellular mediators of the transforming growth factor-β pathway, including SMAD3 (36), but we found only a slow and modest induction of phosphorylation of SMAD2 and nuclear localization of SMAD3 in U87MG or D54MG in response to exogenous SPARC treatment (data not shown). Therefore, it is unlikely that SPARC activates AKT by direct activation of these growth factor receptors that are frequently linked to glioma pathophysiology. SPARC-induced AKT phosphorylation at low doses, suggesting the specificity of this effect (Fig. 4B). Exogenous SPARC treatment also induced an increase in AKT kinase activity as measured by a nonradioactive kinase assay measuring the phosphorylation state of an AKT substrate, GSK3β, after AKT immunoprecipitation (Fig. 4C). As SPARC prepared from different tissues are differentially glycosylated (37) with potential functional differences as a result, we validated that human SPARC prepared either from platelets or recombinant means and SPARC derived from bone (bovine) also induced AKT phosphorylation (Fig. 4D and data not shown). The specificity of the impact of SPARC on AKT phosphorylation was also evident from the fact that a neutralizing anti-SPARC antibody blocked the induction of AKT in response to exogenous human SPARC (Fig. 4D). Of note, the self-limited course of AKT phosphorylation in response to treatment with exogenous SPARC likely explains why exogenously administered SPARC could not directly promote cell survival in the absence of serum (data not shown). Thus, the relationship between SPARC treatment and AKT activation is rapid and specific.

**SPARC-mediated Resistance to Apoptosis Requires Activation of PI3K and AKT**—Both PI3K and AKT have been strongly linked to cell survival and resistance to apoptotic stimuli. As PI3K can activate AKT by increasing the membrane levels of phosphatidylinositol 3,4,5-triphosphate to promote recruitment of AKT to membrane signaling complexes, we sought to define the contribution of PI3K activity to the impact of SPARC on both AKT phosphorylation and the resistance to apoptosis as a result of SPARC expression. Parental glioma cell lines were preincubated with the small molecule PI3K inhibitors, LY294002 or wortmannin, and treated either with control conditions or exogenous SPARC. Both of the PI3K inhibitors lowered the base-line phosphorylation of AKT, and more importantly, PI3K inhibitors completely blocked the impact of SPARC treatment on AKT phosphorylation (Fig. 5A). Further-
more, wortmannin treatment restored the same sensitivity to apoptosis as a result of serum starvation in glioma cell lines expressing SPARC relative to control lines as measured by caspase 3/7 activity (Fig. 5, B and C). The dependence of SPARC-mediated resistance to serum starvation-induced apoptosis on AKT was dissected by using both the expression of a kinase-dead AKT (AKT-KD, a kind gift of Robert Abraham, Burnham Institute) and treatment with a selective small molecule AKT inhibitor (SH-5). Both of these approaches reversed the relative resistance to apoptosis induced by serum withdrawal in the THR cell line expressing SPARC relative to the control line measured both by decreased annexin V staining and fluorimetric caspase 3/7 activity (Fig. 6, A–D). We confirmed these results in both the D54MG and U87MG cell lines expressing SPARC as well (Fig. 6, E and F). As AKT activity is necessary for the effects mediated by SPARC on apoptosis, we next showed that constitutively active AKT can replace SPARC expression in the ability to suppress apoptosis upon serum starvation (Fig. 6G). In summary, both PI3K and AKT activity are required for the contribution of SPARC to improved survival in serum-starved conditions.

**DISCUSSION**

We have now shown that SPARC, a protein overexpressed in gliomas relative to normal brain, promotes increased cell survival in the face of cell stress induced by serum withdrawal. The apoptotic rate of SPARC-expressing glioma cell lines is reduced relative to control lines and is associated with activation of the anti-apoptotic kinase, AKT, but not that of ERK, the latter of which is more closely linked to mitogenesis. The activation of AKT by SPARC appears to be a proximal effect as exogenous SPARC treatment rapidly induces AKT phosphorylation and activity. As expected, the impact of SPARC on AKT activation is dependent on PI3K activity as measured by the ability of PI3K antagonists to block AKT phosphorylation and
increased survival imparted by SPARC. AKT activity is necessary and sufficient for the pro-survival effects of SPARC as measured by treatment with a specific AKT inhibitor or expression of a kinase-dead AKT. The impact of SPARC expression on apoptotic resistance does not include chemotherapy resistance as our glioma cell lines expressing SPARC did not exhibit any relative resistance to apoptosis induced by either treatment with cycloheximide or etoposide at 100 μM (data not shown).

Tumors grow through the imbalance of cellular proliferation and death. The apoptotic indices of gliomas correlate with tumor proliferation and negatively associate with survival (38–40). These findings suggest that gliomas encounter increased apoptotic stimuli as they progress in malignancy, i.e., proliferation and progression require concordant loss of normal apoptotic mechanisms. The PI3K-AKT pathway represents one of the most potently pro-survival signaling pathways frequently activated in malignant gliomas. The importance of AKT in gliomas is apparent from the frequent increase in active AKT detected in human tumor specimens associated with activation of upstream growth factor receptor pathways, inactivation of the negative regulator Pten, or (less commonly) amplification or overexpression of AKT family members (30–32). Recently, expression of phosphorylated PI3K and AKT in patient glioma specimens has been inversely correlated with survival (41). Small molecule compounds that block the kinase activity associated with PI3K or AKT have demonstrated efficacy against human tumor xenografts (42, 43). Thus, mechanisms by which AKT activation is increased would be expected to provide advantages to tumors.

The tumor microenvironment is regulated not only by neoplastic cells but also by endothelial cells, inflammatory cells, and stromal elements. Elegant studies suggest that non-neoplastic tumor components provide critical cell-matrix, cell-cell, and vascular support (reviewed in Refs. 2 and 4). The matricellular proteins, a family of proteins that contains thrombospondin, tenascin-C, and -X, and SPARC, are a class of nonstructural extracellular matrix proteins that are structurally diverse but promote cell detachment and motility (44). These proteins play diverse roles in cancers but can induce cell rounding and an ablation of focal adhesions, a state associated with increased motility (44). Cell attachment is permissive for cellular proliferation and survival, thus decreased adhesion may limit cell proliferation. Signals from the extracellular matrix are derived from the engagement of integrins with specific matrix proteins to stimulate cell survival signals including AKT (45). In contrast, extreme loss of cell attachment induces apoptosis through a process termed anoikis, which is regulated by the PI3K/PTEN/AKT pathway (46). Serum represents another potent stimulus to activate AKT. Withdrawal of serum from cells may decrease proliferation and increases apoptosis that may mimic some aspects of the inhospitable microenvironments that invading cells may face. The partial protection afforded by SPARC to serum withdrawal-induced apoptosis mediated through the activation of AKT supports an additional
mechanism by which SPARC may promote tumor progression. Invasion and metastases are the most common causes of cancer-induced morbidity and mortality. Despite its importance, little is understood of the regulators of tumor spread. The physical processes of invasion involve cellular disengagement from the local microenvironment, followed by degradation of surrounding matrix, cellular movement, and re-establishment of the local microenvironment at a new location. The teleological explanation for the cellular advantage gained through migration of tumor cells has included migration to areas in which the metabolic environment is more favorable. However, this cannot explain why tumor cells will move into many areas that appear less favorable for cellular survival. Gliomas commonly migrate as single cells along white matter tracts that offer little mechanical resistance (13) but also provide few interactions with cell bodies at which external survival signals are present. As tumor cells commonly secrete growth factors and other soluble factors that stimulate proliferation, resistance to apoptosis, and angiogenesis, significant pro-tumorigenic benefits would be expected to occur in highly cellular regions of tumors. Migrating tumor cells may therefore encounter hostile microenvironments. The ability of tumor cells to survive under stress may represent a critical aspect of glioma invasion (47). It is then not surprising that intracellular signal transduction pathways contributing to survival are active in invasive and metastatic cancers. In particular, the loss of the Pten tumor suppressor gene or gain of activity of the proto-oncogene Akt provides both anti-apoptotic and pro-invasive cellular effects to cancer cells (33, 34). In the absence of genetic disruption of Pten or amplification of Akt, growth factor receptor pathways, including IGF1R, EGFR, and PDGFR, frequently activate Akt in cancer cells. However, it remains likely that other mechanisms may be invoked to activate Akt either in concert or independent of growth factor receptor pathways to provide pro-survival signals.

Although receptors have been discovered for some of the matricellular proteins, it remains unclear how the secreted protein SPARC interacts with the cell surface to induce its effects. Recent work has suggested that integrins αβ2 and α6β1 may mediate some effects of SPARC (48). However, we found that both blocking antibodies to these integrins and circularized RGD peptides failed to inhibit either the induction of AKT phosphorylation in response to exogenous SPARC treatment or the increased cell survival of glioma cell lines with forced phosphorylation in response to exogenous SPARC treatment or circularized RGD peptides failed to inhibit either the induction of AKT and PI3K remains to be elucidated.

AKT and PI3K have been recognized as potential targets in cancer therapy. The contributions of SPARC to activation of AKT in glioma cultures may provide further rationale for the inclusion of SPARC as a similar target. Caution must be exercised, however, as some xenografts grown in transgenic mice with targeted disruption of SPARC exhibit a growth advantage over those grown in a wild type background (20, 21), suggesting that SPARC may act in an anti-proliferative manner in some cancer types or some stages of cancer. Indeed, we are currently investigating important cell type differences in the impact of SPARC on cancer cells.3 The relationship between SPARC and AKT may have direct implications for future glioma patient treatment as targeting AKT activity may provide additional therapeutic benefits if combined with blockade of SPARC function or expression. It also remains to be investigated the degree to which the invasive aspects of SPARC relate to the AKT or other signal transduction pathways that may create additional therapeutic synergies.

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