INTRODUCTION

Loss of the p53 tumor suppressor gene has been described as an early event in low-grade astrocytoma formation and progression to secondary glioblastoma (GBM) with estimates of 59%-77% p53 mutation when studies consider only astrocytomas or secondary GBMs (24–26, 29). Mutations or deletions of p53 were also found in 76.5% of glioma cell lines, often in combination with the loss of other tumor suppressor genes (14). Re-introduction of p53 in glioma cell lines that have mutant or absent p53 results in reduced cell survival and reduced tumor size. The loss of p53 would result in reduced cell survival and tumor formation and increased tumor immunogenicity in an in vivo xenograft brain tumor model. In vitro, the loss of p53 in p53-null astrocytes resulted in an increase in cell proliferation, but a loss of tumorigenicity. At 7 days after intracranial implantation, Sparc-null tumors had decreased tumor cell survival, proliferation and reduced tumor size. The loss of Sparc promoted microglia/macrophage activation and phagocytosis of tumor cells. Our results indicate that the loss of p53 by deletion/mutation in the early stages of glioma formation may cooperate with the induction of SPARC to potentiate cancer cell survival and escape from immune surveillance.

Abstract

Both the induction of SPARC expression and the loss of the p53 tumor suppressor gene are changes that occur early in glioma development. Both SPARC and p53 regulate glioma cell survival by inverse effects on apoptotic signaling. Therefore, during glioma formation, the upregulation of SPARC may cooperate with the loss of p53 to enhance cell survival. This study determined whether the loss of Sparc in astrocytes that are null for p53 would result in reduced cell survival and tumor formation and increased tumor immunogenicity in an in vivo xenograft brain tumor model. In vitro, the loss of Sparc in p53-null astrocytes resulted in an increase in cell proliferation, but a loss of tumorigenicity. At 7 days after intracranial implantation, Sparc-null tumors had decreased tumor cell survival, proliferation and reduced tumor size. The loss of Sparc promoted microglia/macrophage activation and phagocytosis of tumor cells. Our results indicate that the loss of p53 by deletion/mutation in the early stages of glioma formation may cooperate with the induction of SPARC to potentiate cancer cell survival and escape from immune surveillance.
analyzed the MG/MP content of p53-null/Sparc-wt and p53-null/Sparc-null astrocyte xenograft tumors to assess the role of SPARC in glioma-induced immunosuppression in a model of early glioma formation.

We found that the loss of Sparc in p53-null astrocytes results in an increase in cell proliferation; however, there is an inhibition of growth in soft agar. When the astrocytes are grown intracranially, the loss of Sparc results in decreased tumor cell proliferation and a reduced tumor size at 7 days post-implantation and promotes MG/MP activation and the phagocytosis of tumor cells.

METHODS

Mice and breeding

Sparc-null mice on a mixed 129SV/C57BL/6 background were originally obtained from Dr. Chin Howe at the Wistar Institute, Philadelphia, PA, USA. The null mice were backcrossed with B6129SF1/J (Jackson Laboratory, Bar Harbor, ME, USA), which retained the C57BL/6 background. Heterozygotes were obtained to derive the Sparc-wt (wt) controls and Sparc-nulls (null). Homozygous C57BL/6 TSG-p53 knockout mice (P53N12-M) were obtained from Taconic (Oxnard, CA, USA). The p53-null and Sparc-null mice were mated to generate heterozygotes. Heterozygotes were then mated to generate neonates.

Astrocyte isolation

Using Institutional Animal Care and Use Committee (IACUC) approved protocols, 1–2-day-old neonates were sacrificed and the meninges were removed, the medium was aspirated and the brain tissue was minced and disaggregated using trypsin at 37°C for 30 minutes, vortexing every 5 minutes. Dulbecco’s modified Eagle’s medium (DMEM) + 10% fetal bovine serum (FBS) was then added and the tissue was centrifuged at 1000 rpm for 5 minutes. The supernatant was aspirated and the cells were resuspended in fresh DMEM + 10% FBS. The cells were trituated by pipette and then by syringe through a 21-gauge needle. Next, the cells were transferred to T75 flasks precoated with 12.5 μg/mL poly-L-lysine, supplemented with DMEM + 10% FBS and incubated at 37°C. Astrocytes were isolated from cerebral cortex cultures as previously described (23). Briefly, the mixed cultures were grown for 10 days, then agitated by shaking at 100 rpm overnight to remove oligodendrocytes and pulsed twice with Ara-C (20 μM) to kill dividing cells. The remaining cells were replated and defined as passage 1. Four p53-null/Sparc-null clones were expanded and designated #2, #6, #11 and #30. The Ast11.9 and Ast11.9-2 cell lines are genetically matched p53-null/Sparc-wt controls and were provided by Dr. Oliver Böglér.

Astrocyte p53 genotyping

Southern blot analysis was used for p53 genotyping of astrocytes. DNA was isolated from mouse tails using the DNeasy Blood & Tissue kit (69504, Qiagen, Valencia, CA, USA). Equal concentrations of DNA from each mouse were digested with BamHI and then electrophoresed on a 1% agarose gel. Gels were then transferred onto Hybond N membrane (GE Healthcare, Pittsburgh, PA, USA), after denaturing in NaOH/NaCl for 2 × 25 minutes, followed by neutralization in Tris/NaCl for 2 × 30 minutes. Transfers were done with 20× saline sodium citrate buffer overnight. Membranes were rinsed briefly in 2× SSC buffer, dried on filter paper and ultraviolet crosslinked in a Stratagene Crosslinker (Agilent Technologies, Chicago, IL, USA). Blots were prehybridized for 4 h and then hybridized overnight with a P12 random primer labeled murine p53 cDNA as a probe as previously described (6). Knockouts were verified by loss of the wild-type p53 band (~5-kb) and presence of the p53 mutant band (~6.5-kb).

Astrocyte Sparc genotyping

Sparc genotyping was assessed using polymerase chain reaction (PCR) with 0.2 μM primers including the Sparc forward primer: GAT GAG GGT GGT CTG GCC CAG CCC TAG TCT CAC, the Sparc reverse primer: CAC CCA CAC AGC TGG GGG TGA TCC AGA TAA GCC AAG and the Neomycin reverse primer: GGT GTG CCC AGT CAT AGC CGA ATG GCC TCT CCA CCC AAG. PCR reactions were performed with 100 ng of mouse genomic DNA in a 25 μL reaction including PCR buffer [10 nM Tris-HCL (pH 9.0), 50 mM KCl, 2.5 mM MgCl2 and 0.1% Triton X-100], 0.5 units of Taq DNA polymerase and 0.2 mM of deoxynucleotide triphosphates. The reaction was carried out with an initial 1 minute at 96°C, followed by 40 cycles of denaturation at 96°C for 45 s, annealing at 70°C for 45 s, and extension at 72°C for 6 minutes, followed by 10 minutes at 72°C and then a 4°C hold. The PCR product sizes are approximately 300 bp for the wild-type allele and 550 bp for the Sparc-null insertion.

Western blot analysis for SPARC

Protein was isolated using a single detergent lysis buffer as reported (12, 30), and protein concentration was determined using the bichinchoninic acid protein assay (Thermo Scientific, Rockford, IL, USA). DMEM was conditioned by cells for 24 h as previously reported (19). Equal concentration of protein or volume of conditioned medium were evaluated by Western blotting as previously described (30) using anti-mouse SPARC antibody (AF942, R&D Systems, Minneapolis, MN, USA).

Proliferation assay

For each cell type, 1.5 × 10⁴ cells were seeded into each of the five wells of a 96-well, white-walled microplate (#3610, Fisher Scientific, Chicago, IL, USA). DMEM was conditioned by cells for 24 h as previously reported (19). Equal concentration of protein or volume of conditioned medium were evaluated by Western blotting as previously described (30) using anti-mouse SPARC antibody (AF942, R&D Systems, Minneapolis, MN, USA).

Colony formation in soft agar

Triplicate wells were plated with 1 × 10⁴ or 5 × 10⁴ cells per well in DMEM + 10% FBS with 0.4% low gelling temperature agarose (A9045, Sigma-Aldrich, Atlanta, GA, USA). The cell suspension was overlaid onto a presolidified base layer of DMEM + 10% FBS and incubated
with 1.0% agarose. Fresh media was added every 2–3 days. After 10 days in culture, live images were captured at 10× and 25× magnification.

**Astrocyte implantation**

Cells (5 × 10^5 cells/5 μL phosphate buffered saline (PBS)) were implanted into the brains of nude rats as previously described (36) using an IACUC-approved protocol. Tumors were allowed to grow for 7 or 49/50 days. At sacrifice, the rats were anesthetized, perfused with sterile 0.9% saline via cardiac puncture, and then fixed with 10% formalin. Formalin-fixed rat brains were sliced into 2 mm blocks which were routinely processed, paraffin-embedded and serially sectioned at 5 μm as previously reported (36). Animals/group: for day 7: Ast11.9 has n = 12 and #11 has n = 12; for day 50: Ast11.9 has n = 5 and #11 has n = 8.

**Immunohistochemistry**

Adjacent serial sections were routinely stained with hematoxylin and eosin (H&E), immunohistochemically stained for SPARC (1:200 for 45 minutes; AF942, R&D Systems), cell proliferation marker Ki-67 (1:250 for 40 minutes; M7249, Agilent Technologies) and macrophage/histiocyte marker CD68 (1:200 for 30 minutes; MCA341R, BioRad, Los Angeles, CA, USA) as previously reported (36). Additional adjacent sections were subjected to the periodic acid Schiff (PAS) reaction with or without diastase (liver tissue was positive control) as previously described (43) to demonstrate phagocytic activity (histiocytes). Images of stained sections were captured on either a Nikon Eclipse E800 microscope with a Nikon DXM1200C digital camera using ImagePro 6.0 Plus (Media Cybernetics, Rockville, MD, USA) software or a Nikon Eclipse Ni microscope with a Digital Sight DS-U3 digital camera using NIS-Elements AR 4.20 (Nikon, New York, NY, USA) software. Composite figures were prepared using Adobe Photoshop CS6 software.

**In vivo analysis of tumor area**

For day 7 tumors, every 10th section of the tumor was stained with H&E. However, as some of the tumors were very small with only a few H&E sections per tumor, tumor volume could not be calculated for all of the tumors, and therefore the tumor area of the largest section through the tumor was imaged at 2× magnification and used to assess tumor size. Nikon NIS-Elements AR 4.20 software was used to outline the tumor and calculate the area in mm^2 (n = 8 for Sparc-wt, n = 11 for Sparc-null).

**MIB-1 proliferation index**

One section per tumor, chosen from the sections having the largest tumor cross-section, was stained for Ki-67 with MIB-1 antibody (Dako) and was used to calculate the proliferation index. Using Adobe Photoshop CS5 software, 40× images (one to three depending on the extent of staining) were overlaid with a grid and total and MIB-1-positive cells were counted. The proliferation index was calculated by dividing the number of MIB-1-positive cells by the total cell number and multiplying by 100 (n = 9 for Sparc-wt, n = 8 for Sparc-null).

**CD68 staining density quantitation**

The sum density of CD68 staining was measured from one 40× image per tumor of the region with the highest staining density using Nikon NIS-Elements AR 4.20 software. Automated measurement was used to detect the staining with intensity thresholding from 0 (low) to 130 (high). Animals/group: n = 6 for day 7 and n = 5 (Sparc-null) or 8 (Sparc-wt) for day 50.

**Statistical analysis**

The Wilcoxon rank-sum test was used to compare the probability that Sparc-wt tumors are consistently higher, or lower, than Sparc-null tumors for percentage of MIB-1 stained cells and for tumor area. Probability is estimated as the c-index from a logistic regression model. Confidence intervals were estimated by DeLong’s method. Proliferation rates were estimated using linear regression with generalized estimating equations to account for the correlation (auto-regressive-1) within technical replicate. Exponential growth was assumed and a log2 transformation was used. Given that growth is estimated relative to the zero time, the modeled lines were required to pass through the origin.

**RESULTS**

**Confirmation of the loss of p53 and Sparc in knockout mouse astrocytes**

Homozygous p53-null and Sparc-null mice were mated to generate heterozygotes which were then mated to generate double-null neonates. In astrocytes isolated from the mice, PCR and Western blot were used to confirm the loss of Sparc (Figure 1A,B), whereas Southern blot analysis was used to confirm the loss of p53 (Figure 1C). Four double-knockout (KO) astrocyte cell lines were generated and designated #2, #6, #11 and #30.

**Loss of Sparc increases p53-null astrocyte proliferation but inhibits growth in soft agar**

The growth characteristics of p53-null/Sparc-wt and p53-null/Sparc-null astrocytes were assessed by proliferation assay and growth in soft agar. Analysis of proliferation over 5 days showed that the three p53-null/Sparc-null clones had an increased proliferation rate (15%–33%, P < 0.01) when compared with p53-null/Sparc-wt cells (Figure 2A). Daily fold change in cell count is estimated to be 1.87 for Ast11.9 and 2.48, 2.41 and 2.14 for clones #2, #11 and #30, respectively. However, the loss of Sparc inhibited the ability of the two p53-null/Sparc-null clones used (#11 and #30) to form colonies in soft agar (Figure 2B). Based on the similarity of results for the different Sparc-null clones, the following in vivo studies were performed using clone #11.

**Loss of Sparc decreases the in vivo survival and proliferation of p53-null astrocytic tumor cells resulting in a reduced tumor size**

To determine the effects of Sparc loss on tumor development in vivo, p53-null/Sparc-wt and p53-null/Sparc-null astrocytes
Sparc Loss Promotes Phagocytosis of p53-null Cells

Figure 1. Generation of p53-null/Sparc-wt and p53-null/Sparc-null mouse astrocytes. Homozygous p53-null and Sparc-null mice were mated to generate heterozygotes which were then mated to generate double-null neonates. Four p53-null/Sparc-null astrocyte cell lines were generated and designated #2, #6, #11 and #30. Ast11.9-2 is the control p53-wt/Sparc-wt astrocyte cell line. A. PCR and B. Western blot analysis were used to confirm the loss of Sparc. The PCR product sizes are approximately 300 bp for the wt allele and 550 bp for the Sparc-null insertion. The Western blot results show SPARC expression in lysate and conditioned medium (CM). C. Southern blot analysis was used to confirm the loss of p53. Knockouts were verified by loss of the wild-type p53 band (~5 kb) and presence of the p53 mutant band (~6.5 kb).

were implanted in the brains of nude rats and intracranial xenografts were assessed at 7 and 50 days post-implantation (Figures 3–5). At day 50, both Sparc-wt and Sparc-null tumors had few or no tumor cells remaining; however, there was still histo-

logical evidence of an immune response (Figure 4). Therefore, day 7 tumors were assessed for tumor size, proliferation rate and SPARC expression and representative images from two different tumors per genotype are illustrated in Figure 3. The Sparc-null tumors were significantly smaller than the Sparc-wt tumors \( P = 0.0091, \text{median (range)} = 0.709\text{mm}^2 \) (0.176–1.099) for Sparc-wt (n = 8), and 0.240mm \(^2\) (0.079–0.539) for Sparc-null (n = 11) (Figures 3 and 5A). In addition, the Sparc-null tumors had a significantly lower proliferation index than the Sparc-wt tumors \( P = 0.0345, \text{median (range)} = 8.6\% \) (0–21.8) for Sparc-wt (n = 9), and 0.4\% (0–1.7) for Sparc-null (n = 8) (Figures 3 and 5B).

The day 7 Sparc-wt tumors had a central area of necrosis, nuclear changes evident of tumor cells undergoing apoptosis, and were invasive (Figure 3). Tumor cells were seen infiltrating along vessels at the brain tumor interface as well as into adjacent normal brain tissue. In comparison, Sparc-null tumors were smaller in size with apoptotic tumor cells and prominent karyorrhexis throughout the lesions (Figure 3). These tumors were better circumscribed than Sparc-wt tumors, and no tumor cells were seen invading along vessels at the periphery. A comparison of the number of tumor cells undergoing apoptosis based on nuclear changes in H&E stained sections revealed that there was no significant difference in the number of apoptotic cells between Sparc-wt and Sparc-null tumors at day 7 (data not shown).

Loss of Sparc promotes macrophage activation and phagocytosis of tumor cells

Staining with CD68 shows that day 7 Sparc-null tumors had a massive infiltration of large phagocytic cells with bubbly cytoplasm. Phagocytic MG/MP were still present at day 50, after most of the tumor cells had been cleared (Figure 4). The day 7 Sparc-wt tumors had MG/MP scattered throughout the tumor area, however, most of the MG/MP had an activated but not phagocytic appearance. By day 50, there were only a few remaining MG/MP in the area where the tumor cells had previously been located (Figure 4). The staining density for CD68 + MG/MP was quantitated using Nikon NIS-Elements AR 4.20 software. When a 40x image of the region with the most intense CD68 staining was analyzed, there was a significant increase in staining density in Sparc-null tumors compared with Sparc-wt tumors at both 7 and 50 days post-implantation \( P < 0.0001, \) Figure 5C). This difference in the amount of staining is evident in the images in Figure 4 and indicates that the MG/MP that are present in Sparc-null tumors are enlarged in size which is consistent with the phagocytic morphology.

Further evidence of a phagocytic process in the Sparc-null tumors is shown by PAS staining +/- diastase. Sparc-null tumors had increased PAS staining at both days 7 and 50 compared with Sparc-wt tumors (Figure 4). The PAS stain detects glycogen, glycoproteins, glycolipids and mucins, which when abundant in lysosomes is a feature characteristic of phagocytic cells. The remaining PAS stain after diastase treatment shows that the glycogen is trapped in lysosomes and not available for metabolic function which is another indication that the cells have become phagocytic.
DISCUSSION

To assess the cooperation of p53 loss/mutation and SPARC upregulation as early events in glioma formation, we compared the in vitro phenotype and intracranial growth of p53-null/Sparc-wt and p53-null/Sparc-null astrocytes. In vitro, we found that the loss of Sparc in p53-null astrocytes resulted in an increase in cell proliferation, but an inhibition of growth in soft agar. When the astrocytes were grown intracranially, the loss of Sparc resulted in decreased tumor cell proliferation and a reduced tumor size at 7 days post-implantation and promoted macrophage activation and phagocytosis of tumor cells.

The in vitro data demonstrate that the loss of Sparc increased the proliferation rate of the p53-null astrocytes. This finding is consistent with our previously published results that the overexpression of SPARC in glioma cells suppresses cell proliferation (30, 40, 41). Despite the increase in proliferation with the loss of Sparc, the cells demonstrated an inability to form colonies in soft agar. These results suggest that the in vitro growth of Sparc-null cells is more anchorage-dependent than that of Sparc-wt cells.

The in vivo intracranial growth of the p53-null/Sparc-wt and p53-null/Sparc-null cells demonstrates that by day 7 there was a reduction in tumor size and cell proliferation for Sparc-null tumors compared with Sparc-wt tumors. This data appears to contradict our previous xenograft results in which SPARC-expressing glioma tumors have a reduced tumor volume and proliferation rate compared with tumors that do not express SPARC (36, 40). However, as our previous xenograft models used p53-wt tumorigenic glioma cells, the different genetic background of the cells, including the loss of p53, may influence the function of SPARC. We know from our previous work that the status of the tumor suppressor protein PTEN in glioma cells can influence the function of SPARC. We have shown that the reconstitution of PTEN in SPARC-expressing glioma cells was able to inhibit SPARC-induced glioma cell migration in vitro and tumor invasion in vivo (40). Thus, the genetic background of the astrocytes, including the presence or absence of tumor suppressor genes, has the ability to influence the function of SPARC.

The glioma literature provides conflicting results on the role of MG/MP in animal models of glioma progression, with some studies showing tumor enhancement and others showing tumor suppression. The current study supports the role of MG/MP in keeping p53-null astrocyte growth under control as evidenced by the lack of tumor development by day 50. The results clearly demonstrate that the loss of p53 is insufficient to fully transform the astrocytes, and therefore the astrocytes may not yet have acquired an immunosuppressive phenotype. However, Sparc provides some degree of immunosuppression by keeping MG/MP from becoming fully activated to a phagocytic state. This is demonstrated by the increase in phagocytic MG/MP observed in Sparc-null tumors. Therefore, the massive infiltration of phagocytic MG/MP in Sparc-null tumors contributes to the reduction in tumor size and cell proliferation in these tumors.

Figure 2. Loss of Sparc increases p53-null astrocyte proliferation but suppresses growth in soft agar. A, p53-null/Sparc-wt (Ast11.9) and p53-null/Sparc-null (#2, #11 and #30) astrocytes were analyzed for proliferation at 0–5 days timepoints by measuring dsDNA content using a fluorescent assay. The assay was repeated four times and analyzed for differences in growth rate between the clones. Mean ± SEM per day are presented here. Based on the daily fold change in cell proliferation, there is a significant increase in growth for the Sparc-null clones relative to Ast11.9 (* = P < 0.01; ** = P < 0.0001). B, Astrocytes were grown in soft agar and imaged after 10 days. Representative images show a reduction in colony-forming ability for the Sparc-null clones #11 and #30 (inset) compared with the Sparc-wt cell lines Ast11.9 and Ast11.9-2 and the glioma cell line U87MG.
Glioma cells have been shown to express monocyte chemotactic protein-1 (MCP-1) (18), which is a known glioma chemoattractant for MG/MP (27). When glioma cells expressing MCP-1 were grown intracranially, tumors were 3.4-fold larger than control tumors and had a 10-fold increase in microglial cell content (27). Interestingly, exogenous SPARC was shown to inhibit basal and lysophosphatidic acid (LPA)-stimulated MCP-1 production by ovarian cancer cells (33). Therefore, SPARC expression in glioma cells may be involved in regulating MCP-1 expression and the recruitment of macrophages.

Furthermore, some studies provide evidence that MG/MP enhance glioma progression. When MG/MP were ablated locally at the site of tumor injection in a transgenic GL261 glioma model, tumor size was drastically reduced in MG/MP-depleted mice and tumors in depleted mice were almost undetectable by day 42. These results were confirmed when tuftsin, the stimulator of MG/MP, killed the mouse astrocytoma cell lines SMA-560 and MT539MG after direct co-culture or exposure to microglia supernatant by autophagic death (20).

Our model in this study demonstrates that SPARC may play a role in immunosuppression exerted by gliomas. Interestingly, SPARC can bind to the Stabilin-1 receptor which is expressed by alternatively activated macrophages (17). Stabilin-1 is thought to act as a scavenger receptor for SPARC; however, it is currently unknown whether the endocytosis of SPARC by macrophages alters the intracellular signaling or activation status of the macrophages.

Figure 3. Loss of Sparc suppresses the in vivo tumor growth and proliferation of p53-null astrocytes. Intracranial xenografts of p53-null/Sparc-wt and p53-null/Sparc-null astrocytes were assessed at 7 days post-implantation. Brains were harvested, formalin-fixed and paraffin-embedded. Tumor xenograft sections were H&E stained and immunohistochemically stained for mouse SPARC and the proliferation marker Ki-67. Magnifications as indicated. Representative images shown for two Sparc-wt and two Sparc-null animals out of n = 12 animals/group at day 7.
There is an increasing literature suggesting that in tumor models, SPARC has an immunosuppressive function. For example, the administration of melanoma cells with SPARC knockdown in nude mice promoted polymorphonuclear leukocyte (PMN) recruitment and inhibited tumor growth, whereas the \textit{in vitro} migration and cytotoxic capacity of PMN stimulated by SPARC knockdown cells was suppressed by purified SPARC (1). In animal models of ovarian and bladder cancers in which cancer

\textbf{Figure 4.} Loss of \textit{Sparc} promotes macrophage activation and phagocytosis of p53-null astrocytes. Day 7 and day 50 p53-null/\textit{Sparc-wt} and p53-null/\textit{Sparc-null} tumor xenograft sections were immunohistochemically stained for CD68 and for periodic acid Schiff (PAS) +/- diastase (D) to assess the presence of microglia/macrophages. Images were taken at 40x magnification with insets at 60x. Representative images from two animals out of n = 6 animals/group for day 7 and n = 5 (Sparc-null) or eight (Sparc-wt) animals/group for day 50.
cells were grown in Sparc-wt and Sparc-null mice, the tumors in Sparc-null mice had significantly higher macrophage infiltration (32, 34). The chemotactic effect of ovarian cancer cells on macrophages was attenuated when the cancer cells expressed SPARC, and co-cultures of ovarian cancer cells with macrophages had a reduced production of the inflammatory mediators interleukin-6, prostaglandin E2 and 8-isoprostane when the cancer cells expressed SPARC (35). Another study indicating that SPARC regulates macrophage chemotaxis found that pancreatic cancer cells grown in Sparc-null mice had macrophages present throughout the tumor, whereas macrophages were localized to the margin of the tumor in Sparc-wt mice (28). Our current data agree with these studies as the presence of SPARC altered MG/MP activation and delayed tumor cell destruction indicating a role for SPARC in glioma-induced immunosuppression. Further investigation into the interactions between SPARC-expressing glioma cells and glioma-associated MG/MP is warranted.

There is some evidence that SPARC and p53 can regulate one another. The re-expression of p53 in a glioma cell line in which both p53 alleles were lost resulted in a downregulation of SPARC expression in conditioned medium which was not because of the altered mRNA levels (15). On the other hand, several studies have found that SPARC expression inversely regulates p53 protein levels. Expression of SPARC in melanoma cells was shown to reduce p53 expression and inhibit p53 induction in response to the cytotoxic drug actinomycin-D (9, 10). In melanoma and cervical cancer cell lines, knockdown of SPARC with siRNA/shRNA resulted in an induction of p53 (8–10, 13). In melanoma cells, this induction of p53 resulted from increased protein stability because of a reduction in total and phospho-MDM2 (9). Therefore, in the non-cancerous cell, p53 may play a role in keeping SPARC levels under control; whereas in the cancer cell with wild-type p53, SPARC may suppress p53 function.

Our results indicate that in glioma cells in which p53 function is lost by deletion/mutation, the induction of SPARC may cooperate with the loss of p53 to potentiate cancer cell survival and escape from immune surveillance. However, as this early model involves only changes in Sparc and p53 and is not sufficient to produce tumors that last and completely escape immune surveillance, future studies should also address the mechanism by which glioma cells can switch from an immunogenic phenotype to an immunosuppressive phenotype. This model will be useful for determining alterations in cytokine expression that regulate MG/MP activation and phagocytosis. Further studies should also determine whether the inhibition of SPARC in developing p53-null gliomas can lead to anti-tumor activity and a reduction in tumor growth when combined with currently used chemotherapeutic agents.

ACKNOWLEDGMENTS

We thank Dr. Oliver Bögler for providing the Ast11.9 and Ast11.9-2 cell lines and the p53 cDNA probe for Southern blotting. This work was supported by grants CA86997 and CA138401 from the NCI/NIH to S.A.R. The authors are grateful to the Barbara Jane Levy family for their support.
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