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Meeting Report

International hermelin brain tumor symposium on matricellular proteins in normal and cancer cell-matrix interactions

The second of the Hermelin Brain Tumor Center Symposia was held once again at Henry Ford Hospital in Detroit, Michigan on October 24th and 25th, 2003. A public conference was held on the 24th while a closed-door session took place on the 25th. The purpose of these symposia is to bring together experts in a particular field of study with the aim to share information with each other and the public, but then to meet privately to present novel data, hold discussions, and share concepts. While the interaction is intended to benefit all involved, the incentive is the expectation that the shared information will aid researchers at the Hermelin Brain Tumor Center in their quest to identify potential therapeutic targets and explore translational therapeutic strategies for the treatment of patients suffering nervous system tumors.

Increasing evidence indicates that the development and success of future cancer therapies will be dependent upon our understanding of the complex relationship between tumor cells and their surrounding host matrix. The matricellular proteins are key players as they modulate normal cell-matrix interactions, and it is increasingly apparent that abnormal expression of these proteins contributes to different tumor phenotypes. However, the roles these proteins play may differ depending on the specific normal tissue or cancer examined. Of note, the matricellular protein secreted protein acidic and rich in cysteine (SPARC), also known as osteonectin and BM-40, appears to regulate cell proliferation, angiogenesis and tumor invasion. However, its influence on these processes may be shared by some cancer types but be different in others. If so, this would significantly impact SPARC's use for cancer treatment; i.e. use as a therapeutic target, or conversely, as a therapeutic agent. Therefore, the focus of this symposium was on the role of matricellular proteins in normal cells and tumor cells, with an emphasis on the role of SPARC in breast and prostate cancers, melanoma, neuroblastoma and glioma.

The first presentation was by Dr James Rutka who introduced the audience to glial tumors and further described the brain microenvironment. Primary glial brain tumors can be divided into the following subtypes: astrocytoma, oligodendroglioma, ependymoma, ganglioglioma and astroblastoma. Of these, the astrocytoma is the most common primary glial tumor. Malignant astrocytomas can be divided into three subtypes (grades II–IV) based on degree of anaplasia, mitoses, necrosis and endothelial hyperplasia (Kleihues et al., 2002). The most anaplastic astrocytoma is called glioblastoma (GBM) and is characterized by its nuclear and cytoplasmic pleomorphism, necrosis, and diffuse invasiveness into regions of normal brain. This latter histopathological feature thwarts current treatment paradigms, and contributes to the overall poor prognosis of most patients. Most patients with GBM will die within 12–18 months from the time of diagnosis.

The invasiveness of GBM must be placed into context with the microcellular environment of the brain, which includes a better understanding of the extracellular matrix (ECM). The ECM of the brain is comprised of laminin, type IV collagen, fibronectin and proteoglycans at the glial limitans externa and the basal laminae of most cerebral blood vessels. However, the ECM of the cerebral parenchyme is comprised of as yet poorly characterized glycosaminoglycans, such as hyaluronic acid, proteoglycans (e.g. chrondroitin sulfate proteoglycan) and other molecular species. GBMs are known to secrete proteases (such as matrix metalloproteinases, serine proteinases and cathepsins), which degrade the matrix and facilitate tumor cell infiltration. Other mechanisms important for tumor cell migration include tumor cell:ECM interactions, ECM:cytoskeletal dynamics and cell adhesion molecule (CAM) binding. Into these mechanisms of tumor cell migration and invasion, one must factor in matricellular proteins that have the potential to modulate astrocytoma cell motility and invasion.

The ability of matricellular proteins to influence cell proliferation, survival and cell motility results from their ability to influence the level of cell adhesion. Dr Joanne Murphy-Ullrich provided an introduction to the family of matricellular proteins and a summary of the regulation of the intermediate adhesive phenotype, and the conse-

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quent implications for cell motility and survival (Murphy-Ullrich, 2001). The state of intermediate cell adhesion is thought to be an adaptive state that is permissive for increased cell locomotion while maintaining adhesion-dependent cell survival signals. For normal cells in culture, the intermediate adhesive phenotype is characterized by a rapid restructuring of focal adhesion plaques with dispersal of vinculin and α-actinin, and loss of associated actin-containing stress fibers from focal adhesions. Cells remain spread, while the talin and integrins remain clustered. This suggests that integrin-matrix contacts are maintained despite unlinking of the actin cytoskeleton with integrins.

The matricellular proteins, SPARC, tenascin-C, and thrombospondins (TSPs) 1 and 2 induce this intermediate adhesive state in mesenchymal cells. These three matricellular proteins all induce a basically identical phenotype, although they utilize different receptors and signaling mechanisms to achieve this endpoint. Focusing on the TSPs, Murphy-Ullrich's laboratory has identified a sequence in the amino-terminal heparin binding domains of TSP1 and 2 that is sufficient to induce focal adhesion disassembly. A peptide containing this sequence (hep I) has been used in studies to identify the receptor and downstream signaling mechanisms. Using a peptide affinity approach, they isolated calreticulin (CRT) as a hep I-binding protein localized to the cell surface that mediates focal adhesion disassembly. Hep I binds to a specific sequence in the N-terminal domain of CRT. As CRT is a peripheral membrane protein, it requires a transmembrane co-receptor, which they identified as LDL receptor-related protein (LRP). TSP binding to CRT enhances association of CRT with LRP. This binding triggers association of LRP with the pertussis toxin-sensitive heterotrimeric G proteins, which then activates FAK, PI3K and ERK.

Signaling of the intermediate adhesive state not only triggers focal adhesion disassembly, but it also stimulates both directed and random cell motility of endothelial cells and fibroblasts. The adhesive state potentially regulates cell responsiveness to other stimuli, such as FGF-1 and -2. Cell invasiveness through collagen gels is also enhanced by the hep I peptide. Since hep I signaling activates mediators known to be anti-apoptotic, such as PI3K and FAK, they investigated whether hep I signaling was able to prevent cell death on non-adhesive substrata (poly-L-lysine), which does not activate integrin signaling. Their studies showed that hep I activates Akt and that treatment of bovine aortic endothelial cells prevents adhesion-dependent cell death on poly-L-lysine. These studies support their hypothesis that the intermediate adhesive phenotype is an adaptive state influenced by the matricellular proteins and a state that facilitates cell survival under conditions requiring increased cell plasticity.

Importantly, unlike tenascin-C and TSPs 1 and 2, SPARC can also further stimulate cell transition to a weakly adherent state, characterized by cell rounding. SPARC is a highly-conserved protein that belongs to a gene family consisting of hevin/SC-1, QR1, testicans 1-3, SMOC 1 and 2, and Tsc 36. Dr E. Helene Sage provided a review of SPARC as the prototype of this family (Bradshaw and Sage, 2001). SPARC is composed of three protein domains: an N-terminal, acidic, low-affinity Ca2+-binding domain, a follistatin domain containing EGF repeats, two Cu2+-binding sequences, and the majority of the disulfide bonds, and an extracellular Ca2+-binding (EC) domain with two high-affinity Ca2+-binding EF hands. These domains are conserved among all the SPARC family members.

Its de-adhesive function is due in part to its capacity to regulate ECM synthesis and assembly, as well as its ability to diminish focal adhesions in cultured cells as described above. In addition to its de-adhesive function, SPARC is also anti-proliferative for normal cells in vitro. The inhibitory effect of SPARC on the cell cycle is mediated through a number of targets, which in part are cell-type specific and include decreases in cyclin A, cyclin E-associated cdk2 activity, and Rb phosphorylation. Additionally, SPARC can form complexes with several growth factors and/or it can inhibit activation of cognate growth factor receptors.

Importantly, the likelihood that the in vitro observations reflect what is happening in vivo is supported by the study of cells cultured from SPARC-null mice, which exhibit enhanced levels of adhesion and proliferation, relative to wild-type cells. Studies from the Sage laboratory with SPARC-null mice have revealed several major characteristics: the mice have attenuated levels of connective tissue, especially collagen type I, and dermal collagen fibrils are both smaller and more uniform in diameter, in comparison to those of wild-type mice. They exhibit severe osteopenia and excessive adipogenesis, as well as early lenticular opacity and formation of cataracts. The lens capsule basement membrane (comprised mainly of laminin 1 and collagen type IV) is compromised structurally, such that the lens is subjected to increased amounts of ions and solutes that permeate the capsule.

Interesting results have also been obtained with regard to the responses of SPARC-null mice to injury. Enhanced closure of excisional dermal wounds was also noted in SPARC-null animals, a result reflecting in part the attenuated collagen and ECM in the dermis that increase its contractility by stromal myofibroblasts. Subcutaneous tumors grow substantially larger, with higher metastasis, in the absence of SPARC, due in part to compromised encapsulation of the tumor, as well as decreased levels of stromal ECM and invading macrophages.

A thread underlying these (and other) characteristics seen in mice lacking SPARC appears to be a deficiency

in ECM (including collagens I and IV) synthesis and assembly. The interaction of SPARC with cells, to mediate adhesion/migration and cell-cycle progression, is not yet understood, although there are preliminary data supporting the activation of certain integrins, and possibly other cell-surface receptors by SPARC. SPARC may act as an antagonist of ECM-cell surface interactions as well. Identification of specific receptors or binding partners by which SPARC regulates cell-ECM communication (as was previously described for thrombospondin) was emphasized as a requisite priority for defining the various functions of this interesting matricellular protein.

While much work has been undertaken to understand the role of SPARC in normal cells and during development, it has become a focus for cancer researchers because it is upregulated in many cancer types, including breast, brain, esophageal, and prostate carcinomas, and melanomas. Since it normally plays a role in the regulation of cell proliferation and cell motility, its potential to influence tumor cell proliferation and migration/invasion has come under considerable investigation.

Drs Hynda Kleinman and Jennifer Koblinski discussed the role of SPARC in breast and prostate cancers, with an emphasis on its role in tumor metastasis (De et al., 2003). Skeletal metastases occur with high frequency and incidence in patients with breast cancer and cause long-term skeletal morbidity. SPARC is a bone matrix factor (Termine et al., 1981) that is an in vitro chemoattractant for breast and prostate cancer cells (Jacob et al., 1999). Recently, bone extracts derived from SPARC knockout mice were found to have reduced chemoattractant activity relative to wildtype bone extracts demonstrating the role of SPARC as a major active factor for bone metastasis.

To examine the role of SPARC expression in breast cancer cells and its effect on metastasis, in particular to bone, they infected MDA-MB-231 breast cancer cells with a SPARC-expressing adenovirus. Expression of SPARC did not affect MDA-MB-231 cell proliferation or migration. However, in vitro invasion of these infected cells through Matrigel was decreased. High SPARC expression in MDA-MB-231 cells inhibited colony growth on Matrigel, tumor growth in a subcutaneous model, and metastasis especially to bone, in an intracardiac experimental metastasis model. These studies suggest that high endogenous expression of SPARC in breast cancer cells may reduce malignancy.

Dr Erik Thompson followed with his laboratory's investigations of SPARC in breast cancer and its role in the regulation of ECM-degradation. In invasive human breast cancer cells, SPARC enhanced cell surface activation of MMP-2 in an MT1-MMP-dependent manner (Gilles et al., 1998). Analysis of TIMP-2, a biphasic regulator of MT1-MMP-mediated MMP-2 activation, demonstrated that, although SPARC had no effect on MT1-MMP RNA and protein or TIMP-2 mRNA levels, its expression did correlate with reduced TIMP-2 in the conditioned medium. The alteration in TIMP-2 levels provides a mechanism whereby SPARC may regulate ECM degradation and these data suggest that some of SPARC's effects on the invasive phenotype may occur on the cell membrane.

They extended these observations made with *E. coli*-expressed recombinant SPARC and synthetic SPARC peptides to various additional forms of SPARC, including commercial preparations of human platelet-derived and bovine bone-derived SPARC (Haematological Technologies, Essex Junction, VT); SPARC purified from the PYS-2 mouse parietal yolk sac cells (Sigma, St. Louis, MO); SPARC purified from the EHS tumor, and recombinant murine and human forms expressed in HEK-293 cells (Timpl laboratory). In addition, they tested domain-specific mutant forms of human SPARC, which lack domain I (ΔI) or domains I and II (ΔI,II). They also tested recombinant follistatin, which resembles domain II. They found reduction of extracellular TIMP-2 levels with all full length forms, including mouse, bovine and human, and further found that this reduction was lost in any mutant forms lacking domain I. This confirms their previous observation that peptide 1.1, a hydroxyapatite-binding region in the acidic, amino terminal domain I, was active in causing reduced soluble TIMP-2 and increased MMP-2 activation. Although down-regulated TIMP-2 was highly reproducible, the increased MMP-2 activation associated with this was highly variable, presumably due to additional factors.

They next determined whether tetracycline-inducible, endogenously expressed and secreted SPARC was able to mimic exogenously added SPARC in the ability to reduce soluble TIMP-2. Full-length human SPARC was transfected into MDA-MB-231 cells, which have low endogenous SPARC expression (Dhanesuan et al., 2002). SPARC induction (up to 5 μg/ml) in these cells was less than the >25 μg/ml of exogenous recombinant SPARC needed, suggesting that its level of expression in the ECM is important. This is consistent with the results of others that indicate that SPARC effects are concentration-dependent. The mechanisms underlying the ability of relatively high concentrations of the SPARC amino terminal domain, or the corresponding peptide 1.1, to reduce soluble TIMP-2 require further investigation.

The effects of the tetracycline-inducible SPARC expression on various aspects of breast cancer biology were also examined. No effects on morphology or adhesion were detected. In addition, they observed reduced proliferation of SPARC-expressing MDA-MB-231 cells. These data support those seen by Koblinski and Kleinman (see above) with adenovirally expressed SPARC inhibiting MDA-MB-231 cells grown subcutaneously and in bone metastasis, although they saw no effect on in vitro proliferation.

While tumors of epithelial origin appear to express SPARC mainly in mesenchymal tissue (fibroblasts and endothelial cells), in tumors of ectodermal origin (such as melanoma and glioblastoma), SPARC appears to be expressed in both mesenchymal tissue and the malignant cells. Dr Osvaldo Podhajcer reported on their results with melanoma, for which SPARC expression has been associated with malignant progression (Ledda et al., 1997a). Immunohistochemical analysis of tissue sections indicated that all the primary and metastatic melanomas expressed SPARC, whereas part of dysplastic nevi and none of the nevocellular nevi expressed SPARC. In an effort to elucidate the role of SPARC in the progression of human malignant melanoma, malignant cells were transfected to express the full-length cDNA in the antisense orientation. This approach led to a strong decrease in SPARC expression in different cell lines. Isolated clones lost their in vitro capacity to adhere and invade Matrigel and were unable to migrate in transwell systems (Ledda et al., 1997b). Importantly, suppression of SPARC expression induced the complete loss of tumorigenic capacity in a process that involved the recruitment of a strong inflammatory infiltrate to the site of tumor cell injection. During the last year, they have tried to elucidate the importance of the inflammatory infiltrate in the outcome of the in vivo tumor growth and whether this inflammatory infiltrate is regulated by SPARC.

The next three presentations focused on neuroblastomas, peripheral nervous system tumors, and glioblastomas, central nervous system tumors. These tumors are in stark contrast with respect to SPARC expression and function. Dr Susan Cohn presented her work on SPARC in neuroblastoma (NB), a common pediatric neoplasm demonstrating a broad spectrum of clinical behavior (Alvarado et al., 2000). A number of clinical and genetic features are predictive of outcome, although the biologic basis underlying the disparities in tumor growth and response to therapy remain largely unknown. Recently, several clinical and laboratory studies have implicated angiogenesis in the regulation of NB growth, and preclinical studies have shown effective reduction of NB tumor growth in vivo by a variety of anti-angiogenesis agents. NB tumors consist of two main cell populations, neuroblastic/ganglionic cells and Schwann cells, and tumors with abundant Schwannian stroma display a more benign clinical behavior than stroma-poor tumors. Several studies suggest that Schwann cells influence NB tumor growth by secreting molecules that serve as antiproliferative and pro-differentiation factors for neuronal cells. The Cohn laboratory and others have shown that Schwann cells produce several angiogenesis inhibitors, including pigment epithelial-derived factor (PEDF) and tissue inhibitor of metalloproteinase-2 (TIMP-2). Thus, Schwann cells may also affect the growth of Schwannian stroma-rich/stroma-dominant NB tumors by restricting angiogenesis.

Using 2-step chromatography, they recently isolated SPARC from Schwann cell-conditioned media. In NB, SPARC expression is inversely correlated with the degree of malignant progression (Chlenski et al., 2002). In NB cell lines, SPARC mRNA levels were significantly higher in non-tumorigenic NB subclones than in tumorigenic subclones and NB cell lines, and SPARC protein levels in conditioned media collected from the cells paralleled the mRNA levels. In addition, SPARC was detected by immunohistology in Schwann cells and in differentiating neuroblasts/ganglion cells in favorable histology NB tumors associated with good prognosis. In contrast, Schwannian stroma-poor tumors composed predominantly of undifferentiated neuroblasts showed minimal to no staining for SPARC.

SPARC has previously been shown to inhibit endothelial cell proliferation stimulated by vascular endothelial growth factor and basic fibroblast growth factor (bFGF), indicating that it may function as an inhibitor of angiogenesis. Dr Cohn's studies confirmed that SPARC inhibits bFGF-induced endothelial cell migration, and they showed for the first time that SPARC also induces endothelial cell apoptosis. Furthermore, using the rat corneal neovascularization assay they found that purified SPARC potently blocked bFGF-induced angiogenesis in vivo. The addition of anti-SPARC antibody fully restored angiogenesis by bFGF, indicating that this inhibitory effect was indeed due to SPARC. The effect of SPARC on NB growth in vivo was tested in a mouse xenograft model where SPARC was delivered continuously for 3 weeks using osmotic pumps. Following SPARC treatment, the average tumor volume in mice was significantly smaller than that observed in control animals. Furthermore, histologic comparison revealed decreased vascularity in SPARC-treated tumors compared to control tumors as assessed by the number of structures that stained positively with an anti-CD31 antibody. Thus, their studies indicate that SPARC is one of the key contributors to the anti-angiogenesis activity associated with Schwann cells, and purified SPARC potently inhibited angiogenesis in both in vitro and in vivo assays. Therefore, in NB, SPARC expression is inversely correlated with the malignant phenotype, and treatment with SPARC results in impaired tumor growth in vivo. Importantly, their data suggest that SPARC may be an effective candidate for the treatment of children with clinically aggressive, Schwannian-poor NB tumors.

In contrast with SPARC's role in NB, Dr Sandra Rempel demonstrated that SPARC expression is highly increased in the invasive human glioblastoma tumor cells, as well as in the endothelial cells (Rempel et al., 1998). To determine whether SPARC functionally regulated the proliferative or invasive phenotype, they transfected the non-invasive U87MG cell line with SPARC using the tetracycline-off inducible system and analyzed

tumors resulting from the in vivo xenograft implantation of the parental clone, designated as U87T2, two empty vector control clones, and three SPARC-transfected clones (Schultz et al., 2002). In agreement with the results of others, SPARC had a suppressive effect on proliferation. However, the suppressive effect was not completely inhibitory as further investigation indicated that SPARC does not inhibit growth but rather appears to slow cell cycle progression. A decrease in cell cycle regulatory gene expression (cyclins D1 D3, A and B) was consistent with the decreases in cell growth and tumor volume observed, and in agreement with the observation of reduced cyclin A, as discussed previously. The suppression of growth does not appear to be influenced by growth on brain and blood vessel basement ECMs. In addition, the SPARC-induced delay in tumor cell cycle progression appears to be biphasic, consistent with its effects reported for normal cells in culture.

Interestingly, the SPARC-induced delay in growth was accompanied by the induction of glioma invasion into the adjacent brain, along white matter tracts, and along blood vessel basement membranes. The morphology of the tumors and patterns of invasion were representative of the morphology and invasion patterns demonstrated by human GBMs.

Since tumor invasion is a complex process requiring both adjacent tissue ECM degradation and tumor cell motility into adjacent tissue, the Rempel laboratory examined SPARC's role in these two processes. Consistent with observations by Thompson's group (Gilles et al., 1998); increased SPARC expression was accompanied by increased expression of MMPs, including MT1-MMP and MMP-2, suggesting that ECM degradation contributes to the invasive phenotype. These observations are consistent with the decrease in MMP-2 by melanoma cells after antisense inhibition of SPARC expression reported by Podhajcer's group (Ledda et al., 1997b). In addition, SPARC-induced changes in glioma motility appear to be influenced both by ECM proteins and the amount of SPARC secreted into the ECM. As was observed for proliferation, SPARC's effects on invasion also appeared to be biphasic. These data suggest that SPARC suppresses growth and increases invasion, but its ability to do so is tempered by the level of secretion into the ECM.

While data suggest that SPARC contributes to tumor invasion, they do not address whether it is sufficient to induce invasion. Dr Jeremy Rich and his group have undertaken a genetic approach to answer this question (Rich et al., 2003). They have generated a genetically defined human glioma model through the serial introduction of simian virus 40 early T antigen, human telomerase catalytic subunit, and an oncogenic ras into normal human astrocytes. These cells form tumors when implanted into immunocompromised rodents with pathology similar to malignant gliomas with the exception of tumor invasion. They explored the phenotypic consequences of introducing SPARC into these tumors. The SPARC-expressing cells showed no change in cellular proliferation or apoptosis, but exhibited an invasive phenotype in in vitro Matrigel assays associated with increased expression of specific matrix metalloproteinases. While MMP-2, -3, and -9 were all increased in expression, only MMP-3 expression was induced by treatment of parental cells with exogenous SPARC and only a specific MMP-3 inhibitor blocked in vitro invasion. These results further support the hypothesis that SPARC acts, in part, through regulation of elements that degrade surrounding microenvironment.

Intracranial tumors formed by cells expressing SPARC exhibited areas of invasion often associated with hypertrophied vessels. Of further note, these cells underwent a high rate of spontaneous metastasis when grown in a subcutaneous location. Three other human glioma cell lines exhibited identical behavior with both in vitro and in vivo invasion and metastasis.

It would seem then that SPARC definitely plays a role in cancer, but its influence depends on the cancer type. Understanding its role in each cancer type is, therefore necessary for future therapeutic approaches based on SPARC. However, whether the approach is to use it as a therapy (NB, breast cancer) or as a therapeutic target (melanoma, glioma), its use either way would be enhanced by understanding how the transcription of the gene is regulated. Although this protein has been studied for many years, the regulation of its expression in human cells is just being understood, and the c-jun proto-oncogene is implicated. Overexpression of c-Jun in MCF-7 breast cancer cells results in a variety of phenotypic changes related to malignant progression, including a shift to estrogen independence and increased motility and invasion (Smith et al., 1999). Dr Timothy Bos demonstrated that concomitant with these phenotypic effects are changes in the expression of multiple gene targets, one of which is the SPARC gene (Rinehart-Kim et al., 2000). They demonstrated that SPARC expression is undetectable in the MCF-7 parental cell line, even by RT-PCR, but highly induced in response to stable c-Jun overexpression in three independent c-Jun/MCF-7 cell clones. Since SPARC is associated with tumor cell invasion in a variety of different cancers, they examined its role in mediating the phenotypic changes induced by c-Jun in their model MCF-7 system. They found that antisense-mediated suppression of SPARC dramatically inhibited both motility and invasion in this c-Jun/MCF-7 model (Briggs et al., 2002). In contrast, stable overexpression of SPARC in the parental MCF-7 cell line was not sufficient to stimulate cell motility or invasion. Examination of the promoter region of the human SPARC gene revealed three non-canonical AP-1 sites. They demonstrated that one of these sites binds c-Jun/

Fra1 heterodimers in vitro, but that this and the other AP-1-like sites are dispensable with respect to c-Jun stimulated SPARC promoter activation. Deletion analysis identified a region between −120 and −70 as a c-Jun responsive element sufficient to induce maximal promoter activation. This region does not contain any AP-1 sites but does mediate binding by SP1/SP3 'like' complexes. Because of the dramatic induction of SPARC gene expression in response to c-Jun, they hypothesized that the SPARC locus was silenced by DNA methylation in MCF-7 cells. In support of this, treatment of MCF-7 parental cells with a DNA methyltransferase inhibitor, 5-aza-2′-deoxycytidine (5-aza-dC), resulted in SPARC activation. Methylation mapping of the SPARC promoter in MCF-7 and c-Jun/MCF-7 cells revealed a dramatic but localized c-Jun-induced demethylation of SPARC in the proximal promoter region. These results demonstrated the ability of an oncogenic transcription factor, c-Jun, to activate SPARC gene expression through a mechanism involving localized epigenetic changes. It remains to be seen whether these same mechanisms regulate SPARC expression in other cancer types. It will then be important to determine what regulates the localized epigenetic changes to devise strategies to modulate SPARC's expression.

In summary, SPARC's effects on DNA synthesis, angiogenesis, and tumor growth appear to be dependent upon cell type, concentration, and the presence of full-length SPARC vs. cleavage fragments. In part, these differences may be dictated by the cell of origin (i.e. epithelial vs. mesenchymal). This may also account for the differences observed between NB and gliomas, as NB is an embryonal tumor of neural crest stem cell origin and gliomas are of neuroepithelial origin. Further insight may come from the identification of SPARC's putative receptor, or the delineation of intracellular vs. extracellular functions, that may be governed differently in the various cancer types.

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