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A Prototypic Matricellular Protein in the Tumor Microenvironment—Where There's SPARC, There's Fire

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Abstract Within the tumor microenvironment is a dynamic exchange between cancer cells and their surrounding stroma. This complex biologic system requires carefully designed models to understand the role of its stromal components in carcinogenesis, tumor progression, invasion, and metastasis. Secreted protein acidic and rich in cysteine (SPARC) is a prototypic matricellular protein at the center of this exchange. Two decades of basic science research combined with recent whole genome analyses indicate that SPARC is an important player in vertebrate evolution, normal development, and maintenance of normal tissue homeostasis. Therefore, SPARC might also play an important role in the tumor microenvironment. Clinical evidence indicates that SPARC expression correlates with tumor progression, but tightly controlled animal models have shown that the role of SPARC in tumor progression is dependent on tissue and tumor cell type. In this Prospectus, we review the current understanding of SPARC in the tumor microenvironment and discuss current and future investigations of SPARC and tumor–stromal interactions that require careful consideration of growth factors, cytokines, proteinases, and angiotropic factors that might influence SPARC activity and tumor progression. J. Cell. Biochem. 104: 721–732, 2008. © 2008 Wiley-Liss, Inc.

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Within the tumor microenvironment, the stromal response through the production of growth factors, cytokines, and proteinases can determine tumor progression, invasion and metastasis [Witz and Levy-Nissenbaum, 2006; Albini and Sporn, 2007; Li et al., 2007; Potter, 2007]. In the setting of an established tumor, the network of extracellular and cellular components surrounding a tumor can facilitate or hinder tumor progression. Recent evidence also indicates that non-malignant, normal mesenchymal stromal cells (fibroblasts and myofibroblasts) can sufficiently alter the microenvironment of normal epithelial cells to predispose them to malignant transformation or carcinogenesis [Olumi et al., 1999; Hu et al., 2005; Kalluri and Zeisberg, 2006]. Despite the wealth of information gained from tumor models that manipulate processes within tumor cells, we can no longer ignore the fact that tumor cells grow, not only in a highly interactive environment, but also in discretely different microenvironments. For example, a host response is undoubtedly divergent when human glioblastoma cells are injected into the relatively hypoxic, nutrient-poor dermis of a mouse, as opposed to implantation of these cells into the nutrient-rich, immune-privileged brain parenchyma of a syngeneic host.

Due to the complexity of interactions at the stroma–tumor interface, two distinct approaches are currently employed. First, systems biologists, using in silico analyses based on high throughput technologies that include whole genome single nuclear polymorphism (SNP) arrays, serial analysis of gene expression (SAGE), phage display, DNA microarray, tandem mass spectrometry proteomics, and tissue microarrays, are modeling tumor–host interactions to identify key, "rate-limiting" players in the tumor microenvironment [Kitano, 2002; Anderson et al., 2006]. Although terabytes of

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uncurated data are publicly available, current bioinformatic resources suffer from a fairly low signal-to-noise ratio [Buchman, 2002; Aderem, 2005; Goh et al., 2007]. Only restricted closed-system models at this time appear to reflect complex biologic systems [Aldridge et al., 2006; Karsenti et al., 2006].

The second approach uses strict controls to model tumor–host interactions in a manner that minimizes dependent and independent variables. Syngeneic and orthotopic tumor models employing traditional transgenic knock-out or conditional knock-out mice (*cre/loxP* site- and tissue-specific DNA recombination or tetracycline-responsive regulatory systems) can provide precise analyses of 'effector' and 'target' transgenes in the tumor microenvironment [Lewandoski, 2001]. This slow and tedious approach has resulted in tremendous insights into tumor–host interactions, but only in specific tumor microenvironments [Ruiter et al., 2002; Chu et al., 2007]. Unfortunately, these interactions are often irreproducible or contradictory when repeated in a different microenvironment. The tumor–host interaction is both host tissue-specific and cancer cell-specific. Moreover, the host microenvironment undoubtedly evolves as the tumor undergoes clonal selection due to this complex exchange.

In this Prospectus, we have chosen a well-described component of the tumor microenvironment, secreted protein acidic and rich in cysteine (SPARC), to explore the experimental challenges of studying tumor–stromal interactions. We also discuss how the integration of bioinformatics, systems biology, and carefully designed tumor models can provide novel insights to guide us in the study of the tumor microenvironment.

STRUCTURE AND FUNCTION OF SPARC

SPARC is the prototypic gene for a subgroup of Ca^{+2}-binding glycoproteins included in the larger family of matricellular proteins. Although structurally diverse, matricellular proteins (Table I) do not contribute significantly to the structure of the extracellular matrix (ECM), but act to modulate cell–cell and cell–matrix interactions and are expressed during morphogenesis, development, tissue injury, and tissue remodeling [Bornstein, 1995].

The sub-family of SPARC-related proteins share three modular domains: (1) an N-terminal acidic and low-affinity calcium-binding domain; (2) a disulfide-bonded, copper-binding follistatin domain (homologous to the transforming growth factor-beta (TGF-beta) inhibitors activin and inhibin), and (3) the C-terminal extracellular calcium-binding domain. Unlike many structural components of the ECM (fibrillar collagens, fibronectin, laminin, entactin),

TABLE I. Matricellular Proteins

Gene	Alternative names
Secreted protein acid and rich in cysteine (SPARC) family	
SPARC	BM-40, osteonectin
SPARC-like 1	Hevin, Mast9, Ecm1, SC1, PIG33
Testican 1	SPARC/osteonectin, CWCV and Kazal-like domains proteoglycan-1 (SPOCK-1), Ticn1
Testican 2	SPOCK-2, granule cell antiserum positive 26 (Gcap26)
Testican 3	SPOCK-3
SPARC-related modular calcium binding (SMOC) 1 and 2	
CYR61, CTGF, Nov (CCN) family	
CCN1	Cysteine-rich, angiogenic inducer 1 (CYR61)
CCN2	Connective tissue growth factor (CTGF)
CCN3	Nephroblastoma overexpressed (Nov)
CCN4	Wnt-induced secreted protein-1 (WISP-1)
CNN5	WISP-2, connective tissue growth factor-like protein (CTGF-L)
CNN6	WISP-3, Gm735
Thrombospondin family	
Thrombospondin 1 (THSP1)	TSP1
Thrombospondin 2	TSP2
Thrombospondin 3	TSP3
Thrombospondin 4	TSP4
Thrombospondin 5	TSP5, Epiphyseal dysplasia 1 (EPD1), cartilage oligomeric matrix protein (COMP)
Other	
Osteopontin (OPN)	Secreted phosphoprotein 1 (SPP1), Bone sialoprotein I (BSPI), Early T-lymphocyte activation 1 (ETA-1)
Tenascin-C	Hexabrachion
Tenascin-X	

SPARC is counter-adhesive. Studies in vitro have shown that SPARC impairs cell attachment to the ECM in a concentration-dependent fashion [Sage and Bornstein, 1991]. Upon exposure to SPARC, primary cultured cells lose focal adhesions and exhibit impaired cell spreading. This modulation of cell shape does not rely on the extracellular calcium-binding capacity of SPARC [Sage, 1992]. Alterations in cell shape and attachment indicate that SPARC plays an instrumental role in cell rounding during proliferation and differentiation, cell migration and chemotaxis in acute and delayed immune responses, angiogenesis, wound closure, tumor cell invasion and metastasis, hematopoiesis, and tissue remodeling [Ledda et al., 1997b; Rempel et al., 1999; Yiu et al., 2001; Rich et al., 2003; Framson and Sage, 2004].

The deadhesive properties of SPARC might result from a direct interference of SPARC with the binding of cell-surface integrins to components of the ECM. SPARC also influences cell shape through its interaction with growth factors [De et al., 2003], and both binds to and decreases the mitogenic potency of platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF). SPARC has also been found to impair the proliferative activity of basic fibroblast growth factor (bFGF) by its inhibition of FGF-receptor 1 phosphorylation. In a broader context, the expression and activity of TGF-beta are enhanced by SPARC.

SPARC is rapidly degraded by cathepsins, stromelysin and other matrix metalloproteinases (MMP), elastases, and serine proteinases [Motamed, 1999]. With enhanced production of SPARC in tissue remodeling and the rapid turnover of SPARC by enzymatic degradation, we hypothesized that breakdown products of SPARC are functionally active. Through the systematic analysis of synthetic SPARC peptides, we have demonstrated that degradation products of SPARC have unique domain-specific activity, but studies in vivo are necessary to confirm these highly controlled findings in vitro (see Jendraschak and Sage, 1996 for review of SPARC peptide activity).

SPARC IN EVOLUTION

SPARC is the product of a highly conserved, single-copy gene first isolated from bone and from cultured endothelial cells by Termine et al. [1981] and Sage et al. [1984], and later cloned by Mason et al. [1986] from mouse embryo parietal endoderm cells. In mice, the *SPARC* gene is located on chromosome 11 and contains 10 exons separated by nine introns that span 26.5 kb; in humans, the *SPARC* gene is located on chromsome 5. Sequence alignment studies have identified homologous proteins in many mammals (human, mouse, dog, cow), amphibia (*Silurana tropicalis*, *Xenopus laevis*), bird (quail, chicken), fish (medaka, trout, zebrafish, fugu), fly (*Drosophila melanogaster*), nematode (*C. elegans*), and crustacean (*Artemia franciscana*) [Tanaka et al., 2001; Kawasaki et al., 2004].

As the most abundant non-collagenous bone ECM protein, SPARC has attracted biologists interested in the evolution of vertebrate tissue mineralization, particularly the divergence of exoskeleton for protection and endoskeleton for locomotion. Phylogenetic analysis using whole genome data demonstrates that SPARC and SPARC-related proteins diverged through gene duplication 481 million years ago after the emergence of cartilaginous and bony fish [Kawasaki et al., 2004]. The relatively late divergence of SPARC-related proteins indicates that SPARC probably was a key molecule for the initiation of vertebrate tissue mineralization. Given the high level of conservation among vertebrates and invertebrates, and the apparent importance of SPARC in the evolution of mineralized tissue, we would expect SPARC to play a fundamental role in normal development.

SPARC IN DEVELOPMENT AND AGING

In situ hybridization and immunohistochemistry studies indicate that SPARC is spatially and temporally regulated during development and is expressed in all germ layers of the mammal [Lane and Sage, 1994]. SPARC accumulates rapidly in the somites and limb buds of the growing embryo. The majority of SPARC produced in the embryo is located in mineralizing tissue, including developing bone, teeth, differentiating chondrocytes in the hypertrophic zone, and osteoblasts surrounding the spicules of endochrondral bone [Lane and Sage, 1994]. The significance of SPARC in proper development was further appreciated after it was blocked in *C. elegans* and *X. laevis* embryos [Purcell et al., 1993; Schwarzbauer

and Spencer, 1993]. In nematodes, cRNA inference resulted in embryonic lethality. In the frog embryo, blocking SPARC protein with affinity-purified antibodies was not lethal but led to bent embryonic axes and abnormal eye development. Following the targeted inactivation of SPARC in mice by Gilmour et al. [1998] and Norose et al. [1998], embryonic and developmental mouse studies revealed subtle but important changes. It is now known that SPARC-null mice, while not grossly deformed, exhibit a thin dermis, large fat stores, and a kinked tail.

Phenotypic characteristics of SPARC-null mice become increasing apparent with aging. Adult SPARC-null mice manifest a phenotype similar to post-menopausal women, for example, decreased total bone mass, osteopenia, increased skin laxity, thinner dermis, and significantly increased fat stores [Bradshaw and Sage, 2001]. By 6 months, 100% of SPARC-null mice develop cataracts [Yan and Sage, 1999]. In humans, there is no evidence of a familial syndrome or disease linked directly to a deficiency or mutation in SPARC. SPARC-null mice do not have a higher incidence of tumor formation in comparison to wild-type counterparts. Given the expression of SPARC during development and the unique phenotype of SPARC-null mice with aging, we believe that the major role of SPARC lies in the modulation of cell differentiation and homeostasis of normal tissues during periods of injury, stress, or aging [Sage and Bornstein, 1991]. In the context described by Potter [2007], SPARC appears to be a morphogen-like molecule that has a primary role in functional crosstalk among heterogenous cells and in the maintenance of normal adult tissue.

EXPRESSION OF SPARC

Targeted studies of SPARC in development and aging demonstrate the importance of this matricellular protein in many tissues and throughout the normal life cycle. In the embryo, SPARC is pervasive and has been identified in bone, cartilage, teeth, epithelia, dermis, olfactory tract, heart, kidney, lung, testis, thyroid, and gut. In the adult, expression becomes restricted to bone, kidney, testis, hematopoietic tissue, central nervous system, and cochlea. Early studies in vitro recognized SPARC as a "culture shock" protein, given its expression by almost all cell types grown in culture [Sage et al., 1986]. Stress conditions (low density culture, endotoxin stimulation, heat shock) stimulate the expression of SPARC. Specific enhancers of SPARC expression include TGF-beta, interleukin-1, colony stimulating factor-1, progesterone and glucocorticoids [Ng et al., 1989; Nomura et al., 1989].

To understand the "culture shock" response, we employed wound repair and inflammation models in mice [Puolakkainen et al., 1999; Bradshaw et al., 2002; Barker et al., 2005]. These studies indicate that a stressed microenvironment increases the expression of SPARC. Additional experiments in vitro have demonstrated that proteolytic degradation of SPARC by MMP-3 (stromelysin) can generate cleavage products with biological activity, whereas SPARC is not a substrate for MMP-1 (interstitial collagenase), MMP-2 (72 kDa gelatinase), and MMP-9 (92 kDa gelatinase) [Sage et al., 2003].

The expression profile of SPARC is clearly dynamic and dependent on external stressors. Therefore, in the tumor microenvironment, high levels of SPARC likely reflect the loss of normal tissue homeostasis. In this stressed microenvironment, SPARC and its active cleavage products further alter the microenvironment by influencing cell shape, differentiation, attachment, migration, proliferation, and growth factor activity. In the normal healthy adult, tissues that express SPARC are undergoing rapid differentiation, such as hematopoietic cells, or continuous stress and remodeling, as in bone. Altering the concentration and timing of SPARC expression in normal tissue remodeling and differentiation is expected to lead to inappropriate cell proliferation and differentiation; for example, the osteopenia and excess fat deposition in adult SPARC-null mice. Genetic and acquired mutations in SPARC might sufficiently alter normal tissue homeostasis and lead to tumorigenesis. In contrast, expression of SPARC in the established tumor microenvironment might act to restore homeostasis.

TUMORIGENESIS AND SPARC

Animal models investigating the role of SPARC in tumorigenesis have produced interesting results. In a model of chronic ultraviolet (UV) irradiation, SPARC-null mice had fewer papillomas compared to wild-type controls

[Aycock et al., 2004]. Repeated sunlight or UV exposure is both an initiator and promoter of carcinogenesis associated with DNA damage leading to activation of proto-oncogenes (k-Ras) and inactivation of tumor suppressor genes (p53). Altered intracellular signaling contributes to epidermal hyperplasia and gradual development of squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). The role SPARC might play in this multi-step process of tumorigenesis is not clear. Does the attenuated dermis of SPARC-null mice, with its aberrant collagen fibrils, provide protection from UV irradiation? Or, does UV irradiation lead to enhancement of SPARC transcription and the modulation of the ECM by SPARC in wild-type mice? And, is it this altered ECM that synergizes with intracellular mutations to facilitate loss of cell cycle control and subsequent tumorigenesis? Conditional knock-out mouse models would differentiate between a mechanism rooted in developmental differences and an acute response to tissue injury.

In the adenomatosis polyposis coli (APC)$^{min/+}$ mouse model of spontaneous intestinal tumorigenesis, SPARC expression is enhanced twofold in mouse intestinal adenomas, indicating that it might participate in early tumor formation [Sansom et al., 2007]. APC$^{min/+}$/SPARC$^{-/-}$ mice exhibit almost complete suppression of adenoma formation. Sansom et al. [2007] concluded that enhanced migration of SPARC-null enterocytes impairs adenoma formation. However, once the tumor is established, the presence of SPARC does not appear to alter adenoma growth or cell proliferation. In this context, we suspect that the counter-adhesive properties of SPARC impair the cell–cell or cell–ECM interactions required for rapid shedding of enterocytes as seen in the tumorigenic APC$^{min/+}$ mouse model. Recent studies in our laboratory have shown that SPARC alters levels of beta-catenin in preadipocytes. Therefore, in the absence of APC, SPARC might disrupt beta-catenin signaling and contribute to adenoma formation.

In tumorigenesis, acquisition of an invasive phenotype is a key step. Robert et al. [2006] reported that SPARC promotes this phenotype via its induction of an epithelial-to-mesenchymal transition. Overexpression of SPARC in normal human melanocytes suppressed expression of E-cadherin and P-cadherin and induced a fibroblast-like morphology. Unfortunately, this study did not investigate whether melanocytes overexpressing SPARC formed tumors in vivo. More importantly, is it the production of SPARC by melanocytes or by neighboring fibroblasts or myofibroblasts that is necessary for tumorigenesis in this microenvironment? Co-culture systems and tissue-specific models of conditional gene inactivation could provide insight into the role of SPARC as a carcinogenic factor in the normal melanocyte microenvironment.

TUMOR PROGRESSION AND SPARC

Recent DNA microarray and immunohistochemical analyses of human tumor samples indicate relatively high levels of SPARC, particularly at the tumor–stromal interface. Several of these reports have suggested a correlation between the level of SPARC expression and clinical outcomes (Table II). SPARC appears to indicate a poor outcome in adenocarcinoma of the breast, lung, gastrointestinal tract (esophagus, colon, rectum), and pancreas. Conversely, high levels of SPARC were correlated with a better prognosis in neuroblastoma, possibly due to impaired angiogenesis [Chlenski et al., 2002]. Investigation of tumor cell lines with associated in vitro and in vivo studies have further complicated our understanding of the function of SPARC in tumor progression. The perceived controversy is likely a reflection of the function of SPARC in different tumor microenvironments. We have long recognized that SPARC expression in epithelial tumors is variable, with high levels in some tumor specimens and a complete absence in others [Porte et al., 1995; Porter et al., 1995; Reed and Sage, 1996].

Quantitative gene expression levels provided by the National Cancer Institute SAGE Project provide an unbiased view of SPARC in normal and malignant tissues (http://www.ncbi.nlm.nih.gov/sage). The Cancer Genome Anatomy SAGE Project is a public database comprised of over 6 million SAGE tags representing 114 human cell types covering more than 99% of known genes [Boon et al., 2002]. Review of bulk tissue samples submitted to the SAGE Project demonstrates significant variability in *SPARC* gene expression, both in malignant and normal tissues. The highest levels of SPARC are found in astrocytomas, hemangiomas, breast cancer, and pancreatic cancer; in contrast, melanoma

TABLE II. SPARC Immunoreactivity and Expression in Human Tissue Specimens

Tissue	Specimen	Detection	Expression	References
Brain	Meningiomas WHO grade I, II, III tumors	IHC	No association between grade and SPARC expression	Schittenhelm et al. [2006]
Brain	Neuroblastoma	IHC	Inverse correlation between SPARC expression and extent of tumor progression	Chlenski et al. [2002]
Brain	Glioblastoma	RT-PCR, IHC	Higher expression correlated with poor prognosis	Rempel et al. [1999], Rich et al. [2005], Pen et al. [2007]
Thyroid	Normal thyroid, papillary, follicular, and anaplastic thyroid carcinoma	RT-PCR	Higher expression of SPARC in poorly differentiated cancer	Takano et al. [2002]
Lung	Non-small cell lung cancer	IHC, RT-PCR	Strong stromal expression. Improved survival with higher expression	Koukourakis et al. [2003], Schneider et al. [2004]
Esophagus	Normal esophagus, esophageal carcinoma	DNA microarray, Northern blot, RT-PCR, IHC	Higher expression in cancer tissue	Porte et al. [1998], Yamashita et al. [2003], Luo et al. [2004], Che et al. [2006], Xue et al. [2006]
Stomach	Gastric cancer	RT-PCR, IHC, Northern blot	Higher expression correlated with poor prognosis	Wewer et al. [1988], Maeng et al. [2002], Wang et al. [2004]
Liver	Normal and hepatocellular carcinoma	RT-PCR, IHC, ISH	Higher expression in cancerous tissue	Le Bail et al. [1999], Lau et al. [2006]
Ampullary of Vater	Normal pancreas, chronic pancreatitis, ampullary carcinoma	IHC	Strong stromal expression and correlated with poor prognosis	Bloomston et al. [2007]
Pancreas	Normal pancreas, chronic pancreatitis, pancreatic adenocarcinoma	IHC, RT-PCR, ELISA	Stromal expression correlated with cancer and poor prognosis	Guweidhi et al. [2005], Infante et al. [2007]
Colon	Normal colon, Crohn's disease, colorectal carcinoma	IHC, RT-PCR, Northern blot, ISH	Higher expression in colon cancer at epithelial–stromal interface	Wewer et al. [1988], Porte et al. [1995], Lussier et al. [2001]
Kidney	Sarcomatoid and clear-cell renal cell carcinoma	IHC, Northern blot	High stromal expression	Sakai et al. [2001]
Bladder	Bladder carcinoma	RT-PCR	Higher expression correlated with poor prognosis	Yamanaka et al. [2001]
Prostate	Normal prostate, primary and metastatic prostate cancer	IHC, ISH	Higher expression in metastatic foci	Thomas et al. [2000]
Ovary	Normal ovary and ovarian cancer	IHC	Higher expression in malignant tissue	Paley et al. [2000], Yiu et al. [2001]
Breast	Benign and malignant breast lesions	IHC, SAGE, ISH, DNA microarray	Higher expression in malignant lesions	Bellahcène and Castronovo [1995], Iacobuzio-Donahue et al. [2002], Lien et al. [2007]
Bone marrow	Multiple myeloma	ELISA, DNA microarray	Elevated plasma levels correlated with better prognosis	De Vos et al. [2002], Hedvat et al. [2003], Turk et al. [2005]
Skin	Melanoma, head and neck squamous cell carcinoma	IHC, DNA microarray	Positive stain and high expression correlated with poor prognosis	Ledda et al. [1997a], Massi et al. [1999], Chin et al. [2005], Kato et al. [2005]
Musculoskeletal	Osteosarcoma, giant-cell tumor of the bone, malignant fibrous histiocytoma, angiosarcoma, leiomyosarcoma, hemangioendothelioma	IHC	Moderate to strong staining in malignant tissue	Schulz et al. [1988], Porte et al. [1995], Fanburg-Smith et al. [1999]

Reverse-transcription polymerase chain reaction (RT-PCR), immunohistochemistry (IHC), in situ hybrization (ISH), enyzme linked immunosorbent assay (ELISA).

tissue exhibits very low levels of SPARC. Specific studies of breast tumor progression based on SAGE indicate that production of SPARC is increased in advanced malignancy and invasive phenotypes [Porter et al., 2001; Iacobuzio-Donahue et al., 2002; Parker et al., 2004]. Subsequent analyses of specific cell types in normal and invasive breast tissue indicate that synthesis of SPARC is restricted to stromal fibroblasts and activated myofibroblasts [Allinen et al., 2004]. SAGE studies of endothelial cells derived from colorectal cancer have demonstrated a twofold increase in SPARC, in comparison to normal colorectal endothelial cells [St Croix et al., 2000]. Multiple human tissue studies based on immunohistochemistry and real-time polymerase chain reaction (PCR) analyses provide further support for increases in SPARC during tumor progression.

Animal models investigating the role of SPARC in tumor progression indicate that the effect of SPARC is tissue-specific. In the tumor microenvironment, an important crosstalk between tumor cells and the surrounding stroma can promote a permissive or hostile environment for tumor growth and invasion. Because SPARC is a secreted protein with paracrine and autocrine function, we cannot understand its role in the tumor microenvironment unless we identify the cell type from which it is secreted. Recent studies have approached this question by modifying the expression of SPARC by tumor cells, whereas other studies have modeled tumor progression in the SPARC-null mouse [Ledda et al., 1997b; Schultz et al., 2002; Brekken et al., 2003; Chlenski et al., 2006]. When interpreting these studies, one must consider the source of SPARC, the type of host, and the site of tumor growth. Published investigations of SPARC and tumor progression have failed to employ an orthotopic, syngeneic model that tightly controls for the source of SPARC. Furthermore, the mechanisms underlying the capacity of SPARC to promote or inhibit tumor progression in different tumor microenvironments remains under active investigation.

ANGIOGENESIS AND SPARC

Early studies of SPARC in vitro indicated its potential role in angiogenesis, particularly its capacity to inhibit the proliferation of bovine aortic endothelial cells. The apparent angiotropic activities of SPARC are concentration-dependent and specific to individual peptide domains of SPARC [Jendraschak and Sage, 1996]. SPARC alters membrane permeability, cell shape, proliferation, migration, and attachment, all of which influence angiogenesis in the tumor microenvironment.

The influence of SPARC on endothelial cord and tube formation is dependent on the copper-binding *N*-glycyl-L-histidinyl-L-lysine-OH (GHK) sequence in the follistatin domain of SPARC. Peptides containing the GHK sequence have been shown to induce capillary growth in the avian chorioallantoic membrane model of angiogenesis, and early studies from our laboratory demonstrated that enzymatic degradation of SPARC released peptides with the GHK sequence that promoted angiogenesis in vivo [Lane et al., 1994]. Subsequent studies showed that degradation of SPARC by MMP-3 also released angiogenic peptides containing GHK [Sage et al., 2003].

In a subcutaneous sponge model of angiogenesis, SPARC-null mice exhibit increased vascular invasion. In this model, polyvinyl alcohol sponges were implanted into the subcutaneous tissue of otherwise normal mice, and vessel invasion was quantified. Contrary to the activity of the GHK peptides, mouse models of angiogenesis have indicated that SPARC inhibits vessel growth by binding and decreasing the availability of angiogenic growth factors (e.g., bFGF, PDGF and VEGF) [Bradshaw et al., 2001; Nozaki et al., 2006]. Subsequent studies using the subcutaneous sponge model show that the enhanced angiogenesis in SPARC-null mice is diminished with age [Reed et al., 2005]. Targeted peptide studies by Chlenski et al. [2004] indicate that an epidermal growth factor (EGF)-like module of the follistatin domain in SPARC inhibits angiogenesis associated with neuroblastoma. A similar sequence that inhibited endothelial cell proliferation was reported by Funk and Sage [1993]. The inhibitory effects of SPARC appear to be biphasic and dependent on the structural conformation provided by disulfide bonds within this peptide sequence. In a murine model of lung cancer, however, SPARC had little effect on tumor angiogenesis despite the enhanced tumor growth described in SPARC-null mice [Brekken et al., 2003].

The role of SPARC in tumor angiogenesis is clearly dependent on the availability and

activity of the intact protein, as well as its peptide fragments. To understand the function of SPARC in angiogenesis, we will need to characterize the SPARC peptide profile within the tumor microenvironment. Angiogenesis models will also need to evaluate other angiotropic factors and stromal enzymes (e.g., MMP-3) to account for the effect of SPARC on tumor angiogenesis.

DESMOPLASIA AND SPARC

The desmoplastic response to tumor growth and invasion includes proliferation of endothelial cells, activation and proliferation of myofibroblasts, remodeling of the ECM, rapid deposition of collagen, and infiltration of inflammatory cells. This stromatogenic process could facilitate or hinder tumor invasion through the production of growth factors, MMPs, and recruitment of tumor-associated macrophages (TAMs). SPARC is likely to play a central role in this process, due to its geographic restriction to the stroma in the tumor microenvironment. The production of SPARC by tumor cells or their surrounding stromal cells (fibroblasts, myofibroblasts) is proposed to modulate the activity of growth factors and the capacity of inflammatory cells to infiltrate the tumor microenvironment. The profile of inflammatory cells within the tumor microenvironment might also regulate levels of active SPARC. Kzhyshkowska et al. [2006] recently demonstrated that stabilin-1, a scavenger receptor expressed on alternatively-activated macrophages, binds and internalizes SPARC, data indicating a novel mechanism for modulating SPARC concentration within the tumor microenvironment.

Tumor models of melanoma and breast cancer have demonstrated that SPARC impairs leukocyte infiltration, with the sequela of enhanced tumor growth [Sangaletti et al., 2003; Alvarez et al., 2005]. Conversely, TAM infiltration was diminished in the absence of SPARC, and was associated with enhanced tumor growth in ectopic models of pancreatic and lung adenocarcinoma [Brekken et al., 2003; Puolakkainen et al., 2004]. Kelly et al. [2007] recently reported that VCAM-1 (CD106) is the counter-ligand for SPARC on leukocytes. This interaction inhibits leukocyte endothelial transmigration in a concentration-dependent manner [Kelly et al., 2007]. Furthermore, SPARC-null mice exhibit abnormal leukocyte recruitment in an acute inflammatory model. Although, these recent studies provide insight into the role of SPARC within the local tumor environment, SPARC is one of many factors that influence the migration/infiltration of inflammatory cells into this compartment.

In addition to the infiltration of inflammatory cells, the desmoplastic response represents the proliferation of stromal cells and remodeling of the ECM. The stromal component of specific malignancies can be substantial, for example, an aggressive invasion front or the encapsulation and successful isolation of the tumor from the host. Because SPARC modulates type I collagen deposition and assembly, it likely plays an important role in stromal remodeling and tumor encapsulation. In this context, the increased production of SPARC in the tumor microenvironment represents a non-immunological surveillance mechanism by which the stroma can "wall off" a tumor. In other tumor microenvironments, stromal remodeling may actually facilitate loss of the basement membrane barrier and conversion of in situ adenocarcinoma to an invasive phenotype. In a recent murine model of lung adenocarcinoma, SPARC-null mice had a limited desmoplastic response with poor tumor encapsulation that led to enhanced tumor progression [Brekken et al., 2003]. Similarly, collagen deposition and assembly within the stroma surrounding PAN02 cells in a murine model of pancreatic cancer were also diminished by SPARC [Puolakkainen et al., 2004].

Stromatogenesis within the tumor microenvironment represents the host response to tumor cells as well as the continuous exchange between the tumor cell and its surrounding stroma. Unlike the predictable phases of tissue injury and repair, the "non-healing wound" of cancer [Dvorak, 1986] creates a persistent stress stimulus resulting in abnormal expression of SPARC by host stromal cells or by the tumor itself. Because SPARC acts as a modulator of tissue remodeling, the influence of SPARC on tumor progression and invasion is dependent on the location, source, and time course of SPARC expression. To appreciate fully the contribution of SPARC to the desmoplastic response within the tumor microenvironment will require carefully-designed systems and conditional/gene inactivation models that can account for each of these variables.

CONCLUSION AND FUTURE DIRECTIONS

Evidence in vivo and in vitro indicates that SPARC is a key modulator of the tumor microenvironment, but its true impact on tumorigenesis and tumor progression is yet to be determined. The influence of SPARC on proliferation, migration, angiogenesis, and ECM remodeling is undoubtedly greater in the early stages of malignant transformation. The abundance of SPARC in late stages of tumor progression and invasion might well represent a failed attempt to restore tissue homeostasis within the tumor microenvironment. Perpetually active tumor-associated fibroblasts secrete SPARC in this stressed state, but through a reciprocal interaction between tumor and stroma, the tumor evades the effects of SPARC.

As studies of SPARC continue, we will undoubtedly further appreciate the significance of SPARC in the tumor–host interaction. In the setting of an established tumor, researchers can utilize geographic restriction of SPARC expression to target novel imaging and therapeutic interventions. Drug delivery to the stromal component of a tumor might facilitate local-regional treatment of a tumor, as demonstrated by Lopez et al. [2006] with the expression of thymidine kinase under the control of the SPARC promoter. Similarly, Kelly et al. [2006] have developed an in vivo tumor imaging system based on fluorochrome-labeled phage technology that detects SPARC protein within the tumor microenvironment.

Finally, the most influential role of SPARC in the tumor–host interaction could be within the distant metastatic niche, where metastatic tumor cells have to attach and migrate through the ECM. Does SPARC shape the metastatic niche? Does SPARC create a hostile environment for metastatic cells in bone? To understand how matricellular proteins, such as SPARC, influence tumor invasion and metastasis will require carefully designed conditional/gene inactivation models combined with in silico modeling of this complex biologic system.

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