Journal of Cellular Biochemistry 92:679–690 (2004)

PROSPECTS

SPARC and Tumor Growth: Where the Seed Meets the Soil?

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Abstract Matricellular proteins mediate interactions between cells and their extracellular environment. This functional protein family includes several structurally unrelated members, such as SPARC, thrombospondin 1, tenascin C, and osteopontin, as well as some homologs of these proteins, such as thrombospondin 2 and tensascin X. SPARC, a prototypic matricellular protein, and its homolog hevin, have deadhesive effects on cultured cells and have been characterized as antiproliferative factors in some cellular contexts. Both proteins are produced at high levels in many types of cancers, especially by cells associated with tumor stroma and vasculature. In this Prospect article we summarize evidence for SPARC and hevin in the regulation of tumor cell growth, differentiation, and metastasis, and we propose that matricellular proteins such as these perform critical functions in desmoplastic responses of tumors that culminate in their dissemination and eventual colonization of other sites. J. Cell. Biochem. 92: 679–690, 2004.

Key words: angiogenesis; cancer; collagen; desmoplasia; extracellular matrix; hevin; inflammation; matricellular; SPARC; tumor

INTRODUCTION AND HISTORICAL PERSPECTIVE

We shall not cease from exploration
And the end of all our exploring
Will be to arrive where we started
And know the place for the first time.
T.S. Eliot, "Four Quartets"

SPARC and Matricellular Proteins

Recognized as extracellular modulators of cell function, matricellular proteins are defined as secreted macromolecules that interact with cell–surface receptors, extracellular matrix (ECM), and/or growth factors and proteases, but do not in themselves subserve structural roles [Bornstein and Sage, 2002]. SPARC, thrombospondin (TSP) 1 and 2, osteopontin, Cyr61, CTGF (connective tissue growth factor), Nov-1 (CCN-1), and tenascins are structurally unrelated proteins belonging to this functional group that are generally expressed at high levels during development and in response to injury, and that modulate cell adhesion (in most cases, deadhesion, in contrast to the adhesivity of most ECM proteins). The matricellular proteins have modular structures, the domains of which account for functional pleiotropy [Brekken and Sage, 2001; Bornstein and Sage, 2002]. Mice with targeted deletions of most of the matricellular proteins described to date exhibit either grossly normal or subtle phenotypes that are exacerbated upon injury. Predictably, closer examination has revealed both developmental and challenge phenotypes in these animals (e.g., neurological, vascular, wound healing, foreign body response, tumor growth, bone, connective tissue, immune response, and hemostasis) that confirm and extend the significant roles exerted by this group of proteins in the design, maintenance, and repair of most tissues [Bornstein and Sage, 2002].

Recent provocative data indicating interesting (and sometimes unanticipated) roles for SPARC and its ortholog hevin/SC1 in tumor growth, progression, and/or metastasis have underscored the need to reevaluate these matricellular proteins in the context of cancer biology. In this study, we summarize these data and

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Received 13 February 2004; Accepted 1 March 2004
DOI 10.1002/jcb.20091

propose that SPARC (and, in some cases, hevin) fulfills several aspects of Paget's "seed and soil" hypothesis [Fidler, 2003] that enable tumor cells to interact productively with stromal cells and ECM.

Structure and Function of SPARC

SPARC (also known as osteonectin and BM-40) is the prototypic gene for a family recently grouped on the basis of a novel, extracellular Ca^{+2}-binding (E-C) module, a self-folding, crystallizable, bioactive domain that is, with one exception, immediately preceded by a follistatin-like module (Fig. 1) [Hohenester et al., 1996; Brekken and Sage, 2001]. To date the family members are hevin/SC-1, QR1, testicans 1–3, tsc 36, and SMOC-1 [Vannahme et al., 2002]. The follistatin and E-C modules are thought to confer activities common to the family, whereas the uniqueness of each protein could be ascribed to the poorly-conserved N-terminal acidic domains, as well as other modules specific to SMOC-1 and the testicans [Brekken and Sage, 2001; Vannahme et al., 2002; Hambrock et al., 2003].

Three general functions have been attributed to SPARC: deadhesion, anti-proliferation, and regulation of ECM production [Bradshaw and Sage, 2001; Brekken and Sage, 2001]. Whereas the last of these has become apparent in SPARC-null mice, the deadhesive and cell cycle-inhibitory functions have been characterized largely in vitro. Many of the effects of SPARC on cultured cells have been attributed to the E-C domain and to EF-hand 2, a Ca^{+2}-binding loop that is stabilized by a disulfide bond (Fig. 1) [Brekken and Sage, 2001]. For example, peptides comprising EF-hand 2 inhibit cell spreading and proliferation, disassemble focal adhesions, and account for much of the binding activity of SPARC to cells, growth factors (vascular endothelial growth factor (VEGF) and platelet-derived growth factor), and ECM, e.g., interstitial (types I and III) and basement membrane (type IV) collagens.

SPARC, TSP1, and tenascin C induce a state of intermediate adhesion in cultured cells, ostensibly through their disassembly of focal adhesion complexes and subsequent engagement of distinct signaling pathways [Murphy-Ullrich, 2001]. Since a specific cell–surface receptor for SPARC has not been identified, it is likely that SPARC engages extracellular binding partners with low affinity, or acts as an antagonist of known receptor-ligand interactions (e.g., integrin–ECM) [Bornstein and Sage, 2002]. The equivalent of an intermediate state of adhesion in vivo has been proposed as a cell "primer," enabling migration, invasion, growth arrest, and/or engagement of signaling cascades influencing differentiation, all of which have been attributed to functions of SPARC in various cancers [Ledda et al., 1997; Rempel et al., 1999; Yiu et al., 2001; Rich et al., 2003].

It is important to note that several proteinases, including matrix metalloproteinases (MMPs), release bioactive fragments from SPARC that affect angiogenesis and cell behavior [Sage et al., 2003]. Some of these fragments from the follistatin domain contain high-affinity Cu^{+2}-binding sequences that regulate proliferation in vitro and angiogenesis in vivo, whereas others diminish focal adhesions and inhibit the cell cycle [Brekken and Sage, 2001]. The substantial downregulation of collagen I in SPARC-null cells, however, is reminiscent of the thin dermis and reduced amounts of collagen fibrils in these animals [Bradshaw and Sage, 2001; Bradshaw et al., 2003a]. That SPARC affects cellular levels of TGFBeta-1, as well as its receptor activation and certain components of TGFBeta signal transduction,

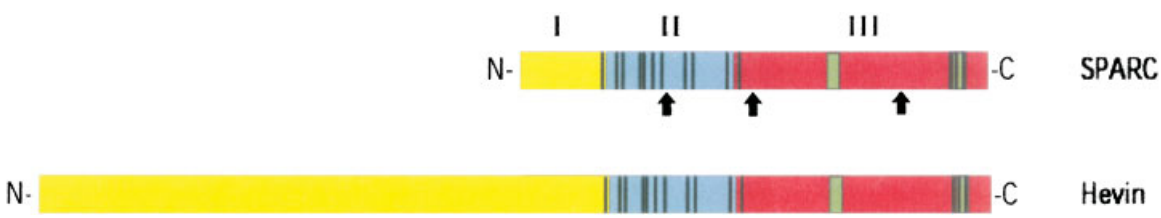

Fig. 1. Domain Organization of SPARC and Hevin. Modules indicated for the two proteins: I (yellow), acidic domain; II (blue), follistatin-like domain; III (red), E-C domain containing two EF-hands (green bars). The total number of amino acids in mouse SPARC is 285, and in mouse hevin, 634, with an overall identity of 53%. Vertical black bars indicate conserved Cys residues. Arrows indicate major MMP-3 cleavage sites in SPARC.

could account in part for the paucity of ECM in SPARC-null mice [Francki et al., 2003; Schiemann et al., 2003].

A summary of the characteristics comprising the phenotype of SPARC-null mice is shown in Table I. The diversity of tissues affected by the targeted deletion of SPARC (adipose, dermis, bone, lens) reflects a common theme: an aberration in the production and/or assembly of ECM. This deficit can be further appreciated in the responses to injury mounted by these mice, most if not all of which represent some sort of compromise in ECM (Table I). Studies on the growth of tumors in SPARC-null mice revealed poor encapsulation and deficiencies in tumor stroma and macrophage accumulation (Lewis lung model), or changes in basement membranes, vasculature, and leukocyte infiltration (mammary carcinoma model) (Table I). Although superficially the results from these two models appear disparate with respect to net tumor size, in our view they are consistent with a role for SPARC in tumor/stromal cell interactions, which in turn are highly dependent on the nature of the connective tissue ECM, including its vascular supply, inflammatory component, and immune response, as well as the nature of the malignancy itself. The interrelationship between SPARC and hevin as matricellular proteins and tumor growth is developed in the succeeding sections.

Revisiting the Seed and Soil Hypothesis

An overview of recent publications in the extended family of ECM, matricellular, and "unaffiliated" proteins and proteoglycans provides ample precedent for SPARC (and/or hevin, see below) as a mediator of cell signaling and function, e.g., elastin [Karnik et al., 2003], collagen I [Davis et al., 2002; Grinnell, 2003], CCNs [Lau and Lam, 1999], TSPs [Murphy-Ullrich, 2001], and fibulins [Timpl et al., 2003]. For many of these proteins, distinctive signaling pathways, effectors, and/or adaptor proteins have been identified, information that is lacking in the case of SPARC. The biological significance of matricellular proteins, particularly SPARC and hevin, and their mechanism of action might be especially evident in models of tumor cell growth and metastasis, and associated angiogenesis [St Croix et al., 2000; Rubin, 2001; Bornstein and Sage, 2002; Iacobuzio-Donahue et al., 2002a]. In a revisitation of Paget's "seed and soil" hypothesis for the metastasis of cancer cells (the "seeds"), Fidler emphasizes that metastases develop only in specific organs (the "soil") and states, "Therapy of metastases, therefore, should be targeted not only against the cancer cells themselves, but also against the homeostatic factors that promote tumor-cell growth, survival, angiogenesis, invasion, and metastasis" [Fidler, 2003].

Proteins such as SPARC that regulate ECM production are good candidates as conditioners of the tumor "soil." For example, solid tumors in SPARC-null mice grew significantly larger than those in wild-type (WT) animals, in part due to a compromised tumor stromal ECM [Brekken et al., 2003]. It is known that native collagen gels induce changes in fibroblast morphology and activation [Grinnell, 2003]. Might any of these changes recapitulate the response of stromal fibroblasts to a tumor? Interestingly, both SPARC and hevin were found to be preferentially produced in desmoplasia: hevin, by host angioendothelial cells in response to pancreatic carcinoma [Iacobuzio-Donahue et al., 2002a], and SPARC, by host juxtatumoral stroma in

TABLE I. Characteristics of SPARC-Null Mice

	Tissue/abnormality	Description	Reference
Development	Lens/early cataract	Defective lens capsule	Yan et al. [2002]
	Skin/laxity & decreased tensile strength	Reduction of dermis; small, regular collagen fibrils	Bradshaw et al. [2003a]
	Adipose/increased	Increase in number & size of adipocytes	Bradshaw et al. [2003b]
	Bone/osteopenia	Severe bone loss	Delany et al. [2000]
	Tail/kinked	CT/disc abnormality?	Bradshaw and Sage [2001]
Injury	Lewis lung carcinoma and B-cell lymphoma	Enhanced growth & metastasis: Decreases in tumor encapsulation, stroma, & macrophage recruitment	Brekken et al. [2003]
	Mammary carcinoma	Reduced tumor growth, massive parenchymal infiltration by leukocytes; decreases in vascularization & collagen IV deposition	Sangaletti et al. [2003]
	Cutaneous wounds	Accelerated closure	Bradshaw et al. [2002]
	Subcutaneous foreign body response	Diminished encapsulation	Puolakkainen et al. [2003]

response to infiltrating breast carcinoma [Iacobuzio-Donahue et al., 2002b]. Regulation of tumor growth and metastasis via host angiogenesis is likely to be one of the consequences of altered ECM synthesis, assembly, and/or degradation. In fact, proteolytic fragments of ECM, basement membrane, matricellular, and other secreted proteins have been shown to influence angiogenic responses in a variety of contexts [Sage, 1997; Kalluri, 2003]. Moreover, the infiltration of leukocytes, especially macrophages, is a critical component in the growth and dissemination of many types of tumors [Dranoff, 2004].

Further points to consider in the 2003 definition of the "seed and soil" hypothesis are: (1) the heterogeneity of cells comprising a primary neoplasm, and the selection for further genetic and adaptive phenotypes contributing to metastatic populations, (2) the contribution of nontumor host cells to both the neoplasm and its metastasis, and (3) the changing microenvironments necessary for the initial growth and differentiation of cancer cells versus the subsequent modulation of cancer cell invasion and site-permissive metastasis [Fidler, 2003; Dranoff, 2004]. Indeed, matricellular proteins such as SPARC and hevin appear to be well-poised as effectors of tumor cell behavior in the context of these parameters.

SPARC AND TUMOR BIOLOGY

Expression and Function of SPARC in Tumors: The ECM Connection

Over 200 publications have described the association of SPARC with many types of cancers. As a product of both tumor and host (stromal, inflammatory) cells, SPARC was particularly prevalent at tumor–stromal interfaces, in fibroplasias and desmoplasia, in angiogenesis and vascular remodeling, and in tumor capsules [Brekken and Sage, 2001]. Recent studies have used SPARC-null mice (Table I), tumor cells expressing different levels of SPARC, or gene profiling of cancers and their associated vascular/stromal components. The latter two groups of recent publications are described in Table II. Several investigators have demonstrated a major role for SPARC in the promotion of glioma cell invasion, as well as inhibition of glioma and neuroblastoma growth in vivo [Chlenski et al., 2002; Schultz et al., 2002], the latter via inhibition of angiogenesis. Additionally, data from gene profiling have identified SPARC as part of an invasion-specific cluster in pancreatic and breast carcinoma [Ryu et al., 2001; Iacobuzio-Donahue et al., 2002b]. These and other profiling studies have produced data consistent with a role for increased levels of SPARC in stromal/desmoplastic compartments of several different tumors and their metastases (Table II).

An important element of desmoplastic ECM is collagen I, which was also found within the invasion-specific cluster in breast and pancreatic carcinoma as a "panstromal" component [Iacobuzio-Donahue et al., 2002a,b], and as an angiogenic marker in colorectal tumors [St Croix et al., 2000]. What is the relationship between SPARC and collagen I? Early studies on the synthesis and distribution of SPARC had shown it to be prominent in tissues undergoing remodeling and repair; moreover, there

TABLE II. Expression and Function of SPARC in Tumors

Classification	Description	Reference
Glioma	Expression in tumor cells associated with invasion and growth inhibition	Schultz et al. [2002]
	Expression enhances invasion	Rich et al. [2003]
Neuroblastoma	Expression by Schwannian stroma inhibits angiogenesis and impairs tumor growth	Chlenski et al. [2002]
Melanoma	Enhanced expression in tumor cells promotes metastasis	Ledda et al. [1997]
Adenocarcinoma (pancreas)	Aberrant methylation in tumor; expression in stroma	Sato et al. [2003]
Carcinoma (pancreas)	Part of invasion-specific cluster	Ryu et al. [2001]
Carcinoma (breast)	Part of invasion-specific cluster, expressed in juxtatumoral stromal cells	Iacobuzio-Donahue et al. [2002b]
	Fold change: 65↑ in highly-invasive lines, and 2↓ in weakly invasive lines	Zajchowski et al. [2001]
Carcinoma (colorectal)	Expressed preferentially in tumor-associated blood vessels	St Croix et al. [2000]
Non-small-cell carcinoma (lung)	Increased expression in tumor cells after coculture with normal fibroblasts	Fromigue et al. [2003]
	Downregulated with tumor growth	Bendik et al. [1998]
Carcinoma (ovary)	High levels produced by tumor cells promote their apoptosis	Yiu et al. [2001]

was coincident expression of SPARC and collagen I associated with fibrosis and angiogenesis [Bradshaw and Sage, 2001; Brekken and Sage, 2001]. In SPARC-null mesangial cells, levels of alpha-1 (I) collagen were reduced nearly 50%, and skins from these mice contained half the collagen of WT counterparts and exhibited reduced tensile strength. Moreover, the collagen fibrils in the attenuated dermis were small and highly uniform in size (i.e., "immature") [Bradshaw et al., 2003a]. Clearly, SPARC affects the synthesis, assembly, and quality of collagen I-containing ECM, a function perhaps recapitulated in the excessive growth of Lewis lung carcinoma in host animals lacking SPARC (Table I).

The many influences of ECM structure on tumor progression include the diffusivity of the tumor and stroma, as facilitated by an intervening capsule or ECM, survival of tumor and stromal cells, modulation of growth factor availability, regulation of angiogenesis and vascular remodeling, trans-endothelial trafficking, and structure of the vascular basement membrane. Extensive synthesis of ECM is a hallmark of many tumors and their associated stroma. ECM can increase tumor cell resistance to either drug therapy or natural defenses by its modulation of macromolecular diffusion [Netti et al., 2000], by provision of anti-apoptotic signals [Sethi et al., 1999], or by sequestration of growth factors [Margosio et al., 2003]. The ECM also furnishes important (including apoptotic) signals for endothelial cells during angiogenesis [Stupack and Cheresh, 2003] and contributes to vascular basement membrane abnormalities in tumors [Baluk et al., 2003]. These processes present additional opportunities for the involvement of SPARC in tumor progression.

SPARC and Tumor Growth: The Immune Connection

Both studies in SPARC-null mice suggest a role for SPARC in cancer immunity (Table I). Brekken et al. [2003] found decreased macrophage infiltration of tumors in SPARC-null mice. Sangaletti et al. [2003] found that expression of SPARC by cells of bone marrow origin was associated with reduced leukocyte infiltration into the tumor, and with enhanced tumor growth. Conceivably, SPARC might function either in the bone marrow stroma to modulate the release of hematopoietic cells into the circulation, or in the tumor stroma, where it could modulate infiltration, differentiation, and survival of immune cells. Interestingly, other matricellular proteins, including osteopontin, tenascin-C, and TSP1, confer immunomodulatory functions. In this section we describe published studies relevant to a role for SPARC in the context of immune cell function, and draw an analogy between cancer and inflammation, including that driven by autoimmunity [Coussens and Werb, 2002].

The bone marrow stroma. The bone marrow includes two compartments: a stroma rich in fibroblasts, osteoblasts, and adipocytes, with a complex ECM, and resident hematopoietic cells for which the stroma is essential. The marrow contributes at least four populations of circulating cells relevant to tumor progression: circulating endothelial progenitors, myeloid progenitors, megakaryocytic progenitors, and hematopoietic stem cells [Rabbany et al., 2003], and their release has been characterized in the context of responses to traumas that include wounding, ischemia, and tumor growth. The release process reflects marrow-localized action of hematopoietic cytokines (e.g., KitL), angiogenic switch factors (VEGF, placental growth factor (PGF), and angiopoietin), adhesive factors including VCAM1-VLA4 and ICAM1-LFA1, and MMPs. In contrast to VEGF receptor(R)2+ endothelial progenitors, hematopoeitic stem cells are VEGFR1+, and are mobilized to the circulation in a PIGF-dependent fashion. Release of hematopoietic cells into the circulation is believed to involve remodeling of the hematopoietic stromal ECM, possibly by MMPs. MMP-9 is produced by hematopoietic stem cells, mediates their release from marrow to circulation, regulates their migration through ECM in vitro, and enhances levels of soluble KitL, the bioavailable form of this cytokine. SPARC regulation of MMP-9 expression [Brekken and Sage, 2001] could be one mechanism by which SPARC could mediate the release of hematopoietic cells.

The dynamic of marrow release of circulating cells provides several other possible points of influence by SPARC: modulation of VEGFR1 signaling, deadhesion of hematopoietic precursors, and modulation of ECM architecture in the marrow stroma [Brekken and Sage, 2001]. As described later but appropriate to mention here, hevin is expressed by mouse marrow stromal cells and could also play a role in the release of

circulating cells from the marrow [Oritani et al., 1997]. Furthermore, recombinant fusions incorporating hevin with the immunoglobulin constant region augmented the proliferation of mature B cells and the cloning efficiency of pre-B cells, but not that of myeloid progenitor cells. Additional observations reduced the portion of hevin with lymphopoietic activity to an N-terminal sequence sharing no identity with SPARC (Fig. 1).

Cells infiltrating the tumor stroma from peripheral blood. Monocyte/macrophages, T cells, B cells, granulocytes and NK cells, and dendritic cells all have described functions in cancer immunity. Macrophage and neutrophil infiltration has been described in the two SPARC-null models of tumor progression, but the influence of SPARC upon infiltration by the other cell types has not been addressed. The discussion below singles out three tumor-infiltrating cell types: circulating mesenchymal cells, because they can differentiate into endothelial cells and fibroblasts; macrophages, which are key in two locales where the SPARC-null phenotype is manifest (tumors and fat deposits); and dendritic cells, because they are central to efforts to harness tumor immunity.

Circulating mesenchymal cells. Two cell types that fit this description are the fibrocyte and the mesenchymal precursor cell (MPC). The former is a mesenchymal cell that can be cultured from peripheral blood and demonstrates monocytic and fibroblastic characteristics [Abe et al., 2001]. The roles of fibrocytes in fibrosis, antigen presentation and other immune functions, and angiogenesis have been reported [Hartlapp et al., 2001]. Their combination of surface markers (collagen I+, CD11b+, CD13+/CD34+/CD45RO+/MHCclassII+/CD86+) is consistent with connective tissue and immune roles, as well as progenitor identity. Barth et al. [2002] observe that fibrocyte frequency is higher in stroma from chronic human pancreatitis than in that from pancreatic adenocarcinomas or endocrine pancreatic tumors, and propose fibrocytes as a target for distinguishing purely inflammatory from cancerous processes.

The MPC (also termed mesenchymal progenitor cell or pannocyte, from "pannus") is a resident of postnatal bone marrow stroma supports selected arms of hematopoiesis, and can itself be stimulated to generate bone, cartilage, fat, muscle, and fibrous tissues. MPCs can also be detected in normal circulation. In rheumatoid arthritis, afflicted synovium bears a population of these cells greatly expanded compared with normal synovium [Jorgensen et al., 2001]. Speculation concerning their function is based on the following data: (1) these cells express genes characteristic of the developing limb bud: members of the Wnt, hedgehog, and homeobox families, and members of the fibroblast growth factor, bone morphogenetic protein (BMP), and BMPR families; (2) the expansion of the population of these cells in joint synovium during the initial phase of rheumatoid arthritis precedes infiltration by lymphocytes and neutrophils. Perhaps the expression of limb bud genes is part of a program of abortive tissue remodeling and repair, and would moreover engender an innate immune response, in which MPCs play a vital role of chemokine and cytokine production. The innate immune response, rather than the more highly-subscribed adaptive (T-cell centric) response, would predispose the organism to rheumatoid arthritis. A role for MPCs in tumors or tumor stroma is unreported; however, hedgehog family members have been implicated in tumorigenesis in the pancreas [Thayer et al., 2003] and digestive tract [Berman et al., 2003]. Whether or not circulating fibroblast progenitors play a role in tumor progression is unknown, but is suggested by their description in the cancer and autoimmune processes noted above. Whether SPARC could influence their infiltration into tumor stroma now becomes an important question.

Macrophages: At the crossroads of fat metabolism and tumor progression. Macrophages modulate immune responses, kill pathogens, stimulate angiogenesis, and are involved in several aspects of tissue repair. Monocytes, their circulating precursors, are attracted to wounded and pathogen-compromised areas and to tumors by growth factors and chemokines, which in turn provoke their differentiation to tissue-resident macrophages [Pollard, 2004]. Two lines of inquiry indicate tumor-associated macrophages (TAM) potentiate tumor progression. First are the epidemiologic correlations between TAM abundance and poor prognosis—particularly for breast, prostate, ovarian, and cervical cancer. The levels of two factors with strong macrophage tropism—monocyte-chemoattractant-1 and colony-stimulating factor (CSF)-1—correlate with poor

prognosis in a number of prevalent cancers. Secondly, animal models providing modulation of macrophages through CSF-1 show that diminution of TAM reduces tumor progression.

The role of macrophages in fat metabolism has been inferred from several observations [Wellen and Hötamisligil, 2003]. Obese conditions are associated with a chronic inflammatory response in adipose tissue, with aberrant cytokine production, increased acute-phase species, and increased inflammatory signaling. The identity of the cells orchestrating this dynamic is unknown, but roles in complement activation and inflammatory cytokine production implicate T cells and macrophages, whereas tissue localization implicates fat stroma, the major residents of which are macrophages, preadipocytes, and cells of the vasculature. Of the immune cell types, macrophages are more highly implicated due to a gene profile strikingly shared with that of adipocytes, which includes transcription factors, cytokines, inflammatory molecules, fatty acid transporters, and scavenger receptors. Of the two stromal residents, macrophages are more highly implicated by F4/80 marker studies and their bone marrow origin. However, if preadipocytes also differentiate into macrophages, this discrimination may be mute [Charrière et al., 2003].

The picture emerging from these observations is one of macrophage contribution to two inflammatory milieus, obesity and cancer, and raises the following questions: does macrophage accumulation contribute to the increased adiposity of the SPARC-null phenotype (Table I)? In subcutaneous Lewis Lung carcinoma, the SPARC-null phenotype was associated with reduced tumor infiltration [Brekken et al., 2003], whereas in the case of mammary tumors, it was associated with increased tumor infiltration [Sangaletti et al., 2003]. Do these findings reflect differences in the adiposity of the tissue hosting the tumor? And, independently of tumor locale, do macrophages and adipocytes coassociate in tumors or tumor stroma?

Dendritic cells: Tipping the scale of tumor immunity toward tolerance or rejection. Dendritic cells are central to cancer vaccine strategies because of their ability to process and present both class I and II antigens with a full repertoire of costimulatory signals, and their ability to migrate, bearing antigen, to lymph nodes, where they position themselves to sample circulating T cells for rare ones that can be activated with antigen specificity. Dendritic cell activation of T cells is thought to occur in the peripheral lymph nodes, where immune tolerance or reactivity is dictated [Sallusto and Lanzavecchia, 1999].

Dendritic cell-mediated immunity requires tissue-resident dendritic cell differentiation, antigen activation, and mobilization to draining lymphatics and to the lymph node medulla, where they will activate antigen-specific T cells, which are in turn mobilized through a multistep process to the tumor. These processes likely rely on cellular shape change, deadhesion, and dynamic interaction with the ECM and/or matricellular proteins. Contexts for SPARC involvement are, therefore, numerous. An example of matricellular protein participation in this context is provided by TSP1. Expressed by dendritic cells, TSP1 inhibits their release of interleukin (IL)-12, tumor necrosis factor (TNF)-alpha, and IL-10 through interactions with CD36 and CD47 on the dendritic cell surface [Doyen et al., 2003]. The downregulation of these cytokines is parcel to the dendritic cell's becoming refractory to restimulation. Hence, this dynamic provides one means by which TSP1 contributes to resolution of inflammation, which in turn is consistent with the multiorgan inflammation seen in TSP1-null mice. Also consonant with this phenotype is the observation that dendritic cell CD47 ligation by a TSP1-derived peptide provoked dendritic cell apoptosis [Johansson et al., 2004]. Such dendritic cell–TSP1 interactions summon related inquiries with respect to SPARC.

STRUCTURE AND FUNCTION OF HEVIN—IS IT A TUMOR SUPPRESSOR?

Characteristics and Potential Functions of Hevin

Hevin has been described by several different laboratories as synaptic cleft (SC)-1, and ECM 2. Since "SC1" also denotes an integral membrane adhesion protein that is structurally unrelated to hevin/SC-1 [Tanaka et al., 1991], we have chosen to use "hevin." The modular structure of hevin, as a member of the *SPARC* gene family, is shown in Fig. 1. Although the follistatin and E-C domains are well-conserved between SPARC and hevin, the N-terminal acidic region of hevin shows low identity with SPARC and accounts for a molecular mass nearly double that of SPARC (approx. 71,000

vs. 32,000 Da, excluding posttranslational modifications).

Originally cloned (as SC-1) from rat brain [Johnston et al., 1990], hevin is expressed widely in neurons and glia during development. Evidence for its being an important secreted glycoprotein of brain tissue comes from a recent study showing its identity with RAGS-1, a terminator of neuronal migration [Gongidi et al., 2004]. Girard and Springer [1996] cloned hevin from a human high endothelial venule (HEV) library and showed that the recombinant protein inhibited adhesion and focal adhesion formation in endothelial cells. Other functions attributed to hevin include its enhancement of B-cell lymphopoiesis [Oritani et al., 1997], as mentioned earlier, and its interaction with interstitial collagen I fibrils [Hambrock et al., 2003], both of which underscore the participation of hevin in non-neural cell–ECM interactions, i.e., in bone marrow and connective tissue. Consistent with this premise, we showed by in situ hybridization that hevin mRNA was present in a variety of murine tissues and cells and was prominent in certain types of vessels [Soderling et al., 1997]. Although there is partial coincidence in the expression of both hevin and SPARC transcripts in normal fetal and adult tissues, there is also a striking bias toward hevin in neural tissue and toward SPARC in connective tissue. Both proteins have been implicated in the regulation of tumor cell proliferation, invasion, and/or metastasis [Sullivan and Sage, 2004]. Consistent with the phenotypes of most matricellular gene-targeted mice, hevin-null mice are viable and appear grossly normal. However, our data indicate changes in ECM and collagen: compared to WT mice, hevin-null animals exhibit a disordered dermal architecture and accelerated closure of dermal wounds (M. Sullivan et al., in preparation).

Is *Hevin* a Tumor-Suppressor Gene?

SAGE (serial analysis of gene expression), performed with endothelial cells derived from blood vessels of normal and malignant colorectal tissues, identified hevin as the second most abundant pan-endothelial marker [St Croix et al., 2000]. In contrast, tumor cells, including prostatic and non-small-cell lung carcinomas, appear to down-regulate their production of hevin, and Claeskens et al. [2000] showed inhibition of HeLa cell growth by expressed hevin, observations suggesting an anti-proliferative and/or tumor-suppressor role for this protein [Bendik et al., 1998; Nelson et al., 1998; Claeskens et al., 2000]. Several recent studies using gene profiling techniques have short-listed hevin as an angiogenesis-associated gene [Peale and Gerritsen, 2001], as part of an invasion-specific cluster in pancreatic cancer [Ryu et al., 2001], and as an angioendothelial marker in the desmoplastic response to invasive pancreatic carcinoma [Iacobuzio-Donahue et al., 2002a]. Most of this evidence is correlative and in itself does not make a strong case for hevin as a regulator of tumor cell proliferation, metastasis, or angiogenesis. However, the deadhesive and cell-cycle modulatory roles of hevin strengthen the claim considerably.

Hevin: At the crossroads of tumor immunity and autoimmunity? Hevin could offer a probe of dendritic cell-mediated immunity through its expression in HEV, especially those of the peripheral lymph node [Gretz et al., 1997] and within sites of chronic autoimmune inflammation. In the former site HEV deliver circulating lymphocytes to the cortex of lymph nodes in the human body at an estimated rate of 5×10^6 cells/s [Girard and Springer, 1996]. T cells within this flux are of principal interest from the standpoint of immune tolerance or rejection of tumors. T cells pass from the HEV to a T cell cortex comprising a network of corridors running through an ECM-rich fibroblastic reticular cell network. Antigen-primed dendritic cells enter the lymph node through afferent lymphatics but position themselves within the T cell cortex near the venules, consistent with efficient dendritic cell sampling of the T cell repertoire.

Hevin is expressed by the specialized endothelial cells of the HEV, on their apical, lateral, and basolateral surfaces [Girard and Springer, 1996]. Its anti-adhesive and anti-spreading properties observed with cultured endothelial cells led these authors to speculate that hevin is part of the endothelial cell apparatus mediating lymphocyte extravasion from the venule. Does hevin contribute to the architecture of the HEV, and of the lymph node T cell cortex? Does hevin mediate lymphocyte extravasion into the lymph node cortex? Does hevin modulate the positioning of dendritic cells within T cell cortices near the HEVs? Lastly, does hevin modulate tumor immunity mediated by the dendritic cell–T cell axis?

At sites of chronic inflammation, the HEVs coincide with organized aggregates of dendritic and T cells and are the products of "lymphoid neogenesis" [Hjelmströom, 2001]. These neolymphoid structures have been characterized in rheumatoid arthritis, autoimmune thyroiditis, and autoimmune diabetes, both in humans and in animal models, and could provide high efficiency, localized antigen presentation that contributes to the chronic inflammatory state. We might, therefore, ask whether hevin contributes to lymphoid neogenesis and, if so, is chronic inflammation modulated as a consequence of the perturbation of hevin expression?

Hevin and SPARC—Unique or redundant functions? It is interesting that hevin mRNA or protein has not been detected in normal cultured cells, including endothelial cells [Soderling et al., 1997; St Croix et al., 2000], whereas several tumor cell lines secrete hevin in vitro [Hambrock et al., 2003], because the opposite has been published with respect to SPARC: referred to as a "culture shock" protein, SPARC is expressed at high levels by almost all cultured cells and cell lines [Brekken and Sage, 2001]. Although the exon/intron boundaries of hevin (mouse chromosome 5) and SPARC (mouse chromosome 11) indicate divergence from a common ancestral gene [McKinnon et al., 1996], their promoters are substantially different. Virtually nothing is known about the regulation of the *hevin* gene. However, given their structural similarities and selective coincidence of expression, hevin could compensate for some functions of SPARC, especially those associated with the follistatin and E-C modules (Fig. 1).

Understanding at a fundamental level how SPARC and hevin regulate cell adhesion, proliferation, and ECM production will enable us to evaluate experimental outcomes in models of tumor progression and metastasis in which gain- or loss-of-function approaches are used. For example, as homologous matricellular proteins, do SPARC and hevin exhibit complementary functions (i.e., gene compensation), and are there unique domains or sequences in each protein to which we can assign specific functions? Evidence to date indicates that compensation by hevin in SPARC-null mice bearing subcutaneous Lewis lung carcinomas is unlikely [Brekken et al., 2003]: *hevin* has been described as a tumor-suppressor gene that inhibits tumor cell proliferation, whereas SPARC-null mice can support extensive growth of tumors, in comparison to WT mice, due in part to diminished production of ECM, poor encapsulation of the tumor, and reduced influx of macrophages. However, the anticipated increases in cell proliferation and angiogenesis were not found in tumors grown in SPARC-null hosts. Alternatively, compensation between hevin and SPARC might be tissue-specific or protein domain-specific. The latter possibility would predict that the cleavage of hevin and SPARC by, e.g., MMP-3 would produce peptides with similar activities [Sage et al., 2003].

SUMMARY AND PROSPECTS

Recognition of the site-specificity of tumor metastases has validated Paget's seminal "seed and soil" hypothesis and has furthermore refined our definition of the soil: a partial list would include ECM and matricellular proteins; endothelial cells and stromal cells, and their paracrine and endocrine growth factors/inhibitors; MMPs and their activators or inhibitors; immune cells and their cytokines; macrophages and mast cells; microvessels and angiogenesis factors. Indeed, many types of tumors exhibit a reciprocity with respect to these criteria and respond in kind with altered properties of adhesion (cell–cell or cell–ECM), motility, and proliferation, facilitated in part by aberrant ECM structure or composition and a compromised vasculature [Padera et al., 2004]. It is now generally accepted that a continually changing interplay between malignant cells and their immediate stromal compartment is necessary for tumor progression [Rangarajan and Weinberg, 2003]. A testable hypothesis based on these observations is that components of the tumor stroma, e.g., certain of the matricellular proteins demonstrating functions in collagen assembly and vascular growth/remodeling, will influence tumor growth. A shortlist of these proteins would include SPARC and hevin.

In this article we have presented arguments that SPARC and hevin affect the stromal or desmoplastic response to malignant tumors. The deadhesive functions of SPARC and hevin on endothelial cells, as well as the established effects of SPARC and its cleavage products on the endothelial cell cycle, would likely contribute to temporal regulation of the "angio-endothelial" compartment of desmoplasia, with

subsequent modulation of tumor cell invasion. Of equal import might be the effect of SPARC (and possibly hevin) on ECM production and assembly. Fibrillogenesis of collagen I, a major ECM player in desmoplasia, is sensitive to the presence of "accessory" proteins (e.g., SPARC, TSP2, the proteoglycan decorin, and other collagens) [Bornstein and Sage, 2002]. Alteration of collagen fiber morphology and tissue characteristics is predicted to have consequences which might become especially apparent during responses to injury or pathology, when the need for high levels of collagen I is acute and timely. Since the ECM (and collagens in particular) is a known provider of morphogenetic cues for the growth of blood vessels [Dvorak, 2003], changes in its composition are also likely to affect vascular quality and angiogenic response. Jain and colleagues have recently demonstrated, by a fluorescence-based imaging technique, that fibrillar collagen was modulated among various tumors, observations with significant importance to drug delivery within tumor masses [Jain, 2003]. This study complements nicely our findings regarding enhanced tumor growth in SPARC-null mice: a major difference between tumors grown in WT vs. SPARC-null animals was the poorly-developed capsule and stromal compartment (collagen I and decorin) in the absence of SPARC, thus confirming in a pathologic setting the compromised production of collagen I in these animals [Brekken and Sage, 2001].

Clearly, productive tilling of tumor and organ-specific soil, including its various angiogenic, inflammatory, immune, and resident stromal cell components, will contribute substantially to our basic understanding and therapeutic design of anti-cancer drugs. As the Homestead Act encouraged new settlers to farm the original lands, we continue to develop and work from Paget's provocative hypothesis.

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