The role of SPARC in extracellular matrix assembly

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Abstract SPARC is a collagen-binding matricellular protein. Expression of SPARC in adult tissues is frequently associated with excessive deposition of collagen and SPARC-null mice fail to generate a robust fibrotic response to a variety of stimuli. This review summarizes recent advancements in the characterization of the binding of SPARC to collagens and describes the results of studies that implicate a function for SPARC in the regulation of the assembly of basal lamina and fibrillar collagen in the ECM. Potential cellular mechanisms that underlie SPARC activity in ECM deposition are also explored.

Keywords BM-40 · Collagen · Fibrillogenesis · Osteonectin · Review

Introduction

Secreted Protein Acidic and Rich in Cysteine (SPARC, osteonectin, BM-40) is a highly conserved matricellular protein that is expressed in *C. elegans*, *Drosophila*, honey bee, brine shrimp, zebra fish, chicken, and mammals (Bradshaw and Sage 2001; Tanaka et al. 2001; Kucharski and Maleszka 2002; Rotllant et al. 2008). Worms and flies, each with one gene encoding SPARC, do not survive abrogation of SPARC expression (Fitzgerald and Schwarzbauer 1998; Martinek et al. 2008). Ectopic expression and inhibition of SPARC expression in amphibians leads to developmental arrest (Purcell et al. 1993; Damjanovski et al. 1997). SPARC-null mice however, are viable and reproduce perhaps due to compensation from other SPARC family members such as hevin/SC1 and SMOC-2 (Sullivan and Sage 2004; Liu et al. 2008). Although a number of different activities are associated with SPARC, including processes regulating proliferation, growth factor activity, matrix metalloproteinase (MMP) activity, and migration—this review will focus on the interaction of SPARC with collagens and mechanism(s) by which SPARC might influence the assembly and deposition of the ECM (Brekken and Sage 2001).

SPARC binding to collagens

SPARC consists of 3 modular domains (Brekken and Sage 2001). The N-terminal region contains a low-affinity, high-capacity Ca$^{2+}$-binding domain. The central portion of the protein includes a region with homology to follistatin, whereas the C-terminal, third domain contains two high-affinity Ca$^{2+}$-binding EF hands. The domain of SPARC that binds collagens and cell surfaces is localized to the third domain (Hohenester et al. 1997).

Although SPARC is reported to bind a number of ECM proteins, SPARC binding to collagens is the best characterized of these interactions. The binding of SPARC to collagens is Ca$^{2+}$-dependent and requires non-denatured, triple helical collagen (Sasaki et al. 1998). The affinity of SPARC for collagen IV, the most
abundant collagen in the basal lamina, exhibits a $K_d$ of $\sim$1-2 $\mu$m (Sasaki et al. 1998). SPARC also binds to fibrillar collagens I, II, III, and V with a similar, or in some cases, a slightly reduced affinity (Sasaki et al. 1998). Cleavage of SPARC by MMPs at amino acid L197, or by an unidentified protease in tissues at residue L198 in helix $\alpha$C of the collagen-binding region, results in a ten-fold increase in the affinity of SPARC for collagens I, II, III, IV and V (Sasaki et al. 1997). As helix $\alpha$C partially masks the collagen-binding site in helix $\alpha$A, protease cleavage is predicted to relieve this masking effect thereby increasing collagen binding. A recombinant form of SPARC (rSPARC), with aa 196–203 deleted mimics the effects of protease digestion and generates an “activated” form of recombinant SPARC (SPARC $\Delta\alpha$, $\alpha$C; SPARC FS-EC $\Delta\alpha$C) which engages collagen with a 10-fold higher affinity than full-length rSPARC (Sasaki et al. 1997). Tissue-specific differential glycosylation of SPARC also influences SPARC binding to collagen. For example, the SPARC that is expressed in bone is modified with high-mannose structures and exhibits a higher affinity for collagen I than SPARC from platelets that does not have the high-mannose modification (Kaufmann et al. 2004).

The SPARC-binding site on collagen I is described in two separate studies. Wang et al. (2005) identified SPARC binding sites on collagen I with atomic force microscopy using rSPARC (generated in insect cells) and rat tail collagen I or recombinant procollagen I. Similar binding to procollagen I and collagen extracted from rat tail (with propeptides removed) was demonstrated. Although evidence of SPARC binding to collagen I appeared all along the protein, two preferred sites of interaction with SPARC, a primary and a secondary site, were reported. The primary site localized to amino acid residues $\sim$650–800 on collagen I $\sim$87.5–125 nm from the C-terminus of procollagen I whereas a secondary site mapped nearer the N-terminus, $\sim$237.5–262.5 nm from the C-terminus, corresponding to aa 100–200. The primary site at aa 650–800 overlaps a previously reported $\alpha_1\beta_1$ integrin recognition site, as well as binding sites for cartilage oligomeric protein (COMP/thrombospondin 5) and fibronectin, whereas the secondary site overlaps a potential $\alpha_2\beta_1$ integrin interaction site (Wang et al. 2005).

In a second study, Giudici et al. (2008), assessed SPARC collagen interactions by rotary shadowing, followed by electron microscopy. Using rSPARC made in mammalian cells with the helix $\alpha$C deleted to generate “activated” SPARC $\Delta\alpha$, $\alpha$C and human procollagen produced in vitro by skin fibroblasts, these authors identified a major SPARC binding site $\sim$180 nm from the C-terminus of collagen I. Likewise, a nearly identical region in fibrillar collagens II and III was shown as the most favored for interaction of SPARC in these studies.

Lesser sites of SPARC binding, in order of preference, were located at $\sim$80 nm and $\sim$240 nm from the C-terminus. Giudici et al. (2008) obtained a synthetic triple-helical peptide representing homotrimeric collagen III aa residues 397–423 in the major binding site ($\sim$180 nm from the C-terminus) and found that SPARC binds this peptide with an affinity similar to that of full-length procollagen III. The analogous domain of collagen I is known to contain binding sites for von Willebrand factor (vWF) and Discoidin Domain Receptor (DDR) 2.

The primary disagreement between the two SPARC collagen I binding studies is that Wang et al. did not find a primary interaction site at the $\sim$180 nm region, containing the previously characterized vWF/DDR 2 binding domain. Both studies identified the $\sim$80 nm site and the lesser site $\sim$240 nm from the C-terminus as SPARC-binding regions. Whether differences in the source of SPARC (rSPARC from insect cells versus rSPARC $\Delta\alpha$, $\alpha$C from mammalian cells) underlie these results is currently not known. The source of human procollagen, versus recombinant procollagen, is perhaps another factor that influences the location of binding sites. Nonetheless, the identification of SPARC-binding sites on fibrillar collagens suggests that SPARC can influence the interaction of collagen with cell-surface collagen receptors of the integrin and/or DDR family, as well as sites of collagen interaction with other ECM components including fibronectin and/or collagen-binding proteoglycans.

A recent study by Hohenester et al. (2008) reports the crystal structure of activated rSPARC FS-EC $\Delta\alpha$C bound to a 33-residue peptide from collagen III representing aa residues 564–590, the major binding site identified by Giudici et al. $\sim$180 nm from the C-terminus. SPARC bound the GVMGFO motif and buried a total of 720 A$^2$ of solvent-accessible collagen surface. Engagement of collagen by SPARC is predicted to alter substantially the conformation of SPARC resulting in a deep pocket that accommodates the phenylalanine on the trailing collagen chain (Hohenester et al. 2008). In contrast, the triple helix of the collagen molecule does not undergo significant changes upon SPARC binding. Based on data from the crystal structure, the authors devised a sequence rule that predicts SPARC interaction sites elsewhere in the collagen molecule (Hohenester et al. 2008). A second site is predicted at the GATGFO sequence which is located at $\sim$80 nm from the C-terminus at residue $\sim$700, as shown previously by atomic force microscopy and by rotary shadowing (Wang et al. 2005; Giudici et al. 2008). Using the same sequence rule, Hohenester et al. (2008) predict four SPARC-binding sites in collagen IV, [ $\alpha_1$(IV)]$_2\alpha_2$ (IV). Of these, one site at position aa 480 in COL4A1 is in close proximity to a previously identified integrin $\alpha_1\beta_1$ binding site (Kern et al. 1993).
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SPARC and collagen ECM assembly

Fibrillar collagens

The fact that SPARC binds to collagens suggests that SPARC might influence the assembly of collagen in the ECM. In fact, the absence of SPARC in mice results in significant differences in collagen fibril morphology, as well as substantial decreases in adult tissue concentrations of collagen. For example, the skin of SPARC-null mice has ~ half the amount of collagen, as measured by hydroxyproline analysis, in comparison with wild-type skin (Bradshaw et al. 2003b). Similar decreases in interstitial collagen are apparent in heart and in fat depots of SPARC-null mice (Bradshaw et al. 2003a; Bradshaw et al. 2009). Collagen fibrils formed in the dermis in the absence of SPARC are smaller and more uniform in diameter than those of wild-type animals. Decreases in the average diameter of collagen fibrils in SPARC-null skin are significant at 1 month of age and become more substantial at 6 months of age (Rentz et al. 2007). At 1 month, the frequency of collagen fibrils with diameters of 60–70 nm is significantly elevated in the absence of SPARC (Rentz et al. 2007). This size of collagen fibril is proposed to be an intermediate in collagen fibril assembly that serves as a template that is followed by a fiber growth phase. The addition of either rSPARC or SPARC ΔI, αC following the lag phase, coincident with the middle of the growth phase, has no effect on fibrillogenesis in this assay (Giudici et al. 2008). Hence, the capacity of SPARC to influence fibrillogenesis is implicated during early steps in collagen fibril formation.

Basal lamina

Evidence that SPARC also influences basal lamina assembly, perhaps through an interaction with collagen IV, is supported by studies both in invertebrates and in vertebrates. SPARC labeled with green fluorescent protein localizes to basal lamina structures in C. elegans (Fitzgerald and Schwarzbauer 1998). Abrogation of SPARC expression in flies results in a lack of collagen IV in the basal lamina of certain structures in the embryo, notably in the ventral nerve chord, and results in embryonic lethality (Martinek et al. 2008). SPARC mutant flies display defects similar to those of collagen IV mutants suggesting an inter-dependency of these two proteins in embryonic basal lamina formation in flies (Martinek et al. 2008).

The phenotype of premature cataractogenesis in SPARC-null mice indicates that SPARC also contributes to basal lamina assembly in vertebrates (Gilmour et al. 1998). Immunohistochemistry of the basal lamina produced by lens epithelial cells shows that laminin and collagen IV are disorganized in the lens capsule of SPARC-null animals (Yan et al. 2002). Interestingly, whereas the cell membrane of wild-type epithelial cells forms a tight, linear surface adjacent to the basal lamina, SPARC-null epithelial cells appear convoluted, with abundant membrane protrusions extending into the basal lamina (Yan et al. 2002). Immunohistochemistry reveals that these cellular protrusions are β1 integrin-positive. Perhaps SPARC bound to collagen IV diminishes interaction with cell-surface receptors, such as α1β1, so that, in SPARC-null mice, increased receptor engagement tethers collagen IV to cell surfaces interfering with appropriate basal lamina assembly. The collagen IV network in the lens basement membrane is less stabilized by non-covalent and covalent forces in comparison to other basement membranes and therefore might prove more susceptible to alterations in structural integrity in the absence of SPARC (Vanacore et al. 2004). Results generated from SPARC-null lens epithelial cells also reveal an activity of SPARC in the regulation of laminin deposition as its absence results in an increase in amounts of laminin in the lens capsule and an increase in laminin deposited by SPARC-null versus wild-type lens epithelial cells in vitro (Yan et al. 2005). A recent report from Greiling et al. (2009) revealed a disruption in proper circulation of fluids and ions in...
SPARC-null lens that suggests the cellular basis of cataract formation in the absence of SPARC is likely influenced by multiple factors.

**Cellular mechanisms of SPARC in ECM assembly**

Regulation of collagen binding to cell surface receptors

SPARC purified from media conditioned by parietal yolk sac (PYS) cells was the first form of SPARC shown to induce rounding of endothelial cells in culture, and this together with subsequent studies have led to the characterization of SPARC as a counter-adhesive protein (Sage et al. 1989). Exogenous SPARC rounds cells but usually does not lead to cell lifting and subsequent cell death by anoikis, i.e. apoptosis induced by cell detachment. Removal of exogenous SPARC reverses rounding and cells reattach without significant cell death. The counter-adhesive activity of SPARC is consistent with an activity of SPARC that interferes with cell-ECM interactions. Along similar lines, the analysis of collagen interaction with primary dermal fibroblasts reveal an increase in procollagen, intermediates of procollagen processing, namely pC collagen I, pN collagen I, and processed collagen I, in SPARC-null versus wild-type cells (Renz et al. 2007). Given the binding sites of SPARC to collagen, one might predict that SPARC bound to collagen diminishes cell-surface receptor engagement and thus reduces cell adhesion, whereas collagen synthesized in the absence of SPARC readily associates with cell receptors. Alternatively, SPARC has also recently been shown to affect integrin-linked kinase (ILK) activity that is predicted to influence integrin ligation independent of SPARC: collagen interactions (Barker et al. 2005; Nie and Sage 2009). The role of SPARC in the modulation of ILK function and downstream pathways is covered in more detail in the review of SPARC in adipocyte biology by Nie and Sage in this series.

If SPARC functions to limit collagen engagement with cell-surface receptors, then could the phenotypic alterations in ECM assembly, noted in SPARC-null mice, result from an increased interaction of collagen with its receptors? The primary SPARC-binding site on collagen described by Guidici et al. (2008) and further assessed in Hohenester et al. (2008), is coincident with the DDR2-binding site on collagen I. DDR2 was shown to inhibit collagen fibrillogenesis in cultured cells (Mihai et al. 2006; Vogel et al. 2006). Hence, if the absence of SPARC promotes engagement of collagen by DDR2, then collagen fibril formation might be reduced and/or altered. Reductions in collagen concentration and compromised collagen fibril assembly in SPARC-null mice might result from an increase in DDR2 to collagen interactions.

The ligation of collagen-binding integrin receptors, namely $\alpha_1\beta_1$, $\alpha_2\beta_1$, and $\alpha_11\beta_1$, may also be diminished by SPARC binding to collagen (White et al. 2004). In contrast to DDR2, integrin collagen receptors are thought to contribute to the formation and/or stability of collagen fibrils. In vitro, fibronectin assembly precedes and is required for robust collagen fibril assembly (Velling et al. 2002). Collagen-binding integrin receptors enhance fibronectin-dependent collagen fibrillogenesis in culture. For example, in vascular smooth muscle cells, a decrease in collagen fibrils is observed on cells treated with function-blocking anti-$\alpha_2\beta_1$ IgG versus control IgG (Li et al. 2003). Similarly, in mouse embryonic fibroblasts, fibronectin is required for collagen fibrillogenesis while $\alpha_11\beta_1$ and $\alpha_2\beta_1$ are shown to enhance this process (Velling et al. 2002). Therefore, an increase in integrin engagement might be predicted to facilitate collagen fibril assembly and increase collagen concentrations in tissues, a result not in line with the ECM phenotype of SPARC-null tissues.

In addition to its functional contributions to ECM assembly, integrin $\alpha_2\beta_1$ is also known to mediate collagen phagocytosis (Lee et al. 1996). In this case, the lack of SPARC expression is anticipated to increase collagen turnover at the cell surface through elevated uptake of collagen by $\alpha_2\beta_1$ engagement. A plausible scenario might be one in which SPARC, a protein that supports collagen deposition, interferes with receptor interactions on collagen that inhibit fibril formation and/or promote collagen turnover (Fig. 1).

**Regulation of processing of procollagen propeptides**

Whether SPARC acts exclusively in the extracellular space to modulate collagen interactions with cell-surface receptors or acts intracellularly in collagen trafficking and post-translational modification remains to be elucidated. SPARC might influence collagen I production and deposition at a number of different steps. A series of post-translational modifications of collagen I occur in the endoplasmic reticulum, the cis-Golgi, and possibly in trans-Golgi compartments, that produce hydroxylated amino acids, sugar modifications with carbohydrates, trimeric chain assembly, and finally secretion of correctly assembled procollagen I (Prockop and Kivirikko 1995). SPARC is also present in the secretory pathway raising the possibility that intracellular interaction of SPARC with collagen I might influence collagen I modifications and assembly. The hypothesis that SPARC serves as an intracellular collagen chaperone that is important in the latter stages of collagen secretion was put forth by Martinek et al. (2007) based primarily on results from experiments with *Drosophila* and collagen IV. Along these lines, chaperone folding activity is exhibited by SPARC in assays performed *in vitro* (Emerson et al. 2006).
However, at least in dermal fibroblasts, intracellular accumulation of procollagen I is not evident in SPARC-null fibroblasts as would be predicted in the event that SPARC were critical for post-translational modification and/or sorting of procollagens (Rentz et al. 2007). Once secreted, procollagen I is converted to mature collagen I by N-propeptide proteinases, specifically ADAMTS-2 (A Disintegrin And Metalloproteinase with ThromboSpondin motifs), that excises the N-terminal propeptide to generate pC collagen I, and by the C-propeptide proteinase, Bone Morphogenic Protein (BMP)-1, that excises the C-propeptide to produce pN collagen I (Porter et al. 2005; Hopkins et al. 2007). The collagen on primary murine dermal fibroblast cell surfaces is therefore comprised of four different forms: procollagen I, pC collagen I, pN collagen I, and collagen I. In the absence of SPARC, in addition to an increase in total amounts of cell-associated collagen I, processed collagen I (with both propeptides removed) represents a greater proportion of total collagen than in wild-type cells (Rentz et al. 2007). Hence, SPARC appears to serve a function in the regulation of procollagen processing that is generally considered to occur in the extracellular space. Although in embryonic tendon, some intracellular processing of propeptides is observed indicating that, at least in some cell types, procollagen processing can occur in secretory vesicles prior to secretion (Canty and Kadler 2005). The mechanism by which SPARC modulates procollagen processing, whether intra or extracellular, is actively under investigation.

In some cases, propeptides are retained following incorporation into fibrils (Fleischmajer et al. 1988). During development in the skin of chicks, antibodies against the N-propeptide of collagen I were immuno-localized exclusively to the surface of collagen fibrils 20–40 nm in diameter (Fleischmajer et al. 1983). Hence, processing of the propeptides of collagen I represents a possible mechanism in the regulation collagen fibrillogenesis (Holmes et al. 1996). Retention of the propeptides of collagen I (and proteins bound to propeptides) following incorporation into fibrils, for example, could present a steric hindrance to fibril aggregation and thus limit fibril expansion.

Alternatively, some evidence suggests that the N-propeptide acts to enhance collagen I fibril aggregation (Watson et al. 1998). In this case, premature processing of the N-propeptide might limit fibril expansion. In vitro, the absence of SPARC is associated with greater procollagen processing, as judged by propeptide cleavage. In the event that the absence of SPARC in vivo results in a similar increase, perhaps the premature removal of the N-propeptide generates collagen fibrils without N-propeptides that are less efficient in fibril fusion. Given the result that SPARC inhibits collagen fibrillogenesis in vivo, specifically during the lag phase of initiation, one possibility is that SPARC binds nascent monomeric collagen I (and/or procollagen I) and delays its incorporation into nascent fibrils until events such as propeptide processing and/or engagement of proteoglycans (including fibromodulin, lumican, decorin, etc.) occur in a precise spatial and temporal sequence in the pericellular space. In the absence of SPARC expression then, the precise regulation of procollagen maturation to collagen is altered and the efficiency of collagen deposition to the insoluble ECM is compromised.

**SPARC as an anti-fibrotic target or promoter of ECM assembly**

SPARC expression is consistently found coincident with expression of fibrillar collagens and is therefore frequently elevated in adult tissues undergoing wound repair or in tissues with fibrotic disease. Mouse models characterized by renal, cardiac, lung, and dermal fibrosis performed in SPARC-null mice demonstrate reduced collagen deposition versus that in wild-type mice (Strandjord et al. 1999;
Bradshaw et al. 2002; Socha et al. 2007; Bradshaw et al. 2009). Given the significant effects of the loss of SPARC expression on collagen levels in SPARC-null mice, SPARC represents an attractive target for reducing collagen deposition in fibrosis. Scleroderma is a disease characterized by fibrotic deposition of collagen. Targeting SPARC expression by siRNA in fibroblasts from individuals with scleroderma reduces collagen I expression in vitro (Zhou et al. 2006). Furthermore, in a rat model of liver fibrosis, Camino et al. (2008) demonstrated that anti-sense RNA against SPARC, delivered by adenovirus, reduces collagen deposition as measured by histological and by hydroxyproline analysis. These studies suggest that inhibition of SPARC is sufficient to decrease collagen accumulation in tissues and further validate the development of tools that target SPARC activity to alleviate fibrosis in a variety of tissues.

As a reduction in SPARC expression diminishes collagen accumulation in the ECM, an increase in SPARC expression is anticipated to enhance collagen deposition. In heart, myocardial infarction (MI) is associated with rapid synthesis of a collagenous scar to reduce the risk of cardiac rupture. SPARC-null mice are compromised in myocardial collagen formation and exhibit a greater mortality due to cardiac rupture following MI in comparison with wild-type mice (Bradshaw et al. 2009; Schellings et al. 2009). The production of SPARC, delivered by adenoviral expression in SPARC-null animals, improved collagen fiber assembly and reduced the mortality of SPARC-null animals with infarctions to that of wild-type mice (Schellings et al. 2009). Importantly, over-expression of SPARC by adenoviral delivery in wild-type animals subjected to infarction further enhanced collagen assembly in these mice and improved cardiac function (Schellings et al. 2009). Similar results from experiments using injected glioma cells demonstrated increased collagenous ECM production in vivo, that was associated with cells that over-expressed SPARC, in comparison with the use of control cells (Yunker et al. 2008). Taken together, these studies provide evidence that increased expression of SPARC stimulates increased collagen deposition and assembly into the ECM.

The evidence that SPARC influences TGF-β activity has been reported in several different cell types. In epithelial cells and in mesangial cells, SPARC was shown to enhance TGF-β1 activity through an as yet undefined mechanism (Schiemann et al. 2003; Francki et al. 2004). In addition, resident cells of the infarct scar in wild-type mouse hearts with over-expression of SPARC demonstrated increased levels of activated Smad2, a down-stream element in the TGF-β1 signaling cascade (Schellings et al. 2009). Hence, there is concern that long term exposure to elevated levels of SPARC in a therapeutic setting might give rise to unexpected deleterious outcomes associated with increased TGF-β activity. Further characterization of the mechanism(s) by which SPARC modulates TGF-β1 signaling will provide insight in this regard.

Conclusions

In this review, a summary of results that support a function for SPARC as a critical factor in the formation of a collagenous ECM has been presented. In particular, SPARC is shown to play a prominent role in the deposition of fibrotic collagen in adult tissues. As such, analyses to elucidate the functional basis of SPARC activity in the regulation of collagen assembly have the potential to provide new insight into synthesis of the ECM and to suggest ways to control the formation of a pathological ECM. Conversely, in instances in which collagen deposition is required, for example in the synthesis of bioengineered tissues, an increased expression of SPARC can be expected to facilitate and enhance ECM assembly.

SPARC can influence the interaction of collagen with the cell surface possibly through modulation of receptor binding. In addition, purified SPARC inhibits collagen fibrillogenesis in a cell autonomous manner during early stages of fibril initiation in vitro. Thus SPARC has at least two roles in collagen fibril assembly—one that modulates interaction of collagen with the cell surface and another that regulates collagen incorporation to fibrils. Whether similar mechanisms involving the interaction of SPARC with collagen IV regulate basal lamina assembly is currently not known. Much remains to be learned from future studies that will hopefully discover the precise mechanisms that govern SPARC activity in ECM formation. These studies will no doubt bring novel and exciting ideas to the field of collagen and matricellular biology.

Acknowledgements ADB is supported by a Merit Award from the Veteran’s Administration and by P20 2P20RR017696-07 from the NIDCR, NIH.

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