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Role of SPARC in Bone Remodeling and Cancer-Related Bone Metastasis

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ABSTRACT

There is a growing socioeconomic recognition that clinical bone diseases such as bone infections, bone tumors and osteoporotic bone loss mainly associated with ageing, are major issues in today's society. SPARC (secreted protein, acidic and rich in cysteine), a matricellular glycoprotein, may be a promising therapeutic target for preventing or treating bone-related diseases. In fact, SPARC is associated with tissue remodeling, repair, development, cell turnover, bone mineralization and may also participate in growth and progression of tumors, namely cancer-related bone metastasis. Yet, the function of SPARC in such biological processes is poorly understood and controversial. The main objective of this work is to review the current knowledge related to the activity of SPARC in bone remodeling, tumorigenesis, and bone metastasis. Progress in understanding SPARC biology may provide novel strategies for bone regeneration and the development of anti-angiogenic, anti-proliferative, or counter-adhesive treatments specifically against bone metastasis. J. Cell. Biochem. 115: 17–26, 2014.

KEY WORDS: SPARC; BONE REMODELING; BONE METASTASIS; TUMORIGENESIS

SPARC (secreted protein, acidic and rich in cysteine), also termed osteonectin or BM-40, is a major bone matrix non-collagenous protein and a component of the extracellular matrix (ECM) of multiple tumor types. It is a member of a larger family of SPARC-related proteins that modulate cell interaction with the extracellular milieu [Termine et al., 1981]. SPARC is in matricellular class of secreted glycoproteins that exhibit counter-adhesive effects that lead to cell developing round shape and other changes in cell morphology and disruption of cell–matrix interactions [Sweetwyne et al., 2004].

Other members of the SPARC family include testican-1, -2, and -3, tsc 36 (transforming growth factor beta (TGF-β) stimulated clone 36), SPARC-like 1 also known as hevin/SC1 (synaptic cleft 1), Mast9 or ECM2, and SPARC-related modular calcium-binding (SMOC)-1 and -2 [Bradshaw, 2012].

SPARC, as a multifunctional calcium-binding matricellular glycoprotein, participates in tissue remodeling, morphogenesis, and bone mineralization and is secreted by many different types of cells, such as osteoblasts, fibroblasts, endothelial cells, and platelets [Termine et al., 1981; Brekken and Sage, 2000; Alford and Hankenson, 2006].

SPARC is a single-copy gene with a high degree of evolutionary conservation, with a molecular weight of 32.5 kDa that can be divided into three distinct modules as shown in Figure 1.

Module I (NH_2-terminal) contains immune dominant epitopes and binds to hydroxyapatite (HA). The NH_2-terminal domain is an acidic region rich in asparagine (Asp) and glutamate (Glu), which can bind to 5–8 calcium ions, through a different mechanism found in a large family of calcium-binding proteins, the helix-turn-helix structural domain (EF-hand motifs). It is also the region that is the most distinct from other members of the SPARC gene family.

Module II, Cysteine-rich, is homologous to a repeated domain in follistatin (FS). It contains bioactive peptides that exert different

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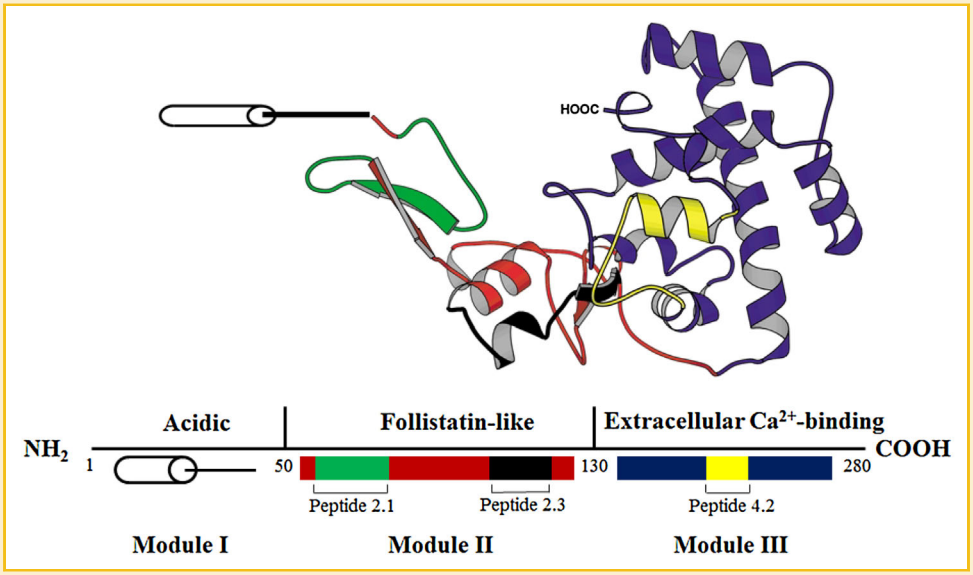

Fig. 1. Structure of human SPARC protein. A ribbon diagram derived from crystallographic data shows the three modular domains of SPARC. The follistatin-like domain, aa 53–137 shown in red (except the peptide 2.1), aa 55–74, and the peptide 2.3 (aa 114–130) shown in green and black, respectively. Module III aa 138–286 is shown in blue (except the aa 255–274, peptide 4.2 shown in yellow). Adapted from Hohenester et al. [1997].

effects on endothelial cells. Peptide 2.1 with an identical structure to epidermal growth factor (EGF)-like S hairpin, inhibits the proliferation of endothelial cells. Peptide FS-E, corresponds to EGF-like module in FS domain of SPARC. It potently inhibits endothelial cell migration in vitro and angiogenesis in vivo in a conformation-dependent manner [Chlenski et al., 2004]. Peptide FS-K has an inhibitory effect on endothelial proliferation. On the contrary, peptide 2.3 has a stimulatory effect on endothelial cell proliferation and angiogenesis [Lane and Sage, 1994]. Additionally, the NH_2-terminal region of module II may bind to heparin or to proteoglycans [Hohenester et al., 1997]. Module III binds to extracellular Ca^{2+} ions through EF-hand motifs. This module contains the peptide 4.2, which stimulates endothelial cells migration but inhibits their proliferation [Kupprion et al., 1998]. The fibril-forming collagen types I, III, and V, and the basement membrane collagen type IV, bind to module III in a Ca^{2+} dependent fashion. Cleavage of SPARC by matrix metalloproteinase 3 (MMP-3) produces a peptide Z-1 containing a Cu^{2+} binding sequence that exhibits a biphasic effect on endothelial cell proliferation and stimulates vascular growth. In contrast, peptides Z-2 and Z-3 inhibit endothelial cells proliferation but stimulate their migration. Different regions of SPARC (designated peptides 1.1–4.2) are presented in Figure 2.

In vitro experiments provided evidence that SPARC:

(a). Has a counter-adhesion effect on cells, since it disrupts cell adhesion to the ECM through its interaction with ECM components such as collagen and vitronectin [Yan and Sage, 1999].
(b). Promotes changes in cell morphology and cell differentiation [Yan and Sage, 1999].
(c). Inhibits cell cycle progression, namely by stalling cells in the G1 phase of cell cycle [Funk and Sage, 1991; Tremble et al., 1993; Yang et al., 2007].
(d). Regulates the activity of growth factors, such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), or vascular endothelial growth factor (VEGF) [Kupprion et al., 1998; Yang et al., 2007].
(e). Regulates ECM and matrix metalloprotease production [Funk and Sage, 1991; Tremble et al., 1993].
(f). Strongly binds to type I collagen and synthetic HA and mediates mineralization of the type I collagen [Termine et al., 1981].
(g). Inhibits adipogenesis and promotes osteoblastogenesis [Nie and Sage, 2009].

In addition to the described functions in vitro, SPARC-null mice are born with no obvious abnormalities, but shortly after birth these mice undergo progressive early-onset cataractogenesis [Anselme and Bigerelle, 2005]. Thus, the SPARC gene is required for lens transparency.

Also SPARC-null mice exhibit an increased accumulation of white adipose tissue (WAT) and show osteopenia. This fact based on in vitro studies could be a consequence of the up-regulation of catenin signaling and altered regulation of collagen expression and deposition [Nie and Sage, 2009]. Interestingly, the overproduction of SPARC by the adipose tissue of obese mice contributes to increased plasminogen activator inhibitor 1 (PAI-1) levels in conditions associated with obesity. SPARC is highly related to body mass index as an autocrine and or/paracrine factor of the adipose tissue that may affect key functions of this tissue and may influence bone metabolism [Tartare-Deckert et al., 2001].

Furthermore, SPARC controls important mechanisms involved in cancer development and progression. These include the regulation of the epithelial-to-mesenchymal transition (EMT), apoptosis, angiogenesis, and also the regulation of the inflammatory response. These mechanisms are relevant in the metastatic dissemination capacity of several cancer cells into bone tissue. However, the function of SPARC

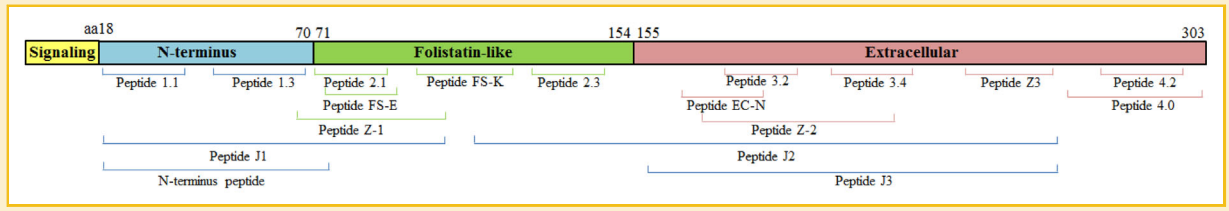

Fig. 2. The regions spanned by different SPARC peptides identified in various studies, adapted from Tai and Tang [2008].

in such phenomena is contradictory. SPARC seems to be a key factor in biological processes, such as bone remodeling, tumorigenesis, and bone metastasis due to all the activities performed by this protein in Figure 3.

This review explores the correlation between SPARC expression/function in bone remodeling and in tumorigenesis, particularly in cancer-related bone metastasis. Ultimately, we aim to contribute towards filling a gap in the literature on the association of SPARC with bone metastasis and encourage further research and progress on novel strategies for bone regeneration and the development of anti-angiogenic, anti-proliferative, or counter-adhesive treatments for metastatic bone tumors.

SPARC AND BONE REMODELING

Bone is a dynamic tissue that combines chemical, cellular, biophysical, and hormonal processes, which undergoes constant turnover. Modeling is a process that sculpts the shape and sizes of bone by the coordinated processes of bone formation and resorption. Modeling process is critical during growth but becomes relatively ineffective after skeleton maturity. Remodeling, on the other hand, is a process by which the skeleton is continuously renewed. It results in the turnover of lamellar bone without causing significant changes in bone quantity, geometry, or size. The purpose of remodeling is to adjust the skeleton to changes in mechanical demands, to prevent accumulation of fatigue damage, to repair microfractures, to ensure the viability of the osteocytes, and to allow the skeleton to participate in the mineral homeostasis [Walsh et al., 2003]. The bone resorption and formation cycle is a highly orchestrated process carried out by a multicellular unit, called the basic multicellular unit (BMU), which comprises osteoclasts and osteoblasts [Fauci and Longo, 2008]. The determinants of the coupling between bone resorption and formation are not known, but they may include the expression pattern of growth factors and/or proteins that vary spatially and temporally. These polypeptides mediate a number of physiological processes, such as immune response, regulation of hormone secretion, growth and cell differentiation, morphogenesis, the regeneration of tissues, as well as the induction and remodeling of bone [Alford and Hankenson, 2006].

SPARC binds to ECM proteins such as types I, III, IV, and V collagen, thrombospondin, PDGF-AB, and PDGF-BB. SPARC binds strongly to type I collagen and synthetic HA and can mediate the in vitro mineralization of type I collagen [Termine et al., 1981]. It appears that SPARC has a role in connecting collagen fibers to HA crystals by a terminal sequence rich in amino acids. Attachment to collagen, however, has been reported both to promote and inhibit HA formation [Termine et al., 1981; Romberg et al., 1985; Romberg et al., 1986; Doi et al., 1989]. Infrared analysis of the mineral and matrix in bones of SPARC-null mice revealed a decreased number of bone cells, leading to decreased bone formation and resorption, that could hinder the degradation and replacement of mature collagen, thereby maintaining collagen crosslinks [Boskey et al., 2003]. Furthermore, a polyclonal anti-SPARC antibody did not affect on mineralization thus, as previously suggested, SPARC may be more important for regulating matrix formation than mineralization [Boskey et al., 2008]. Crystal structure analysis and site-directed mutagenesis within module III revealed that five residues R149, N156, L242, M245, and E246 are required for collagen binding. In addition, SPARC recognized the hydrophobic GVMGFO motif in collagen [Hohenester et al., 2008]. The conformational change that occurred in SPARC during collagen binding created a deep specificity pocket that was bound to the phenylalanine side chain of the GVMGFO motif. Yet, the functional importance of these structural alterations is still not understood. On the other hand, post-translational modification of SPARC may be controlled in a tissue specific manner and potentially associated with functions of SPARC. Several reports indicated that SPARC participated in the regulation of collagen fibril assembly [Bradshaw et al., 2003; Wang et al., 2005]. More recently, it was shown that wild type matrices had thick collagen fibers organized into longitudinal bundles, whereas SPARC-null matrices had thinner fibers in random networks [Kapinas et al., 2012]. Rentz et al. [2007] speculated that SPARC influenced procollagen processing by modulating integrin engagement and processes that affected collagen deposition and also improved matrix assembly. The functional significance of SPARC interaction with collagens in tissues is not clear. Collagen may serve as a storage site for SPARC in the ECM or might directly modulate the activity of SPARC. Interestingly enough, bone and platelet SPARCs have patterns of glycosylation that appeared to affect collagen-binding activity. Specifically, bone SPARC binds to types I, III, and V collagen and platelet SPARC has no apparent affinity for them [Kelm and Mann, 1991].

Some applications for SPARC have been explored in the development of advanced composite biomaterials for skeletal tissue regeneration. One example concerned the production of nano-hydroxyapatite/collagen/SPARC composites for bone graft applications [Liao et al., 2009]. Others studies have used a glutamic acid-rich peptide derived from SPARC, functionalized with an acrylate group for covalent attachment to the matrix that significantly increased the shear modulus of a bone-mimetic hydrogel/apatite nanocomposite

Fig. 3. SPARC is a key protein in bone remodeling, tumorigenesis, and bone metastasis and its activities associated to these biological processes are respectively indicated.

and improved the dispersion of apatite nanoparticles in aqueous solution [Sarvestani et al., 2008].

SPARC ACTIVITY IN CANCER BIOLOGY

SPARC contributes to the disruption of cell adhesion to ECM by promoting morphologic changes in cell shape. SPARC also reduces the activity of several growth factors, including PDGF, VEGF, and bFGF. In addition, its ability to regulate matrix remodeling via metalloproteinases, together with its ability to inhibit G1 to S-phase cell cycle progression in primary cells, suggests that SPARC might participate directly in tumor progression suppression [Funk and Sage, 1991; Tremble et al., 1993]. SPARC involvement with different tumors is reported to be contextual and attributed to a given microenvironment. Different expression patterns and activities of SPARC are depending on cancer type and upon whether it is expressed by malignant cells themselves or by neighboring stromal cells [Tai and Tang, 2008].

SPARC expression is associated with a favorable prognosis in some studies on human prostate cancer [Welsh et al., 2001; Lapointe et al., 2004; Wong et al., 2007]. In these studies SPARC may indeed function as a tumor suppressor since down-regulation and inactivation of SPARC gene expression enhanced aggressive and metastatic behavior. On the other hand, SPARC can be described as a protumorigenic and prometastatic protein as found in studies of colorectal cancer [Porte et al., 1995]. Moreover, high SPARC expression might have utility as a prognostic marker in human breast cancer [Graham et al., 1997; Lakhani et al., 2005]. However, SPARC has been associated with metastasis of prostate cancer, as

high levels of SPARC were found at sites of bone metastasis [Thomas et al., 2000]. Therefore the specific contribution of SPARC in tumor growth and progression is not clear [Arnold and Brekken, 2009]. Understanding of the mechanisms mediating SPARC's functions in each different cancer-associated process may clarify how this complex multifunctional protein functions in cancer.

One of the important mechanisms involved in cancer development and progression is apoptosis. In fact, defects in apoptotic pathways are now thought to contribute to tumor initiation, progression, and metastasis. SPARC-mediated apoptosis occurs by activating the expression of several members of extrinsic pathways of apoptosis such as caspase 3, caspase 8, caspase 10, and Fas-associated protein with death domain (FADD). SPARC induced apoptosis in ovarian carcinoma cells and enhanced the chemosensitivity of colorectal cancer cells when exposed to chemotherapy either alone or in combination with vitamin D [Yiu et al., 2001; Said and Motamed, 2005; Tai et al., 2005; Taghizadeh et al., 2007; Tang and Tai, 2007]. In contrast, the expression and activity of SPARC in human brain tumors promoted tumor invasion by reducing apoptosis and caspase activity of glioma cells through protein kinase B activation [Vajkoczy et al., 2000; Schultz et al., 2002; Shi et al., 2004].

In tumorigenesis, an invasive and metastatic phenotype is often acquired via induction of an epithelial-mesenchymal transition (EMT) in which epithelial cells lose their polarity and develop a mesenchymal phenotype. This process is characterized by the loss of intercellular adhesion (E- to N-cadherin switch), down-regulation of epithelial markers (cytokeratins), up-regulation of mesenchymal markers (vimentin), and the acquisition of a fibroblast-like motile and invasive phenotype. The transcription factor Snail and other members of its family have been implicated in the promotion of EMT [Cano et al., 2000; Moreno-Bueno et al., 2006]. SPARC intervenes at several stages of EMT, thus contributing to malignant phenotype. For example, expression of SPARC in melanoma cells suppresses E-cadherin and increases N-cadherin and vimentin expression through phosphorylation of focal adhesion kinase (FAK) and/or induction of Snail with the subsequent enhancement of cell migration and invasive capacity [Robert et al., 2006; Smit et al., 2007; Sosa et al., 2007]. SPARC might be involved in a collagen-mediated EMT induction, since it induces collagen expression [Brekken et al., 2003; Prada et al., 2007; Sosa et al., 2007]. Moreover, SPARC was shown to modulate cell survival and invasion of glioma cells through the activation of FAK and integrin-linked kinase (ILK) [Shi et al., 2007].

Another cancer-associated process is inflammation and SPARC likely plays a central role in this process, since its expression by malignant or stromal cells modulates the activity of growth factors and the capacity of inflammatory cells to infiltrate the tumor microenvironment. The suppression of SPARC expression in melanoma cells induced polymorphonuclear leukocyte (PMN) recruitment and inhibited tumor growth through a mechanism that involved the release of chemotactic factors such as interleukin 8 (IL-8) and leukotrienes by inflammatory cells [Ledda et al., 1997; Alvarez et al., 2005].

SPARC has been implicated in angiogenesis, a process of neovascularization that is critical to the survival of tumors. In endothelial cells, SPARC is capable of inhibiting the activity of angiogenic growth factors VEGF, PDGF, and basic fibroblast growth factor (bFGF) [Kupprion et al., 1998]. Furthermore, in animal models of ovarian cancer, the absence of SPARC resulted in high expression of VEGF, VEGFR2, MMP-2, and MMP-9, thereby promoting the angiogenic and metastatic potential of these cancers [Said and Motamed, 2005; Said et al., 2007]. In neuroblastomas SPARC can function as an anti-angiogenic factor produced by Schwann cells being its expression inversely correlated with tumor progression [Chlenski et al., 2002; Nie and Sage, 2009]. 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. For instance, peptides that include the KGHK-Cu^{2+} motif, stimulated endothelial cell cycle and angiogenesis in vivo [Sage et al., 2003]. On the contrary, FS-E peptide, inhibited angiogenesis associated with neuroblastoma, even in the presence of bFGF-stimulation [Chlenski et al., 2004, 2010].

SPARC AND CANCER-RELATED BONE METASTASIS

During tumor progression, malignant cells, initiate a process of attachment and subsequent degradation of nearby stroma, leading finally to the establishment of metastatic *foci* in specific tissues, such as lung, bone, liver, or brain [Liotta and Kohn, 2001]. This contributes to the success of the tumor and consequently to poorer prognosis for patients.

Bone metastases result when cancer cells spread from their site of origin (primary tumor) and settle in a bone to form a secondary cancer. This can affect only one area of the bone or several areas at any one time; complications of bone metastases include pain, increased risk of facture, hypercalcemia (abnormally high levels of calcium in the blood), and a decreased blood cell count. The most common cancer types that show tendency to metastasize in bone include prostate, breast, lung, kidney, thyroid cancer, and multiple myeloma. One of the consequences of bone metastases results from a decrease of osteoblast number. Having reached the bone, malignant cells disrupt the remodeling process that normally occurs. Osteoclast number increase as tumor cells secrete factors such as parathyroid hormone related protein (PTHrP) that stimulates bone resorption and therefore gradually destroy it. This in turn results in the release of breakdown products such as TGF-β which stimulates the growth of malignant cells, thus perpetuating the destructive cycle and enhancing localized tumor growth. Recently improved understanding of these biochemical processes has prompted investigation into whether skeletal events in patients with malignant bone disease may correlate with levels of serum and urine markers of bone turnover, thus facilitating earlier detection or screening for such events. More than 90% of all metastases are found in the back, pelvis, upper leg, ribs, upper arm, and skull. The prognosis of cancers that metastasize to bone is in general very poor and the treatment for bone metastases tend to minimize the symptoms by reducing pain and the risk of fracture. The prevention and the development of therapeutic strategies against metastatic bone tumors lie in understanding the malignant cells preference in certain cancer types (prostate, breast, lung, kidney, thyroid cancer, or multiple myeloma) to metastasize in

bone tissue. Bone microenvironment may provide growth stimulating factors or others proteins that induce proliferation and angiogenesis of cancer cells allowing them to arrest the bone tissue. SPARC has been shown by several studies to be a key protein that attracts prostate cancer cells to bone microenvironment [Jacob et al., 1999; De et al., 2003; Donahue, 2004]. One study showed that prostate cancer cells preferably migrated towards wild type bone extracts when compared to extracts obtained from SPARC null mice. This effect was reversed by restoration of SPARC [De et al., 2003]. The up-regulation of VEGF production by SPARC via αvβ3 and αvβ5 is a prostate cancer specific phenomenon, providing prostate cancer cells with significant growth advantage in bone [Donahue, 2004]. Other work indicates that p45-sErbB3 (a soluble form of ErbB3, pooled in bone marrow supernatant samples from men with prostate cancer that had metastasized to bone) enhances the invasiveness of prostate cancer cells in part by stimulating the secretion of SPARC by bone. Thus p45-sErbB3 may mediate the bidirectional interactions between prostate cancer cells and bone [Chen et al., 2007].

The development and progression of bone metastatic prostate cancer using SPARC-deficient mice infected with RM1 mouse prostate cancer cells showed that bone stromal SPARC inhibited prostate cancer expansion in bone through the regulation of osteoclast maturation and function [McCabe et al., 2011]. Another report, by Podgorski et al. [2009], suggested that cathepsin K modulates the biological activity of SPARC in prostate cancer bone metastasis by cleaving it. In two tumor bone metastases cell lines (derived from clinical, PC3, and experimental MDA-231BO) enzymatic processing of SPARC was reduced by inhibition of cathepsin K. Moreover the presence of a cathepsin inhibitor reduces the GRO (growth-regulated oncogene) secretion, a pro-inflammatory and chemotactic factor regulated by SPARC. On the other hand, SPARC and cathepsin K overexpression and secretion raised GRO secretion [Podgorski et al., 2009].

In a recent study the effect of bone matrix SPARC on PC3 behavior was assessed by using murine osteoblast to create normal and SPARC-null bone matrix in vitro. The results of this study showed that when PC3 cells were grown on the wild type matrices, they presented decreased cell proliferation, increased cell spreading, and decreased resistance to radiation-induced cell death, compared to cells grown on SPARC null-matrix [Kapinas et al., 2012]. DeRosa et al. [2012] recently showed that SPARC gene was highlighted as a potential early marker of poorly differentiated phenotype of prostate cancers and the high SPARC expression at the time of prostatectomy was associated with the development of metastasis.

Based on the two separate transgenic models of prostate and breast cancer (transgenic adenocarcinoma of the mouse prostate and murine mammary tumor virus polyomamiddle T, respectively), using $SPARC^{-/-}$ and $SPARC^{+/-}$ mice, found that loss of SPARC had no significant impact on tumorigenesis [Wong et al., 2008]. Although the loss of SPARC, by itself, neither directly promoted nor inhibited spontaneous prostate or breast cancer progression, SPARC expression could be used as a potential prognostic biomarker of tumor severity and/or aggressiveness.

SPARC might have an indirect effect on breast cancer cell metastasis in bone since its isolation from several sources such as osteoblasts or epithelial cells stimulated motility of human breast cancer cells and also enhanced the chemoattraction of breast cancer cells toward vitronectin (a known chemoattractant protein) [McKnight et al., 2006]. In another study a breast cancer cell line (MDA-231) deficient of SPARC was used in order to determine the endogenous effect of SPARC expression on invasion and metastasis of the breast malignant cells in bone. The induction of SPARC expression in MDA-231 cells did not affect the proliferation, apoptosis, aggregation, or cell migration, but inhibited tumor cell invasion in vitro. Moreover, high expression of SPARC inhibited metastasis to different organs including lung and bone. Exogenous SPARC inhibited the platelet aggregation in vitro and the high expression of this protein in MDA-231 cells reduced tumor cell-induced thrombocytopenia in vivo in relation with control. In conclusion, a high endogenous SPARC expression seems to inhibit MDA-231 breast cancer metastasis by reducing the invasion activity and tumor cell-platelet aggregation [Koblinski et al., 2005].

As already mentioned, the survival rate in the case of patients with bone metastasis is very low. The understanding of cancer metastasis to bone should help the prevention of metastasis and the establishment of a valid therapy in order to improve the patient's life quality and may increase survival rates. It has been described that SPARC is associated to tumorigenicity and metastasis of cancer-related bone metastasis like lung or melanoma cancers [Kato et al., 2000; Zhou et al., 2010]. Nevertheless, there are only few studies regarding prostate and breast cancers that tried to disclose the roles of SPARC in bone metastasis (Table I), and none associated with other cancer types that show a tendency to develop bone metastasis.

CONCLUSIONS AND PERSPECTIVES

Considerable attempts have been made to produce adequate matrices or scaffolds that mimic bone ECM for applications in tissue engineering and regenerative medicine. In this context, several factors must be considered, such as the modification of biomaterial surfaces using growth factors, living bone cells or proteins, to guide cellular responses in bone remodeling, like osteoblast adhesion and long-term functionality expressed as proliferation, synthesis of alkaline phosphatase, and deposition of calcium containing mineral [Manuel et al., 2003]. SPARC, a matricellular glycoprotein associated with tissue remodeling, repair, development, cell turnover, is involved in bone formation, bone initiating mineralization process, and collagen fibril assembly [Termine et al., 1981; Doi et al., 1989; Bradshaw et al., 2003; Wang et al., 2005; Kapinas et al., 2012]. The application of this protein could benefit the development of new valid therapeutic strategies for skeletal tissue regeneration. In addition, the research in this topic is essential since there are very few works involving SPARC and biomaterials for bone tissue regeneration [Sarvestani et al., 2008; Liao et al., 2009].

Furthermore, SPARC controls important mechanisms involved in cancer development and progression including the regulation of epithelial-to-mesenchymal transition, apoptosis, angiogenesis, and also the regulation of the inflammatory response. SPARC is relevant in metastatic dissemination capacity of prostate, breast, lung, kidney, thyroid cancer, and multiple myeloma cancer cells into bone tissue.

TABLE I. Studies Related to SPARC Activity in Bone Metastasis

Tumor type	Expression	Experimental approach	Activity	Refs.
Prostate	High levels of SPARC at sites of bone metastasis	Human prostate cancer cell lines LNCaP, LNCaP-C4–2, PC3, and lacZ-transfected CWR22R (H-clones) SPARC-null mouse model	Attracts prostate cancer cells to bone; Increases VEGF production by metastatic cancer cells and integrin activation	De et al. [2003]
	High expression of SPARC in metastatic prostate cancer	Transcriptomes of laser capture-micro-dissected tumor cells with well- and poorly differentiated (PD) phenotype from primary prostate tumors of patients with 78 months of mean follow-up after radical prostatectomy.	High SPARC expression at the time of radical prostatectomy is associated with an increased risk of tumor metastasis. SPARC gene was identified as a potential early marker of less favorable outcome associated with PD of prostate cancers.	DeRosa et al. [2012]
	SPARC expression is increased in prostate cancer metastases	In vitro system composed by PC3 and mineralized matrices synthetized by wild type and SPARC-null osteoblasts.	Bone matrix-associated SPARC attenuated the growth of PC3, increased cell spreading, and increased their sensitivity to ionization radiation.	Kapinas et al. [2012]
	A low glycosylated SPARC is highly abundant in bone	In vitro studies using human prostate cancer cell lines (DU-145 and PC-3), several human prostate epithelial cell lines as well as a HT1080 fibrosarcoma cell line and a B16-F10 mouse melanoma cell and extracts from various organs of mice and rat	Enhances the invasion and migration by prostate cancer cells; Chemoattractant for bone-metastasizing epithelial cells; Enhances matrix metalloprotease activity in prostate cancer cells	Jacob et al. [1999]
	p45-sErbB3 up-regulated the expression of SPARC	Human prostate cancer cell lines (LNCaP and PC-3)	Enhances the invasiveness of the prostate cancer cell lines PC-3 and C4-2B	Chen et al. [2007]
	Higher SPARC expression in bone metastasis compared to primary tumor	SPARC deficient mice infected with SPARC-expressing syngeneic RM1 mouse prostate cancer cells	Inhibits prostate cancer expansion in bone through the regulation of osteoclast maturation and function	McCabe et al. [2011]
Breast		In vitro MDA-231 breast carcinoma cell line study applying SPARC derived from several sources (MDA-MB-435, MDA-MB-468), osteoblasts (hFOB1.19), non-neoplastic breast epithelial (hTERT-HME1), and vascular endothelial cells isolated from a bone biopsy (HBME-1)	Enhances breast cancer cells chemoattraction toward vitronectin	McKnight et al. [2006]
	High SPARC expression in MDA-231 cells	In vitro human cell line study using SPARC-negative MDA-231 breast carcinoma cell line infected with an adenovirus expressing SPARC; In vivo nude mouse model	No effect on MDA-231 cell proliferation, apoptosis, cell aggregation, or migration; Inhibits breast cancer metastasis by reducing the invasion activity and tumor cell platelet aggregation in vitro; Reduces tumor cell-induced thrombocytopenia in vivo compared with control-infected cells	Koblinski et al. [2005]
Prostate/breast	SPARC is significantly down-regulated in highly metastatic human prostate cancer cells	$SPARC^{+/-}$ and $SPARC^{-/-}$ mice using two separate transgenic mouse tumor models: transgenic adenocarcinoma of the mouse prostate (TRAMP) and murine mammary tumor virus polyoma middle T (MMTV-PyMT)	No effect on prostate or breast cancer with the mouse tumor models tested Useful biomarker of aggressive, metastasis-prone tumors	Wong et al. [2008]
	Up-regulation of SPARC both in vivo in experimental prostate bone tumors, and in vitro in co-cultures of bone marrow stromal cells with PC3 prostate carcinoma cells	In vitro co-cultures of bone marrow stromal cells with prostate (PC3) and breast carcinoma cells (MDA-231BO) Severe combined immunodeficient (SCID) mice human/intrabone model	Bone marrow cathepsin K regulates the biological activity of SPARC in prostate cancer bone metastasis	Podgorski et al. [2009]

Yet, the actual function of SPARC in tumorigenesis and tumor progression is still contradictory and not fully understood. There is not any review article addressing SPARC and cancer-related bone metastasis. Depending on cancer type, different expression patterns and activities of SPARC may be found. This could be explained by the distinct tumor microenvironment established in different types of cancers that translates in terms of local composition of matrix molecules and cytokines and the protease profile. The different proteolytic products (peptide fragments) corresponding to different regions of SPARC have distinct activities and may explain the divergent and inconsistent biological activities observed with native full-size SPARC protein in distinct malignancies. SPARC peptide

models could be a valid strategy to understand SPARC's specific action in mechanisms that occur in tumor invasion and metastasis in bone tissue. Very few attempts, including SPARC peptides combined with chemotherapy and/or drugs, have been performed in this direction up to now [Chlenski et al., 2004, 2010; Gradishar et al., 2005; Von Hoff et al., 2008; Inoue et al., 2010].

Furthermore, the differential function of SPARC in several types of cancers might be dependent upon whether it is expressed by the malignant cells themselves or by neighboring stromal cells. A series of studies have been performed in an attempt to elucidate the actual role of SPARC produced by non-malignant stromal cells [Brekken et al., 2003; Sangaletti et al., 2003; Haber et al., 2008]. SPARC knock-out mice showed low turnover osteopenia [Nie and Sage, 2009], intensive osteoclastogenesis [McCabe et al., 2011], and matrices composed by thinner collagen fibers in random networks [Kapinas et al., 2012] that translated a less stroma and collagen deposition. It was proposed that SPARC is a critical component in the orchestration of the tissue microenvironment, important for metastatic cancer cells to grow and survive in the skeleton (skeletal cancer metastasis). In fact, the expression of many bone-enriched proteins, including SPARC by stromal cells in normal prostate and the up-regulation of VEGF production by SPARC being a prostate cancer specific phenomenon, contributes to the preference and significant growth advantage in bone-like environment by prostate cancer cells. Also the association between SPARC expression pattern and malignancy of prostate and breast cancers may contribute to the use of SPARC as a potential prognostic biomarker of tumor severity and/or metastasis [Wong et al., 2008; DeRosa et al., 2012].

According to the works related to bone metastasis and presented in Table I, SPARC acts as protumorigenic and prometastatic protein, when expressed by stromal cells, trough enhancement of metalloprotease activity, VEGF production, or chemoattraction toward vitronectin [Jacob et al., 1999; De et al., 2003; McKnight et al., 2006; Chen et al., 2007; DeRosa et al., 2012]. On the contrary, when SPARC is produced by malignant cells, it inhibits cancer expansion through regulation of osteoclast maturation/function or by reducing platelet aggregation. The final outcome of SPARC function will undoubtedly be highly context dependent [Framson and Sage, 2004]. The development of SPARC-peptide models, conditional/gene inactivation models [Ledda et al., 1997; Briggs et al., 2002; Smit et al., 2007] or the transcriptional targeting using SPARC promoter [Sato et al., 2003; Suzuki et al., 2005; Kelly et al., 2006; Lopez et al., 2006; Yang et al., 2007; Cheetham et al., 2008] could be a valid strategy to understand how SPARC influences tumor invasion and metastasis and may lead to the development of anti-angiogenic, proliferation or counter-adhesive therapeutic treatment against metastatic bone tumors.

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