RESEARCH ARTICLE

Clinicopathological Significance of Reduced SPARCL1 Expression in Human Breast Cancer

Fang Cao[1&], Kuo Wang[1&], Rong Zhu[1], Yong-Wei Hu[1], Wen-Zheng Fang[2], Hou-Zhong Ding[1*]

Abstract

Secreted protein acidic and rich in cysteines-like protein 1 (SPARCL1), an extracellular matrix glycoprotein, has been implicated in the pathogenesis of several disorders including cancer. However, little is known about the expression and significance of SPARCL1 in human breast cancer. The aim of this study was to determine the expression pattern and clinicopathological significance of SPARCL1 in a Chinese breast cancer cohort. mRNA and protein expression of SPARCL1 in human breast cancer cell lines and breast cancer tissues was detected using the reverse transcription-polymerase chain reaction, real-time quantitative PCR, and Western blotting, respectively. Immunostaining of SPARCL1 in 282 Chinese breast cancer samples was examined and associations with clinicopathological parameters were analyzed. Compared to the positive expression in immortalized human breast epithelial cells, SPARCL1 was nearly absent in human breast cancer cell lines. Similarly, a significantly reduced expression of SPARCL1 was observed in human breast cancer tissues compared to that in normal breast epithelial tissues, for both mRNA and protein levels ($P < 0.001$). Immunohistochemical analysis showed that strong cytoplasmic immunostaining of SPARCL1 was observed in almost all normal breast samples (43/45) while moderate and strong immunostaining of SPARCL1 was only detected in 191 of 282 (67.7%) breast cancer cases. Moreover, down-regulation of SPARCL1 was significantly correlated with lymphatic metastasis ($P = 0.020$) and poor grade ($P = 0.044$). In conclusion, SPARCL1 may be involved in the breast tumorigenesis and serve as a promising target for therapy of breast cancer.

Keywords: Breast cancer - SPARCL1 - RT-PCR - immunohistochemistry

Asian Pacific J Cancer Prev, **14 (1)**, 195-200

Introduction

Breast cancer is by far the most frequent cancer among women which presents one of the dominating burdens of diseases for both developed and developing countries (Long et al., 2010). Annually about 1.3 million women were estimated to be diagnosed with breast cancer all over the world, and more than 450,000 people die of the terrible disease, accounting for 14% of the total cancer-related deaths. Although China is usually regarded as a traditional low risk area, the incidence of breast cancer has obviously increased with the rapid development of soci-economical conditions (Jemal et al., 2011; Eheman et al., 2012; Shen et al., 2012). In the past few decades, great efforts have been made to improve the outcome of breast cancer patients. Multidisciplinary approaches such as radical resection, radiotherapy, chemotherapy, hormone therapy, and targeted therapy, have been performed and remarkable improvement of survival for breast cancer patients has been achieved (Hulvat et al., 2009). However, approximately 30% of breast cancer patients still suffer from recurrence and even metastasis, indicating an unfavorable prognosis (Kataja and Castiglione, 2008). A fuller understanding of the extract molecular pathogenesis of breast cancer would greatly contribute to advances in prevention and treatment of the disease.

Secreted protein acidic and rich in cysteines-like protein 1 (SPARCL1), also named as SC1, magnet-assisted subtraction technique 9 (MAST9) or high endothelial venule protein (Hevin), is one of the ten members of SPARC-related family (Sullivan and Sage, 2004). SPARCL1 is localized to human chromosome 4q22-25 and its cDNA open reading frame includes 1992 nucleotides, encoding 664 amino acids (Isler et al., 2001). As a secreted extracellular matrix glycoprotein, SPARCL1 was discovered first in 1995 and cloned from the high endothelial venule (HEV) endothelial cells in human tonsil lymphatic tissue (Girard and Springer, 1995). The high endothelial venules are special round post-capillary venules as opposed to the flat endothelial

*[1]Department of Surgery, the Affiliated Kunshan First People's Hospital, Jiangsu University, Kunshan, [2]Department of Oncology Fuzhou General Hospital of Nanjing Command, Fuzhou, China [&]Equal contributors *For correspondence: dinghouzhong@hotmail.com*

cells found in regular venules, and enable high levels of lymphocytes in the blood to enter the lymphatic system. SPARCL1 participates in many physiological functions such as cell proliferation and muscle differentiation, and can promote lymphocyte transportation through adjusting the endothelial cell adhesion function (Girard and Springer, 1996; Claeskens et al., 2000). SPARCL1 is expressed in many tissues and organs such as heart, lung, brain, bone, muscle, colon and lymphatic gland, but it has a lower expression in pancreas, spleen, thyroid gland and placental tissue and even SPARCL1 is not expressed in liver, kidney and peripheral blood leucocytes (Girard and Springer, 1995; Hambrock et al., 2003). Recently, a few of studies have reported that SPARCL1 was down-regulated in a wide variety of human malignancies including lung cancer (Bendik et al., 1998; Isler et al., 2004), prostate cancer (Nelson et al., 1998; Hurley et al., 2012), pancreatic cancer (Esposito et al., 2007), and gastric cancer (Li et al., 2012), and might play a role as a tumor suppressor gene (Claeskens et al., 2000). However, little is known about the expression and significance of SPARCL1 in human breast cancer. The aim of this study was to determine the expression pattern and clinicopathological significance of SPARCL1 in a Chinese breast cancer cohort.

Materials and Methods

Cell lines and cell culture

Human immortalized mammary epithelial cell line (MCF-10A) and human breast cancer cell lines (MCF-7, MDA-MB-231 and MDA-MB-453) were obtained from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, China. These cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; GIBCO) at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. Cells were seeded at 1×10^6 cells per well in 6-well plates to extract total RNA and protein for reverse transcription polymerase chain reaction (RT-PCR) and western blot analysis.

Patients and tissue specimens

A total of 282 Chinese breast cancer patients who underwent radical mastectomy from 2007 to 2011 were enrolled in the present study. The cohort was composed of patients from the Second People's Hospital of Kunshan (n = 199) and the Chinese Medicine Hospital of Kunshan (n = 83), Jiangsu, China. The requisite clinicopathological information, e.g. age at diagnosis, tumor location, tumor size, histological grade, nodal status, and distant metastasis, were collected by reviewing the patients' medical records. Additionally, 32 pairs of fresh breast tumors and corresponding adjacent normal tissues were obtained from the Affiliated Kunshan First People's Hospital, Jiangsu University, China, immediately snap frozen in liquid nitrogen and stored at -70°C until total RNA and protein extraction. All patients had not received any anticancer therapy before surgery. The tissue samples were obtained with patient informed consent, and the study was approved by Institutional Review Board on Human Research at Jiangsu University.

RNA extraction and RT-PCR

Total RNA was isolated from the cultured cells and fresh-frozen breast cancerous and matched non-cancerous tissues by using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the protocol of the manufacturer. The concentration and purity of the isolated RNA were measured by the optical densities at 260 nm and 280 nm using a spectrophotometer. Then the RNA was reversely transcribed into cDNA using a PrimeScript™ RT Reagent Kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. PCR amplification was performed using a TaKaRa PCR Amplification Kit. The PCR reaction was initially denatured at 94°C for 30 sec, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and elongation at 72°C for 90 sec, and a final extension at 72°C for 10 min. The amplified PCR products were electrophoresed by 1.5% agarose gel and visualized by ethidium bromide staining. The primers used for detection of SPARCL1 mRNA were 5'-CAACTGCTGAAACGGTAGCA-3' (sense) and 5'-GAACTCTTGCCCTGTTCTGC-3' (antisense), and for β-actin the primes were 5'-GCTGTCACCTTCACCGTTC-3' (sense) and 5'-CCATCGTCCACCGCAAAT-3' (antisense). The expression of SPARCL1 mRNA was normalized to β-actin expression which served as an endogenous control.

Real-time quantitative PCR

Real-time quantitative PCR was performed with the LightCycler thermal cycling system (Roche Diagnostics Corp., Indianapolis, IN, USA) using a SYBR® Premix Ex Taq™ II kit (TaKaRa) as described by the manufacturer. A total reaction volume of 20 μl contained 2 μl of cDNA template, 10 μl of 2×SYBR® Premix Ex Taq™ II, 0.8 μl primers of 10 μmol/L each, and 6.4 μl ddH2O. Negative controls included water instead of cDNA in the PCR reaction and addition of RNA instead of cDNA, and β-actin was used as an endogenous control. The primer sequences were as same as which used in RT-PCR mentioned above and were synthesized by Sangon Biotech Co. Ltd., Shanghai, China. The amplification was run at 95°C for 5 min followed by 40 cycles of 95°C for 15 sec and 60°C for 30 sec. All samples were run in triplicate, and the qPCR data were analyzed by using the Roche Molecular Biochemicals LightCycler software (version 3.5). The specificity of amplification reaction was confirmed by analyzing the corresponding dissociation curves. The quantification of SPARCL1 was normalized to β-actin expression using the $2^{-\Delta\Delta Ct}$ method.

Protein extraction and western blot

Total cellular protein was extracted from the cultured cells and frozen human mammary tissues with a RIPA Lysis Buffer (Beyotime, China) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) for 15 min, followed by centrifugation at 20000 g for 15 min at 4°C. The concentrations of the protein samples were determined by using a BCA protein assay kit (Pierce,

Rockford, IL) according to manufacturer's protocol. The protein samples were mixed with loading buffer, boiled for 10 min, and then resolved in 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred to PVDF membranes (Millipore, Bedford, MA), and blocked with 5% (w/v) non-fat dry milk in 20 mmol/L of Tris-buffered saline with 0.1% Tween (TBS-T) for 1 h at room temperature and then probed with goat anti-human SPARCL1 polyclonal antibody (AF2728, R&D Systems, Inc.; dilution, 1:2000) in 5% milk/TBST at 4°C overnight. Then the membranes were washed with TBS-T and incubated with the horseradish peroxidase-conjugated anti-goat secondary antibody (Jackson ImmunoResearch, Laboratories, Inc, West Grove, PA) at a dilution of 1:3000 for 1 h at room temperature. The membranes were washed extensively with TBS-T and the signals were detected by using an Enhanced Chemiluminescence Detection System (Beyotime, China). Imaging was performed with a ChemiDoc™ XRS+ System (BIO-RAD) and the bands were quantified using the Image Lab™ Software (Version 2.0, BIO-RAD). β-actin was used as a loading control for normalization.

Tissue microarray construction and immunohistochemistry

Tissue microarrays were constructed from the paraffin-embedded tissue blocks using a tissue arrayer (Beecher Instruments, Silver Spring, MD) according to the previous description (Kononen et al., 1998; Kallioniemi et al., 2001). For the patient cohort included in the present study, 282 cancerous cylinders and 45 matched non-cancerous cylinders with a diameter of 1.5 mm were arrayed and consecutive 4 μm sections were cut. Immunohistochemistry assay for SPARCL1 expression was performed using an UltraSensitive™ SP kit (#9709, Maixin, Fuzhou, China) according to the manufacturer's instructions. Briefly, the microarray sections were deparaffinized in xylene, rehydrated with graded ethanol, and subjected to microwave antigen retrieval in citrate buffer (pH 6.0). The sections were subsequently blocked for endogenous peroxide activity with 3% hydrogen peroxide, treated with preimmune goat serum to block nonspecific binding sites, and then incubated with the primary goat polyclonal antibody against human SPARCL1 (AF2728, R&D Systems; dilution 1:100) at 4ºC overnight. The sections were washed and incubated with a secondary biotinylated anti-goat antibody. The immunostaining was visualized with a diaminobenzidine detection kit (DAB-0031, Maixin) and then the sections were counterstained with hematoxylin, dehydrated, cleared, and coverslipped. Negative control of SPARCL1 staining was conducted by omitting the primary antibody.

Evaluation of immunostaining

The stained sections were viewed by two individuals independently using an Olympus CX31 microscope (Olympus, Japan) without knowledge of the clinicopathological information and each other's findings. Sections were considered positive for SPARCL1 staining when more than 30% of tumor cells were stained moderately or strongly in the cell cytoplasm (Li et al., 2012).

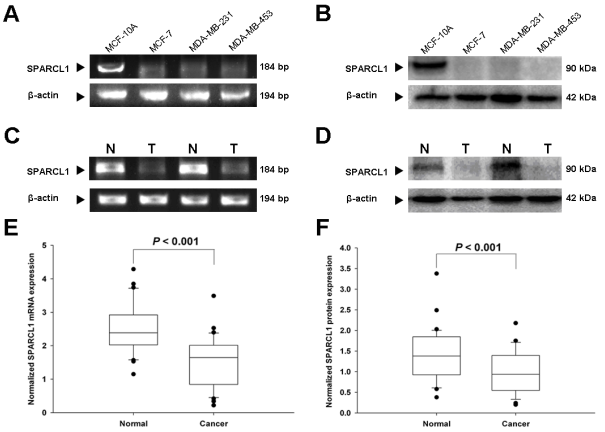

Figure 1. Reduced Expression of SPARCL1 in Breast Cancer Compared to Normal Breast Epithelial Cells. RT-PCR (A) and western blot (B) analysis of SPARCL1 expression in human immortalized mammary epithelial cell line (MCF-10A) and human breast cancer cell lines (MCF-7, MDA-MB-231 and MDA-MB-453). RT-PCR (C) and western blot (D) analysis of SPARCL1 expression in two paired samples of human breast normal (N) and tumor (T) tissues. Data of real-time quantitative PCR (E) and western blot (F) from 32 paired normal and tumor samples are summarized and compared. A significant reduced expression of SPARCL1 in breast tumor tissues compared to corresponding normal breast tissues was determined ($P < 0.001$)

Statistical analysis

The difference in SPARCL1 mRNA and protein expression between paired cancerous and corresponding non-cancerous tissues was examined by Wilcoxon matched-pairs signed-rank test. The associations between SPARCL1 immunostaining and clinicopathological characteristics were determined using Chi-square test. All P values were two-sided and less than 0.05 was considered significant. Statistical analyses were performed by the SPSS 15.0 for windows (SPSS, Chicago, IL, USA).

Results

Reduced expression of SPARCL1 in human breast cancer

The expression pattern of SPARCL1 in human immortalized mammary epithelial cell line and human breast cancer cell lines were determined by RT-PCR and western blot methods. Compared to the positive expression in MCF-10A mammary epithelial cells, SPARCL1 was nearly absent in all the three human breast cancer cell lines (Figure 1A and B).

Similarly, the expression of SPARCL1 in 32 paired human breast cancerous and non-cancerous tissues was also determined on both mRNA and protein levels. As shown in Figure 1C and D, the positive SPARCL1 expression was usually observed in the corresponding adjacent normal breast tissues compared to the absence in paired breast cancerous tissues. The data derived from total 32 paired samples are summarized in Figure 1E and F, which disclosed a significant reduced expression pattern of SPARCL1 in breast cancerous tissues compared to corresponding normal breast tissues ($P < 0.001$), by use of not only real-time quantitative PCR but also western blot analysis.

Immunochemistry staining also showed a significant

Table 1. Associations of SPARCL1 Expression with Clinicopathological Factors of Breast Cancer

Variable	Total patients	SPARCL1 expression		
	(n = 282)	Negative (%)	Positive (%)	P[a]
Age, years				0.54
≤60	217 (77.0)	68 (31.3)	149 (68.7)	
>60	65 (23.0)	23 (35.4)	42 (64.6)	
Tumor size, cm				0.228
≤2 cm	112 (39.7)	31 (27.7)	81 (72.3)	
≤5 cm	83 (29.4)	26 (31.3)	57 (68.7)	
>5 cm	87 (30.8)	34 (39.1)	53 (60.9)	
Tumor grade				0.044
Well	52 (18.4)	11 (21.2)	41 (78.8)	
Moderate	151 (53.5)	58 (38.4)	93 (61.6)	
Poor	79 (28.0)	22 (27.8)	57 (72.2)	
N classification				0.02
pN0 (0)	116 (41.1)	29 (25.0)	87 (75.0)	
pN1 (1-3)	85 (30.1)	25 (29.4)	60 (70.6)	
pN2 (4-9)	60 (21.3)	27 (45.0)	33 (55.0)	
pN3 (≥10)	21 (7.4)	10 (47.6)	11 (52.4)	
Distant metastasis				0.145
No	263 (93.3)	82 (31.2)	181 (68.8)	
Yes	19 (6.7)	9 (47.4)	10 (52.6)	
Estrogen receptor status				0.309
Negative	121 (42.9)	43 (35.5)	78 (64.5)	
Positive	161 (57.1)	48 (39.8)	113 (70.2)	
Progesterone receptor status				0.508
Negative	132 (46.8)	40 (40.3)	92 (69.7)	
Positive	150 (53.2)	51 (34.0)	99 (66.0)	
HER2/neu expression				0.69
Negative	169 (59.9)	53 (31.4)	116 (68.6)	
Positive	113 (40.1)	38 (33.6)	75 (66.4)	
p53 expression				0.911
Negative	135 (47.9)	44 (32.6)	91 (67.4)	
Positive	147 (52.1)	47 (32.0)	100 (68.0)	

SPARCL1, secreted protein acidic and rich in cysteines-like protein 1; [a]Chi-square test

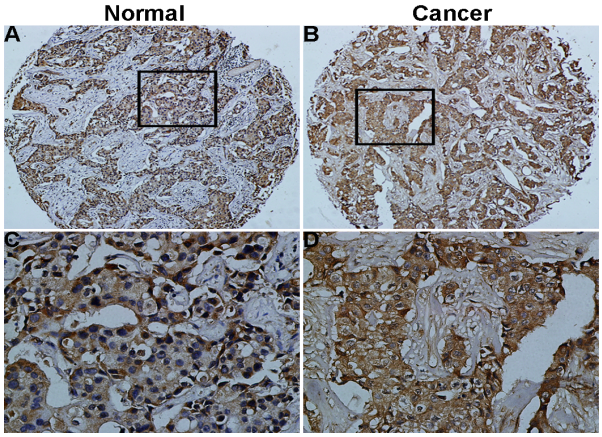

Figure 2. Representative Immunostaining of SPARCL1 in Normal Breast Tissues and Breast Tumors. Positive staining of SPARCL1 in breast normal epithelial tissues (A, C) and breast tumor tissues (B, D) are shown respectively. Original magnification, ×100 for (A) and (B) and ×400 for (C) and (D)

reduced SPARCL1 expression in breast tumor tissues compared to that in adjacent normal breast tissues. Strong SPARCL1 immunostaining was observed in almost all the normal breast samples (43/45), which was mainly located in the cytoplasm (Figure 2A and C). However, moderate and strong SPARCL1 immunostaining was only detected in 191 of 282 (67.7%) breast tumor cases (Figure 2B and D) and the other 91 cases were defined as negative staining ($P < 0.001$). The results were consistent with the findings in the PCR and western blot analysis.

Associations of SPARCL1 expression with clinicopathological factors

Associations of SPARCL1 expression with clinicopathological parameters for 282 breast cancer patients are reported in Table 1. The SPARCL1 expression was significantly related with tumor grade ($P = 0.044$) and N classification ($P = 0.020$). Positive immunostaining of SPARCL1 was more frequently observed in breast tumors with higher grade and less involving lymph nodes.

Discussion

The occurrence of tumor is a complex process involving participation of multiple genes and threading of multiple steps. It can be deemed as a result of cell growth regulation abnormality caused by the inactivation of tumor suppressors and activation of oncogenes (Russo et al., 1993). In recent years, a multitude of relevant molecules have been demonstrated to be implicated in the development and progression of breast cancer, making it possible to propose novel strategies for cancer diagnosis and treatment (Rosman et al., 2007). However, the intrinsic mechanism in pathogenesis of breast cancer, which is the most common malignant disease for women, is not well defined. Therefore, a comprehensive understanding of molecular mechanisms responsible for breast cancer development and progression could contribute to novel treatment approaches. To this end, our research has focused on the crucial molecules involved in breast tumorigenesis.

SPARCL1 is a molecular marker of endothelium related to tumor, and it has the anti-adhesion function and can inhibit cell adhesion and diffusion (Girard and Springer, 1996; St Croix et al., 2000; Hambrock et al., 2003). In the growth and proliferation of various tumor cells, SPARCL1 often presents decreased expression as a negative regulative factor, which may be closely related to the increase of the cell proliferation activity and the cell cycle progression (Claeskens et al., 2000). Particularly, growing evidence shows that SPARCL1 often presents a reduced or absent expression pattern in a variety of human tumor tissues (Bendik et al., 1998; Nelson et al., 1998; Isler et al., 2004; Esposito et al., 2007; Zaravinos et al., 2011; Hurley et al., 2012; Li et al., 2012). However, several studies have also found that an increased expression of SPARCL1 in a few of other type of human tumors derived from liver (Lau et al., 2006), uterus (Mencalha et al., 2008), and colon and rectum (Zhang et al., 2011; Hu et al., 2012). All these conflicting findings suggest that SPARCL1 is indeed implicated in human cancer development and progression, but its expression pattern may be in a tissue-specific manner. Therefore, more studies are needed to elucidate the possible relevance of SPARCL1 to a specific human tumor, for instance breast cancer. In the present study, we characterized the expression of SPARCL1 in human breast cell lines and breast tumor tissues, and analyzed the associations

of SPARCL1 expression with the clinicopathological parameters in breast cancer.

As shown in Figure 1, our data from both PCR and western blot analyses suggested a consistent conclusion that SPARCL1 was significantly decreased in breast cancer compared to normal breast cells. The conclusion was further demonstrated by the results from immunohistochemistry assay which disclosed a reduced positive rate of 67.7% in breast tumor samples compared to 95.6% in normal breast tissues ($P < 0.001$). Meanwhile, our data have further revealed that the absence of SPARCL1 expression was closely associated with tumor grade and lymph node status. The absence of SPARCL1 immunostaining was more frequently observed in breast cancer patients with poor grade tumors and more involving lymph nodes. The result indicates that SPARCL1 expression is negatively related to the malignant biological behavior of breast carcinogenesis. It is supposed that SPARCL1 may inhibit tumor growth and progression which has been confirmed by the findings with respect to SPARCL1 in prostate cancer (Hurley et al., 2012) and pancreatic cancer (Esposito et al., 2007).

The possible reason for the decreased expression and even absent expression of SPARCL1 in human cancer has not been well clarified. The common possible mechanism of tumor suppressor inactivation was usually due to gene mutations, deletion, or epigenetic alteration such promoter methylation and loss of heterozygosity. However, no deletion or mutation that might be responsible for the downregulation of SPARCL1 was found in NSCLC (Isler et al., 2004) and the promoter hypermethylation was also not the key mechanism by which SPARCL1 expression was repressed in pancreatic cancer cells (Esposito et al., 2007). In our previous work, no methylation variable positions and no mutation were observed in gastric cancer but a possible mechanism involving the loss of heterozygosity of SPARCL1 gene was revealed (Li et al., 2012). The specific mechanism for SPARCL1 downregulation in human breast cancer need further confirmed.

In summary, our results showed that the downregulation of SPARCL1 was a common and frequent event in human breast cancer. Moreover, the absence of SPARCL1 expression was significantly associated with lymphatic metastasis and poor grade of breast cancer. SPARCL1 may be involved in the breast tumorigenesis and serve as a promising target for therapy of breast cancer.

References

Bendik I, Schraml P, Ludwig CU (1998). Characterization of MAST9/Hevin, a SPARC-like protein, that is down-regulated in non-small cell lung cancer. *Cancer Res*, **58**, 626-9.

Claeskens A, Ongenae N, Neefs JM, et al (2000). Hevin is down-regulated in many cancers and is a negative regulator of cell growth and proliferation. *Br J Cancer*, **82**, 1123-30.

Eheman C, Henley SJ, Ballard-Barbash R, et al (2012). Annual Report to the Nation on the status of cancer, 1975-2008, featuring cancers associated with excess weight and lack of sufficient physical activity. *Cancer*, **118**, 2338-66.

Esposito I, Kayed H, Keleg S, et al (2007). Tumor-suppressor function of SPARC-like protein 1/Hevin in pancreatic cancer. *Neoplasia*, **9**, 8-17.

Girard JP, Springer TA (1995). Cloning from purified high endothelial venule cells of hevin, a close relative of the antiadhesive extracellular matrix protein SPARC. *Immunity*, **2**, 113-23.

Girard JP, Springer TA (1996). Modulation of endothelial cell adhesion by hevin, an acidic protein associated with high endothelial venules. *J Biol Chem*, **271**, 4511-7.

Hambrock HO, Nitsche DP, Hansen U, et al (2003). SC1/hevin. An extracellular calcium-modulated protein that binds collagen I. *J Biol Chem*, **278**, 11351-8.

Hu H, Zhang H, Ge W, et al (2012). Secreted protein acidic and rich in cysteines-like 1 suppresses aggressiveness and predicts better survival in colorectal cancers. *Clin Cancer Res*, **18**, 5438-48.

Hulvat MC, Hansen NM, Jeruss JS (2009). Multidisciplinary care for patients with breast cancer. *Surg Clin North Am*, **89**, 133-76, ix.

Hurley PJ, Marchionni L, Simons BW, et al (2012). Secreted protein, acidic and rich in cysteine-like 1 (SPARCL1) is down regulated in aggressive prostate cancers and is prognostic for poor clinical outcome. *Proc Natl Acad Sci USA*, **109**, 14977-82.

Isler SG, Ludwig CU, Chiquet-Ehrismann R, Schenk S (2004). Evidence for transcriptional repression of SPARC-like 1, a gene downregulated in human lung tumors. *Int J Oncol*, **25**, 1073-9.

Isler SG, Schenk S, Bendik I, et al (2001). Genomic organization and chromosomal mapping of SPARC-like 1, a gene down regulated in cancers. *Int J Oncol*, **18**, 521-6.

Jemal A, Bray F, Center MM, et al (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.

Kallioniemi OP, Wagner U, Kononen J, Sauter G (2001). Tissue microarray technology for high-throughput molecular profiling of cancer. *Hum Mol Genet*, **10**, 657-62.

Kataja V, Castiglione M, ESMO Guidelines Working Group (2008). Locally recurrent or metastatic breast cancer: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann Oncol*, **19**, ii11-3.

Kononen J, Bubendorf L, Kallioniemi A, et al (1998). Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med*, **4**, 844-7.

Lau CP, Poon RT, Cheung ST, Yu WC, Fan ST (2006). SPARC and Hevin expression correlate with tumour angiogenesis in hepatocellular carcinoma. *J Pathol*, **210**, 459-68.

Li P, Qian J, Yu G, et al (2012). Down-regulated SPARCL1 is associated with clinical significance in human gastric cancer. *J Surg Oncol*, **105**, 31-7.

Long N, Moore MA, Chen W, et al (2010). Cancer epidemiology and control in north-East Asia - past, present and future. *Asian Pac J Cancer Prev*, **11** suppl 2, 107-48.

Mencalha AL, Levinsphul A, Deterling LC, Pizzatti L, Abdelhay E (2008). SPARC-like1 mRNA is overexpressed in human uterine leiomyoma. *Mol Med Report*, **1**, 571-4.

Nelson PS, Plymate SR, Wang K, et al (1998). Hevin, an antiadhesive extracellular matrix protein, is down-regulated in metastatic prostate adenocarcinoma. *Cancer Res*, **58**, 232-6.

Rosman DS, Kaklamani V, Pasche B (2007). New insights into breast cancer genetics and impact on patient management. *Curr Treat Options Oncol*, **8**, 61-73.

Russo J, Calaf G, Sohi N, et al (1993). Critical steps in breast carcinogenesis. *Ann N Y Acad Sci*, **698**, 1-20.

Shen FR, Liu M, Zhang X, et al (2012). Health-related quality of life among breast cancer patients and its Influencing Factor in

a Chinese Population. *Asian Pac J Cancer Prev*, **13**, 3747-50.
St Croix B, Rago C, Velculescu V, et al (2000). Genes expressed in human tumor endothelium. *Science*, **289**, 1197-202.
Sullivan MM, Sage EH (2004). Hevin/SC1, a matricellular glycoprotein and potential tumor-suppressor of the SPARC/BM-40/Osteonectin family. *Int J Biochem Cell Biol*, **36**, 991-6.
Zhang H, Widegren E, Wang DW, Sun XF (2011). SPARCL1: a potential molecule associated with tumor diagnosis, progression and prognosis of colorectal cancer. *Tumour Biol*, **32**, 1225-31.
Zaravinos A, Lambrou GI, Boulalas I, Delakas D, Spandidos DA (2011). Identification of common differentially expressed genes in urinary bladder cancer. *PLoS One*, **6**, e18135.