

SPOCK1 Involvement in Epithelial-to-Mesenchymal Transition: A New Target in Cancer Therapy?

This article was published in the following Dove Press journal:
Cancer Management and Research

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Background: Cancer metastasis is the main obstacle to increasing the lifespan of cancer patients. Epithelial-to-mesenchymal transition (EMT) plays a significant role in oncogenic processes, including tumor invasion, intravasation, and micrometastasis formation, and is especially critical for cancer invasion and metastasis. The extracellular matrix (ECM) plays a crucial role in the occurrence of EMT corresponding to the change in adhesion between cells and matrices.

Conclusion: SPOCK1 is a critical regulator of the ECM and mediates EMT in cancer cells. This suggests an important role for SPOCK1 in tumorigenesis, migration and invasion. SPOCK1 is a critical regulator of some processes involved in cancer progression, including cancer cell proliferation, apoptosis and migration. Herein, the functions of SPOCK1 in cancer progression are expounded, revealing the association between SPOCK1 and EMT in cancer metastasis. SPOCK1 is a positive downstream regulator of transforming growth factor- β , and SPOCK1-mediated EMT regulates invasion and metastasis through the Wnt/ β -catenin pathway and PI3K/Akt signaling pathway. It is of significance that SPOCK1 may be an attractive prognostic biomarker and therapeutic target in cancer treatment.

Keywords: SPOCK1, epithelial-to-mesenchymal transition, extracellular matrix, cancer, metastasis

Introduction

The number of deaths caused by cancer was estimated to be 9.6 million in 2018, and it is anticipated that cancer will be the top cause of death worldwide in the 21st century.¹ Increasing the life expectancy of patients who have cancer with a poor survival rate is a major obstacle, mainly because of early spread of tumors via metastasis or direct invasion.²

Metastasis is the spread of tumors from the primary site to distant sites of the body. The spread of cancer cells is considered to be a vital step in the progression of tumors. Epithelial-to-mesenchymal transition (EMT) is an embryonic program that relaxes cell-cell binding complexes and gives cells enhanced migration and invasion characteristics that can be exploited by cancer cells during metastatic progression. Cancer cells undergoing EMT are more aggressive, showing more invasive, stem cell-like characteristics and resistance to apoptosis.³ As a critical element of the cancer microenvironment, the extracellular matrix (ECM) provides mechanical support for tissues and mediates the interaction between cells and the microenvironment, which is critical to the invasion of cancer cells. The ECM is increasingly taken as a key regulator in cancer.⁴ The ECM plays a crucial role in the occurrence of EMT according to the change in adhesion between cells and matrices. To

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maintain tissue differentiation, the ECM is highly dynamic and continually remodeled.⁵ This process is mediated by a variety of types of peptidases that can degrade the macromolecules of the ECM.⁶ In addition, peptidases play a role in the development of cancer and are involved in physiological functions such as angiogenesis, receptor activation, morphogenesis and apoptosis.⁷ During tumorigenesis, dramatic changes in ECM homeostasis are driven by alterations in peptidases and interruptions in the balance between ECM synthesis and degradation.⁸

SPARC/osteonectin, cwcv and kazal-like domains proteoglycan 1 (SPOCK1), a glycoprotein isolated from human testes, is one of the secreted protein, acidic, cysteine-rich (SPARC) family members and was originally called “testican-1”. SPOCK1 is highly expressed in the brain, cartilage, vascular endothelium, myoblasts, fibroblasts, lymphocytes, and neuromuscular junctions (NMJs), which suggests that the proteoglycan is involved in several physiological functions.^{9–14} It is worthwhile to investigate the effects of SPOCK1 on cancer progression based on the structural similarities between SPOCK1 and SPARC and the role of SPARC in cancer development.¹⁵ SPOCK1 is a critical regulator of the ECM and mediates EMT in cancer cells.¹⁶ This suggests an important role for SPOCK1 in tumorigenesis, migration and invasion.

In recent years, an increasing number of publications have paid close attention to the role of SPOCK1 in cancer diagnosis and treatment. Herein, the functions of SPOCK1 in cancer progression are expounded, revealing the association of SPOCK1 with ECM and EMT in cancer metastasis.

The Structure of SPOCK1

The SPARC family, comprising SPARC, SPOCK1, SPOCK2 and SPOCK3, secretes follistatin-like protein 1, Hevin and modular calcium binding protein 1 and 2.¹⁷ SPARC family members share a basic structure, which is composed of an N-terminus, follistatin-like domain and C-terminus.¹⁶ In contrast to SPOCK2 and SPOCK3, which are pure heparan sulfate proteoglycans, SPOCK1 carries both heparan and chondroitin sulfate chains, SPOCK2 is N-glycosylated and SPOCK3 is composed of several mucin-type O-glycans, suggesting that there are specific physiological roles for each SPOCK.^{18–20}

The 439-amino acid of SPOCK1 is encoded by *SPOCK1*, which is located at 5q31. *SPOCK1* consists of 11 exons. The first, second and fourth osteonectin domains are encoded by the overlap of exons 2, 3, 4 and exon 8. The third osteonectin

domain and the Kazal-like domains are encoded by exons 5 and 6. The CWCV domain is encoded by exon 10.²¹ Thyropin domain homology occurs between residues 310 and 379, comprised of exons 9 and 10.^{22,23}

SPOCK1 is a multidomain protein that is homologous to different protease inhibitors.²⁴ The proteoglycan is composed of the N-terminus, follistatin-like domain, extracellular calcium binding domain and C-terminus containing a thyropin domain and domain V, including two potential glycosaminoglycan attachment (GAG) sites (Figure 1).²³

The unique N-terminus is responsible for the inhibition of a membrane-type metalloproteinase.²⁶ There is similarity between the six-cysteine-follistatin-like domain and the Kazal domains in serine protease inhibitors.^{27–29} The extracellular calcium binding domain is an independent domain that belongs to the SPARC family and is involved in cell adhesion.^{30,31} The thyropin domain is constitutive of 64 amino acids and homologous to protein sequences that inhibit cysteine proteases cathepsin-L.^{22,23} SPOCK1 can be classified in a subset of the osteonectin domains and is a highly conservative chimeric proteoglycan that carries both heparan sulfate and chondroitin sulfate chains.⁹ Due to its modular structure, SPOCK1 is classified as a regulator of the ECM that can interact with cell surfaces.

Physiological Functions of SPOCK1

SPOCK1 was first isolated in human testicular seminal plasma.^{23,32,33} SPOCK1 has different expression levels in tissues, and its expression is highest in specific regions of the brain, including the axons and neuromuscular junctions.^{9,34,35} It has been indicated that there are systemic effects of SPOCK1 because it can also be found in several other tissues and organs, such as lymphocytes and blood (Table 1).^{2,13,14,18,36,37}

The highest expression of human SPOCK1 is in the central nervous system (CNS), including neurons, endothelial cells, and activated astroglia, predominantly in the postsynaptic region of pyramidal cells in the hippocampus CA3 region.^{34,35} Neuronal cell growth and substrate attachment are regulated by SPOCK1, which indicates the role of SPOCK1 in brain development.²⁵ SPOCK1 may participate in diverse steps of neurogenesis and participate in various neuronal processes in the CNS via involvement in brain cell adhesion.^{26,32,38} SPOCK1 plays a role in the behavior of the Rett syndrome phenotype, modulation of dopamine neuron population size and necrosis after brain injury.^{13,39,40}

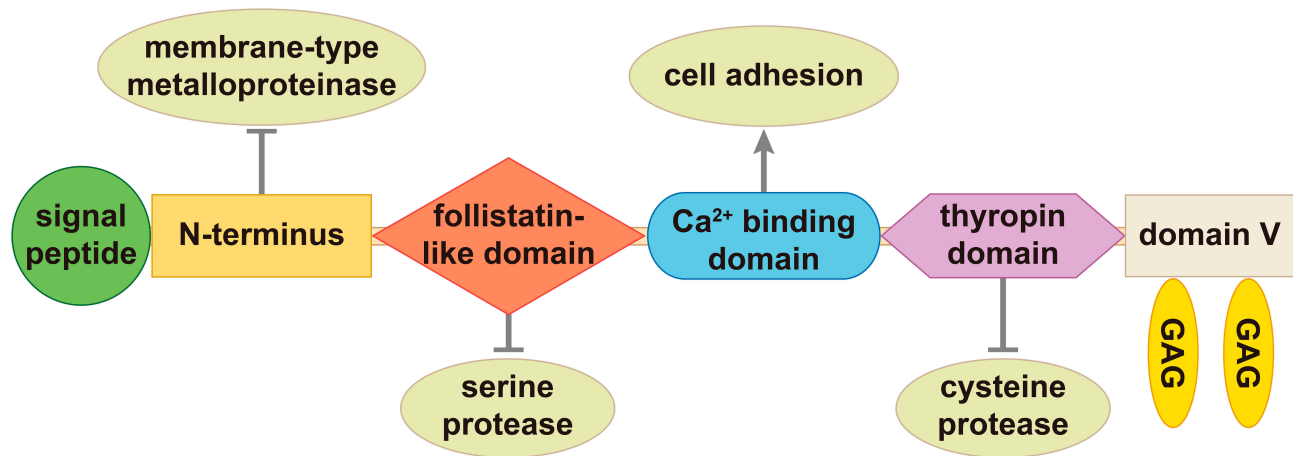


Figure 1 The structure of SPOCK1. SPOCK1 is constitutive of N-terminus, follistatin-like domain, extracellular calcium binding domain and C-terminus containing a thyroprin domain and domain V including two potential glycosaminoglycan attachment (GAG) sites.

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In the peripheral nervous system, SPOCK1 is expressed in axons, Schwann cells and the periphery of the ganglion, suggesting that SPOCK1 is crucial to NMJ immunoreactivity, neurogenesis, tactile and proprioceptive functions and mutations relevant to developmental delay and microcephaly.^{36,41,42}

In addition to its role in the neuronal system, SPOCK1 contributes other physiological functions that are consistent with its tissue distribution. SPOCK1 is a component of the ECM and a regulator of MMP-2. According to the regulation of activity and expression of MMP-2, SPOCK1 has a function of menarche, chronic inflammation and infection, and matrix turnover in cartilage.^{9,14,43,44} In addition, SPOCK1 can participate in other physiological and disease processes, such as embryonic development correlated with MafB, tissue remodeling under spaceflight conditions and coronary artery disease through the low-density lipoprotein-cholesterol pathway.^{31,45,46}

The Role of SPOCK1 in EMT

EMT is a significant process associated with the weakness of cell-cell junctions, loss of cell polarity and epithelial characteristics, resulting in increased motor ability and invasion.⁴⁷ EMT plays a significant role in oncogenic processes, including tumor invasion, intravasation, and micrometastasis formation.^{3,48-50} The EMT process promotes the acquisition of mesenchymal properties of epithelial cells and demonstrates an increase in cohesive intercellular adhesion and motor motile characteristics to migrate towards surrounding tissues, which facilitates subsequent metastases.⁵¹⁻⁵³

In the EMT process, there are a range of ECM factors and membrane receptors involved in alternative signaling pathways that cooperate and compete to promote the connection

between cancer cells and different extracellular clues. EMT is associated with not only loss of cell adhesion and elongation but also latent ECM invasion.⁴⁷ The specific cell ligands in the microenvironment of tumors depend on ECM components that are correlated with cancer cell processes and promote the expression of survival pathways so that cells can escape drug therapy.^{54,55} The development of tumors is often related to the upregulation of different ECM proteins.⁵⁶ The major molecular components of the ECM are proteoglycans and fibrillary proteins.^{37,45} Members of the SPARK family are important regulators of ECM assembly and are also involved in protease activities and cytokine signaling pathways.⁴⁵ Similar to other members of the SPARK family, SPOCK1 is a regulator of the ECM and is crucial for the degradation and remodeling of the ECM, which is critical to ECM homeostasis as well as tumorigenesis.⁸ SPOCK1 has been indicated to regulate the activity and expression of MMPs, such as pro-MMP-2, membrane type (MT)1-MMP or MT3-MMP, MMP3 and MMP9, as a means to modulate ECM degradation and restoration.^{44,47}

The progression of EMT is complicated by the regulation of mediators, such as growth factors and hormones.⁵⁷ EMT is initiated by EMT-inducing transcriptional factors (EMT-TFs), including zinc finger proteins Snail, Slug (Snail2), Zeb1, Zeb2, and Twist.⁵⁸ Stimulated by transforming growth factor- β (TGF- β), Snail controls transcriptional ZEB1 expression by cooperating with Twist and Ets1.⁵⁸ The network of EMT-inducing transcriptional factors is activated and leads to temporal and spatial coordination between EMT biomarkers.⁵⁷ In addition, multiple transcriptional factors and signaling pathways are positively correlated with EMT, including epithelial

markers such as E-cadherin and mesenchymal markers such as vimentin.^{46,52,58} Among the signaling pathways, the signaling pathways of TGF- β , Wnt and Notch are considered to be key to the induction of the EMT phenotype.⁵⁶

TGF- β , the primary enhancer of EMT, plays a crucial role in cancer viability and metastasis. At the transcription level, a range of transcription factors, such as zinc finger proteins Snail (SNAI1) and Slug (SNAI2), can be activated by TGF- β , subsequently leading to EMT.⁵⁹ In addition, TGF- β can be connected to the Wnt/ β -catenin signaling pathway and Notch signaling pathway to promote EMT.⁵² Wnt integrates with other signaling pathways, including RTKs and their downstream regulators MAPK, PI3K and PKB, to promote the progression of EMT.⁵¹ The expression of GSK3 β , Snail, cyclin D1 and c-MYC is associated with Wnt/ β -catenin.⁶⁰ The Notch pathway is a supportive factor that must synergize with other carcinogenic pathways to lead to cell transformation and induce the EMT process. Notch target genes include Akt, mTOR, NF- κ B, cyclin D1, c-myc, VEGF.^{61–63}

Cells are changed to more scattered mesenchymal-like cells with the overexpression of SPOCK1. Several kinds of cancer cells undergo EMT changes according to the induction of N-cadherin and vimentin and the inhibition

of ZO-1 and E-cadherin; these cell types include lung cancer, gallbladder cancer, gastric cancer, prostate cancer, esophageal cancer and glioma cells.^{2,16,50,59,60,64}

It is suggested that several signaling pathways are associated with SPOCK1-mediated EMT. In addition, in the EMT process induced by SPOCK1, the interaction and cooperation between the expression of regulators and signaling pathways have been further discussed. There is a positive correlation between the expression of SPOCK1 and TGF- β in lung and breast cancer, which demonstrates that SPOCK1 is a positive downstream regulator of TGF- β .^{24,59} In addition, SPOCK1 expression is regulated through the Wnt/ β -catenin pathway in NSCLC.⁶⁵ SPOCK1-mediated EMT regulates proliferation and invasion via activation of the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway in gallbladder cancer, colorectal cancer, pancreatic ductal adenocarcinoma and glioma.^{2,60,66,67} It is worth noting that invasion and metastasis are regulated by SPOCK1 through Slug-mediated EMT in gastric cancer (Figure 2).¹⁶

Functions of SPOCK1 in Cancer Progression

In addition to its physiological functions, SPOCK1 is a critical regulator in cancer progression. Accumulating evidence has indicated a role for SPOCK1 in many kinds of

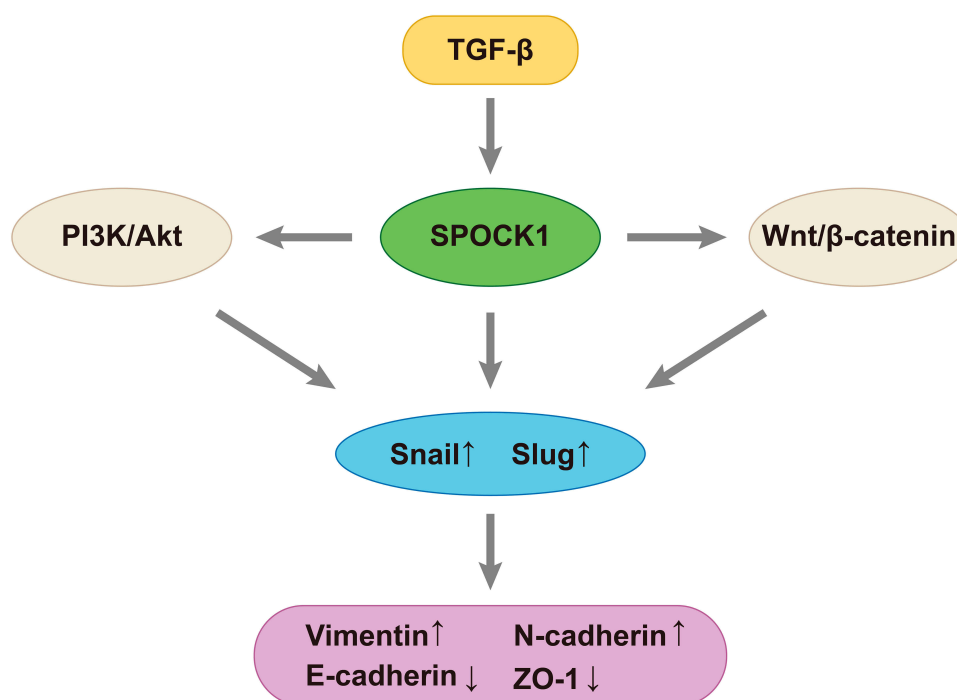


Figure 2 The signaling pathways of SPOCK1 in EMT. SPOCK1 is a downstream regulator of TGF- β . EMT-inducing transcriptional factors Snail and Slug are regulated by TGF- β /SPOCK1 through PI3K/Akt and Wnt/ β -catenin signaling pathways which cause regulation of epithelial markers E-cadherin, ZO-1 and mesenchymal markers vimentin, N-cadherin.

Abbreviations: TGF- β , transforming growth factor- β ; PI3K, phosphoinositide 3-kinase; Akt, v-akt murine thymoma viral oncogene homolog; Wnt, wingless/integrated.

cancers, such as gallbladder cancer, prostate cancer, breast cancer, urothelial carcinomas, colorectal cancer, glioblastomas, ovarian cancer, esophageal squamous cell carcinoma, lung cancer and hepatocellular carcinoma (HCC) (Table 2).^{2,8,24,27,30,51,54,55,59,64,68-70} The importance of SPOCK1 in cancer proliferation, apoptosis, migration and drug resistance has been reported.¹⁵

It is disputed whether SPOCK1 is a regulator of cancer cell proliferation. Some studies demonstrated that SPOCK1 had no effect on gastric cancer cell growth in vivo and in vitro.¹⁶ Other findings showed that SPOCK1 was essential in non-small-cell lung cancer (NSCLC) and osteosarcoma via the

Table 1 The Physiological Functions

Tissues	Physiological Functions	Ref.
Neurons	Subserving tactile and proprioceptive functions	41
Activated astroglia in the hippocampus CA3 region	A possible role in motor or behavioural aspects of the Rett syndrome phenotype	39
Postsynaptic areas, reactive astrocytes	Regenerating axons in gliotic reaction, modulation of dopamine neuron population size and necrosis after brain injury	40
Axons, Schwann cells, neuromuscular junction and the periphery of the ganglion	NMJ immunoreactivity, neurogenesis, tactile and proprioceptive functions and mutations relevant to developmental delay and microcephaly	36
Cartilage	Regulating matrix turnover in cartilage	9
Menarche	Regulation of Age at menarche	14
Goat endometrium	Participating embryo implantation through developing endometrial receptivity	37
Corneal epithelium and stroma	Promoting wound healing after <i>Pseudomonas aeruginosa</i> -Induced Keratitis	44
Mouse lung	Tissue remodeling under spaceflight condition	45,81
Vascular endothelial cells	Promoting coronary artery disease	46

Table 2 The Mechanisms of SPOCK1 in Cancer Progression

Target of SPOCK1	Function of SPOCK1	Kinds of Cancers	Ref.
Induction of EMT	Migration and invasion	Gastric cancer	16
		Urothelial carcinoma	27
		ESCC	55
		Lung cancer	80
PI3K/Akt signaling pathways	Proliferation, apoptosis, migration and invasion	Gallbladder cancer	2
		Colorectal cancer	30
		Colorectal cancer	66
		Glioma cells	60
		HCC	70
		Pancreatic ductal adenocarcinoma	67
Wnt/ β -catenin pathway	Proliferation, colony formation, migration and invasion	NSCLC	65
		Glioma cells	60
Bcl-2, Bax; caspase-3 and PARP	Promoting cell proliferation	Colorectal cancer	30
Cyclin B1, cyclin D1, and Cdc25C	Cell cycle progression	Prostate cancer	64
HSP90a and MMP-2	Invasion and metastasis	HNSCC	76
MMP-3 and MMP-9	Invasion and metastasis	Prostate cancer	64
miR-627-5p	Proliferation, migration and invasion	Glioma cells	78
miR-139-5p, miR-940 and miR-193a-5p	Apoptosis, proliferation and metastasis	HCC	79
miR-130a-3p	Migration	Lung cancer	80

Abbreviations: EMT, epithelial-to-mesenchymal transition; ESCC, esophageal squamous cell carcinoma; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer; HNSCC, head and neck squamous cell carcinoma.

Wnt/ β -catenin and mTOR-S6K signaling pathways, respectively.^{65,71} In addition, the enhancement of tumor cell survival by SPOCK1 may be attributed to its inhibition of apoptosis and regulation of cell cycle progression in cancers, such as colorectal cancer, prostate cancer and gallbladder cancer.^{2,30,64,70} The cell cycle regulators Cdc25C, cyclin B1 and cyclin D1 are regulated by SPOCK1 in colorectal cancer cell lines SW480 and HT29.³⁰ SPOCK1 regulates G0/G1-phase cell cycle arrest and apoptosis in prostate cancer.^{64,72}

SPOCK1 inhibits apoptosis through activation of the PI3K/Akt signaling pathway and subsequently inhibits the cyt c/caspase-9/caspase-3 pathway, which leads to upregulation of Bax and downregulation of Bcl-2.^{51,70,72}

It is noteworthy that SPOCK1 also has effects on drug resistance to chemotherapeutics in cancer cells. SPOCK1 mediates temozolomide (TMZ) resistance in glioblastoma multiforme (GBM) cells and lapatinib resistance in HER2-positive gastric cancer via the Akt and Wnt/ β -catenin signaling pathways, respectively.^{47,54} SPOCK1 is associated with osimertinib resistance in lung cancer, which is correlated with TGF- β 1 and MMP11.⁷³

SPOCK1 plays a critical role in invasion in many cancer types, such as gallbladder cancer, esophageal squamous cell carcinoma and colorectal cancer.^{2,51,66} SPOCK1 interferes with cancer cells by affecting extracellular collagen, subsequently promoting the expansion of cancer cells.¹⁵ SPOCK1 is involved in prostate cancer metastasis via the SPOCK1-Akt-Snail/Slug signaling pathway.⁷⁴

Endothelial protein C receptor (EPCR), plakophilin 1 (PKP1) and chromodomain helicase/adenosine triphosphatase DNA binding protein 1-like (CHD1L) are upstream factors of SPOCK1.^{8,29,70} The high level of EPCR-SPOCK1 expression is associated with 3D growth in vitro, which is consistent with poor clinical outcome in breast cancer patients.²⁹ CHD1L binds directly to SPOCK1 and activates transcription, blocks apoptosis and induces invasion of HCC cells through the Akt signaling pathway.⁷⁰ SPOCK1 is also a target of miRNAs, including *miR-129-5p*, *miR-150-3p*, and *miR-150-5p*, in gastric cancer (GC) cells and head and neck squamous cell carcinoma (HNSCC). The miRNA/SPOCK1 axis is crucial for cancer proliferation, migration and invasion.^{75,76}

Potential Applications of SPOCK1

Because of its high expression in cancer patients, SPOCK1 might be a prognostic marker in cancers. The expression level of SPOCK1 is associated with overall survival in many cancers, such as urothelial carcinoma, pancreatic ductal adenocarcinoma, prostate cancer, gastric cancer, breast cancer, lung adenocarcinoma and lung squamous carcinoma.^{16,24,27,67,73,74} High expression of SPOCK1 is consistent with shorter overall survival time, which indicates that SPOCK1 is associated with poor prognosis in univariate Log rank tests and in the multivariate analysis. There is a link between cancer stage and the expression of SPOCK1. SPOCK1 expression is higher in advanced stages than earlier stages in prostate cancer.⁶⁹ Patients

with upper tract urothelial carcinoma and poor disease-specific survival (DSS) had high SPOCK1 expression.²⁷ HNSCC patients with poor prognoses had high SPOCK1 expression in TCGA database analyses.⁷⁶ The expression of SPOCK1 in gallbladder cancer tissues is higher than that in their nontumor counterparts.² SPOCK1 in lung cancer is significantly upregulated in metastatic tissues compared to nonmetastatic tissues.⁵⁹

Several inhibitors have been identified, indicating that SPOCK1 may be a potential therapeutic target. Apigenin (20–80 μ M), 4',5,7-trihydroxyflavone, can inhibit prostate cancer cell proliferation, migration, and invasion in vivo and in vitro.⁷⁴ The expression of SPOCK1 is downregulated by 100 μ M nitroglycerin in human monocytic leukemia cells to alter atherosclerotic plaque stability.⁷⁷ These results indicated that nitroglycerin could be a candidate SPOCK1 inhibitor. Several endogenous inhibitors have also been identified. The microRNAs mentioned above (*miR-130a-3p*, *miR-129-5p*, *miR-150-3p*, *miR-150-5p*, *miR-627-5p*, *miR-139-5p*, *miR-940* and *miR-193a-5p*) can regulate cancer progression by downregulating the expression of SPOCK1.^{75,76,78-80}

Taken together, these results indicate that SPOCK1 could be a potential therapeutic and prognostic target.

Conclusion

SPOCK1 is a proteoglycan with systemic physiological functions. The anticancer effects have been investigated widely. SPOCK1 plays an important role in cancer proliferation and chemotherapy resistance and especially participates in cancer cell invasion and metastasis via EMT regulation. Patients with low SPOCK1 expression have increased life expectancy compared to those with high expression.⁵⁹ The connection between the expression of SPOCK1 and poor prognosis makes SPOCK1 an effective prognostic factor in cancer. It is of clinical significance that the expression of SPOCK1 in metastatic tumor tissues is significantly higher than that in nonmetastatic tumor tissues.⁵⁹ It is worth noting that multiple signaling pathways of SPOCK1 are involved in tumorigenesis and development. SPOCK1 may be a critical therapeutic target in cancer. Above all, SPOCK1 may be an attractive prognostic biomarker and therapeutic target in cancer treatment.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final

approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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