SPOCK: Master regulator of malignant tumors (Review)

MINGYUAN XIAO[1], JIANCHENG XUE[2,3] and ENLI JIN[1]

[1]Department of Rehabilitation, Shengjing Hospital of China Medical University, Shenyang, Liaoning 110134, P.R. China;
[2]Department of Otolaryngology, Head and Neck Surgery, The Second People's Hospital of Shenzhen, The First Affiliated Hospital of Shenzhen University, Shenzhen, Guangdong 518035, P.R. China;
[3]Shenzhen Clinical Research Center for Otolaryngology Diseases, The Second People's Hospital of Shenzhen, The First Affiliated Hospital of Shenzhen University, Shenzhen, Guangdong 518035, P.R. China

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Abstract. SPARC/osteonectin, CWCV and Kazal-like domain proteoglycan (SPOCK) is a family of highly conserved multidomain proteins. In total, three such family members, *SPOCK1*, *SPOCK2* and *SPOCK3*, constitute the majority of extracellular matrix glycoproteins. The *SPOCK* gene family has been demonstrated to serve key roles in tumor regulation by affecting MMPs, which accelerates the progression of cancer epithelial-mesenchymal transition. In addition, they can regulate the cell cycle via overexpression, inhibit tumor cell proliferation by inactivating PI3K/AKT signaling and have been associated with numerous microRNAs that influence the expression of downstream genes. Therefore, the SPOCK gene family are potential cancer-regulating genes. The present review summarizes the molecular structure, tissue distribution and biological function of the SPOCK family of proteins, in addition to its association with cancer. Furthermore, the present review documents the progress made in investigations into the role of SPOCK, whilst also discussing prospects for the future of SPOCK-targeted therapy, to provide novel ideas for clinical application and treatment.

Correspondence to: Dr Jiancheng Xue, Department of Otolaryngology, Head and Neck Surgery, The Second People's Hospital of Shenzhen, The First Affiliated Hospital of Shenzhen University, 3002 Sungang West Road, Shenzhen, Guangdong 518035, P.R. China
E-mail: thestruggler2022@163.com

Dr Enli Jin, Department of Rehabilitation, Shengjing Hospital of China Medical University, 36 Sanhao Street, Heping, Shenyang, Liaoning 110134, P.R. China
E-mail: 18940254923@163.com

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Content

1. Introduction

Cancer is an ongoing public health challenge globally and currently ranks as the second most common cause of mortality worldwide (1-3). Owing to the heterogeneity, uncertainty and imperceptibility of cancer, coupled with the lack of experimental or clinical research on treatment targets, the mortality rate of cancer remains high. In addition, cancer has become a significant barrier to increasing life expectancy in the 21st century (4). Therefore, genes associated with cancer have become the focus of intense research.

SPARC/osteonectin, CWCV and Kazal-like domain proteoglycan (SPOCK) is a chimeric proteoglycan that is highly conserved. Its modular architecture consists of a core protein, which consists of SPARC/osteonectin domains, CWCV and Kazal-like domains. In total, three members of the SPOCK subfamily, namely *SPOCK1*, *SPOCK2* and *SPOCK3*, which are members of the BM-40/SPARC/osteonectin protein family, have follistatin-like and extracellular calcium-binding domains. SPOCK was initially detected in the seminal human plasma (5), but further study has revealed that SPOCK can be found in certain parts of the nervous system and brain, such as the pyramidal cells of the hippocampus (6). In addition, chondrocytes and endothelial cells have been found to express the SPOCK protein (7).

SPOCK has been previously documented to serve an important role in controlling the physiology of tumor cells (8). Therefore, it may be of benefit to further explore the role of SPOCK proteins in regulating the degradation or repair of the extracellular matrix (ECM) and physiology of tumor cells. In the present review, the most recent research on the mechanism of various isoforms of SPOCK in cancer was summarized to provide a theoretical basis and research direction for the development of diagnostic markers or gene therapy targets.

2. Overview of SPOCK

Structural overview. The SPOCK family consists of cysteine-rich acidic secretory proteins that represent a group of cysteine-rich acidic secretory proteins that are integral components of the ECM (6). The nomenclature of the SPOCK proteins mirrors their modular architecture. SPOCK has been found to interact with both cell surface and ECM molecules (6). In addition, each SPOCK family member has a follicle-like calcium-binding domain. By contrast, the thyroglobulin domains of SPOCK family members can bind to growth factors (9) or interact with the cell matrix (10).

Gene structure and chromosomal localization

Gene structure of SPOCK. The *SPOCK1* gene is the most extensively characterized member of the *SPOCK* family. This gene is located in the long arm of chromosome 5 (5q31), which contains the chondroitin sulphate chain and heparan sulphate chain, between the interleukin-9 and early growth response 1 gene segments. It comprises 11 exons, possessing large introns in the 5' regions (11,12). Based on the size of the non-overlapping exonic genomic clones, exon 1 does not have a transcriptional function, whereas exon 2 begins transcription at the ATG initiation site. There is an overlap among exons 2, 3 and 4 and the first two structural domains of osteonectin. By contrast, exons 5 and 6 encode the third osteonectin- and Kazal-like fields, respectively. Exons 8 and 10 encode the fourth osteonectin structural domain and the CWCV structural domains. The last 42 amino acids and the 3' untranslated region that follows are surrounded by the final exon. Exons 2 and 4 are 46 bp apart, supporting the hypothesis of a single exon. All inline-exon junctions follow the GT/AG rule. This crucial feature in precursor RNA splicing encompasses two conserved sequences at the intron-exon junction: GT at the 5' end and AG at the 3' end. This rule predominantly governs eukaryotic gene splicing sites and underscores a shared mechanism for intron excision. It is important to note that this conservation does not extend to post-transcriptional processing of mitochondrial, chloroplast, and yeast tRNA genes. Analyzing exon 1, which includes the 5' untranslated region, revealed that the sequence covering the first 270 bp of the cDNA appears to be associated with a chimeric gene rather than the *SPOCK* gene. Therefore, the possibility of a small number of spliced exons in this region must be partially ruled out. Because not all introns are at the same stage, it is possible that the alternative splicing of any one of the aforementioned domains can significantly affect reading frame interpretation (13).

The *SPOCK2* gene is located in the long arm of chromosome 10 (10q22) and its corresponding protein binds to glycosaminoglycans (GAGs) to form part of the ECM (14). This gene encodes a protein precursor consisting of 424 amino acids, containing a follistatin-like structural domain, a Ca^{2+}-binding domain, a thyroglobulin type-1 structural domain and two possible glycosaminoglycan-binding sites at the C-terminus. However, the N-terminus shows no homology with other known proteins, except for *SPOCK1*. The cysteine-rich domain (FS domain) is similar to that of follistatin-like proteins. The third domain is homologous to the EC domain of the BM-40 signaling peptide (15). This domain contains two EF-hand motifs, EF-1 and EF-2, which can bind to Ca^{2+}. All essential features exhibited by the EC domains are conserved in *SPOCK2*. Specifically, the EF-hand binding site in the binding domain and the recombinant fragment consisting of a thyroglobulin-like domain can undergo reversible, Ca^{2+}-dependent conformational changes. Following the EC domain is the thyroglobulin type-1 domain (TY domain). The TY domain is stabilized by three conserved disulfide bonds and contains a characteristic CWCV tetrapeptide sequence (16,17). The C-terminal domain of *SPOCK2* is unique to the SPOCK subfamily and has two possible GAG attachment sites.

SPOCK3 has an indistinct characteristic for a *SPOCK* gene. It is encoded by the human chromosome region 4q32.3, which is similar to *SPOCK1* in terms of its structure and function. It has been previously observed to encode multiple isoforms of protein caused by alternative splicing transcript variants (18) (Fig. 1).

Distribution and physiological function of the SPOCKs. *SPOCKs* are widely expressed in brain tissue. *SPOCK1* and *SPOCK2* have been documented to regulate the development and repair of the nervous system, providing ideas for future studies into neurodevelopmental disorders. In terms of tumorigenesis, *SPOCK1* and *SPOCK3* have been reported to mediate oncogenic roles, whereas *SPOCK2* tended to serve as a tumor suppressor (11,13,19,20).

Epigenetic regulation and expression. Sustained hypermethylation commonly occurs at the locus of the gene encoding *SPOCK1* across diverse malignancies leading to transcriptional repression (21). Histone deacetylation along with specific microRNAs (miRs) contribute significantly towards this gene's silencing within neoplastic lesions (11,22). *SPOCK2* is subject to modulation by distinct histone methylation patterns specifically within neuronal tissue. MiRs directed against *SPOCK2* exert profound effects on processes related to neuronal differentiation as well as pathogenesis underlying conditions like autism spectrum disorder and intellectual disability (23-25). Conversely, hypomethylation at the *SPOCK3* promoter region closely associates with heightened expression levels observed across various carcinomas. Epigenetic control mechanisms involving histone acetylation alongside long non-coding RNAs demonstrate pivotal roles during developmental stages within cerebral tissue (26). The clinical relevance pertaining to genetic alterations affecting SPOCK family members remains extensively documented.

Specifically, *SPOCK1* exhibits frequent dysregulation, which is particularly evident within glioblastoma multiforme (27), as well as breast carcinoma (28), whereas *SPOCK3* aberrations predominantly manifest within colorectal cancer (29) and prostate cancer (30). Mutational events impacting *SPOCK2* associate primarily with neurodevelopmental anomalies characterized by perturbed histone modification profiles coupled with miR-mediated regulatory networks (31-33). Recent findings further indicate the involvement of *SPOCK* gene family members in cardiovascular pathology mediated through epigenetic reprogramming occurring within cardiac tissues (34). A comprehensive understanding regarding epigenetically governed control mechanisms dictating expression patterns exhibited by *SPOCK* genes holds paramount significance towards unraveling their

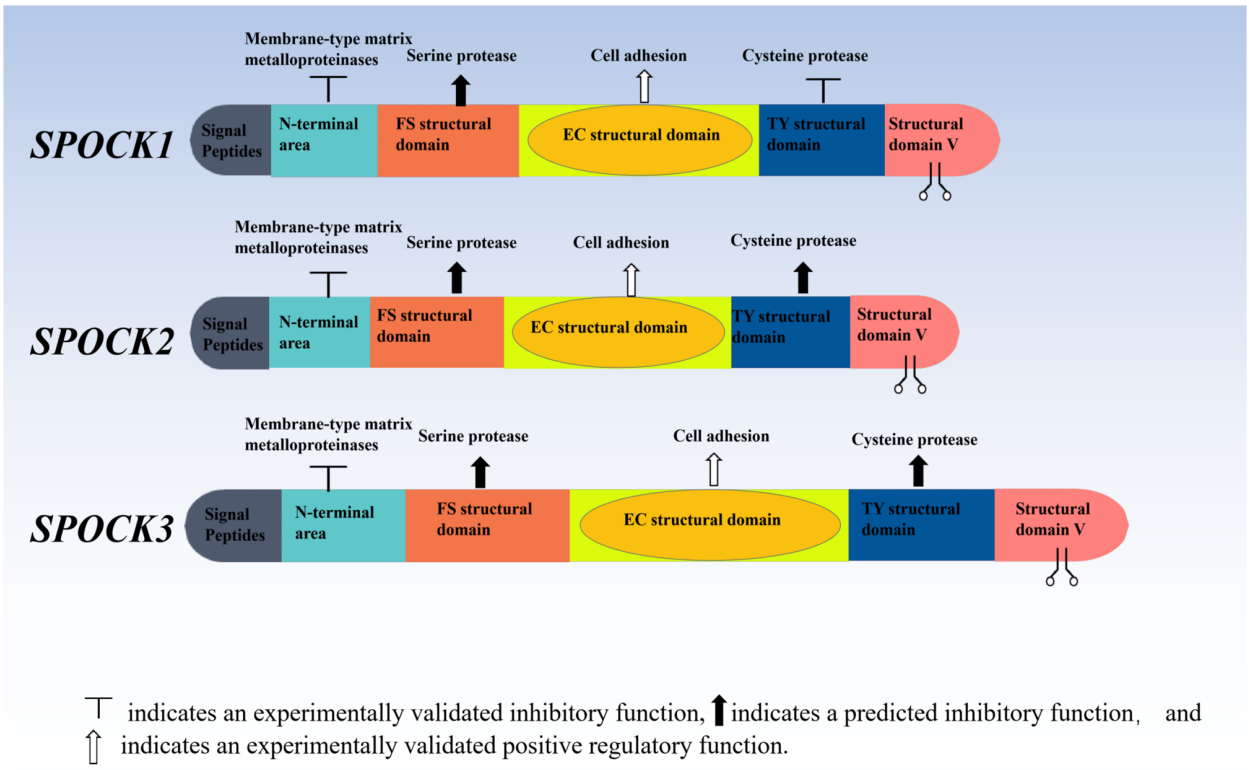

Figure 1.Structure of SPOCK subfamily proteins. SPOCK, SPARC/osteonectin CWCV and Kazal-like domain protein subfamily. TY, thyroglobulin type-1 domain; FS, follistatin.

intricate involvements and spanning physiological homeostasis pathological state. While significant progress has been made, further research is required to fully explore the therapeutic potential of genetically modifiable loci located on *SPOCK*.

Functional insights. The human brain exhibits the highest expression levels of *SPOCK1* (35-37), particularly in the thalamus. Previous cell culture studies have shown that *SPOCK1* can suppress adhesion and facilitates axonal growth (38). It is also associated with axon regeneration following injury. In addition, *SPOCK1* has been reported to affect neuronal system development and interconnectivity (39), whilst being crucial for synaptic plasticity (40,41). Elsewhere, *SPOCK1* is highly expressed in the adrenal gland, endometrium, prostate, heart, kidney, gallbladder and testis, with much lower expression levels in the bone marrow, colon, duodenum, liver, lung, lymph nodes, ovary, pancreas, salivary glands, skin, small intestine, stomach and thyroid tissues. In developing mice, *SPOCK1* is mainly expressed in the central and peripheral nervous systems. By contrast, in adult mice, *SPOCK1* is only detectable in the brain, particularly in the hippocampal neurons.

Human tissues express *SPOCK2* widely, with particularly high expression observed in lung tissues. The brain, adrenal gland, appendix, bone marrow, kidney, lymph nodes, spleen and testes also readily express *SPOCK2* (20,31). It exhibits low expression in the colon, duodenum, endometrium, esophagus, fat, heart, liver, ovary, pancreas, placenta, prostate, salivary glands and skin. Functionally, *SPOCK2* has been documented to regulate various stages of neurogenesis. This suggests that *SPOCK1* and *SPOCK2* form a new family of calcium-binding proteoglycans that participate in various steps of neurogenesis (25). According to its expression pattern, *SPOCK2* may be involved in alveolar formation, regulating the equilibrium between type 1 and 2 epithelial alveolar cells in the lungs. Furthermore, *SPOCK2* may also serve as a susceptibility gene for bronchopulmonary dysplasia (42).

Human tissues express *SPOCK3* at higher levels in the adrenal gland, brain, prostate and ovaries, whilst at lower levels in the bladder, endometrium, appendix, small intestine and colon. Previous studies on *SPOCK3* were mainly performed on animal models. During embryonic development in mice, the *SPOCK3* gene has been observed in the neurons of the vascular system, liver, inner ear and central nervous system (CNS) (43). However, in adulthood, *SPOCK3* is only expressed in the brain, suggesting that it may serve a critical role in the CNS. According to immunohistochemistry analysis, *SPOCK3* expression was found to be the most abundant in adult mouse brain regions, particularly the olfactory bulb, cortex, thalamus, hippocampus and striatum (44). Notably, the thalamus, hippocampus and striatum are regions that have been repeatedly reported to be associated with psychiatric disorders (45-47). Therefore, previous studies have explored the association between *SPOCK3* and defective hyperactivity disorder, which identified single nucleotide polymorphisms in t*SPOCK3* gene loci (48). Mechanistically, SPOCK1 has been reported to function as a potent competitive inhibitor of cathepsin L (CTSL). Its inhibitory activity on CTSL is independent of its chondroitin sulphate GAG and heparan sulphate GAG, which is optimal at pH 5.5 and 7.2. By contrast, CTSL inhibition by SPOCK1 is dependent on its TY homology domain (49). Given

that *SPOCK2* and *SPOCK3* also have TY domains similar to *SPOCK1,* it remains possible that *SPOCK2* and *SPOCK3* can also function as CTSL inhibitors.

3. Regulatory effects of SPOCKs in tumor development

Tumor metastasis is the malignant tumor growth process at other sites secondary to its primary site and is one of the main causes of tumor-related mortality (50,51). The ECM serves as the first barrier to tissue tumor metastasis (52,53). As an ECM glycoprotein, *SPOCK* has been garnering attention because of its possible role in the pathogenesis of malignancies. As the number of in-depth studies on the SPOCK family increased, it became evident that this protein family is associated with the occurrence and progression of solid tumors.

Roles of SPOCK1 in tumor development. *SPOCK1* is the most extensively studied gene in the SPOCK family. *SPOCK1* has frequently been reported to function as an oncogene. A multitude of miRNA-targeted genes have been observed to regulate the occurrence and development of cancer, leading to successful tumor cell metastasis by inhibiting the formation of MMP2. In addition, *SPOCK1* has been previously found to promote epithelial-mesenchymal transition (EMT) in tumor cells to render them more invasive. It can also enhance the malignancy and invasiveness of tumor cells by participating in the Wnt and PI3K signaling pathways. These observations suggest that *SPOCK1* is a regulatory factor in numerous malignant tumors.

Activation of EMT. EMT is the cell transformation from an epithelial to a mesenchymal cell phenotype (54,55). It is involved in various physiological and pathological processes, such as embryogenesis, tissue healing and fibrosis. In particular, EMT leads to the weakening of cellular junctions, which enhances cell motility and invasiveness, promoting cancer development.

EMT is regulated by a number of EMT-inducible transcription factors, such as members of the Zinc finger E-box-binding homeobox, Snail and Slug families. Various other signaling pathways can also regulate the EMT process, where Wnt, TGF-β and Notch are prominent promoters of EMT. In addition, E- and N-cadherins (epithelial markers) and vimentin (mesenchymal markers) are important EMT markers. *SPOCK1* has been reported to promote EMT progression in a variety of cancers by upregulating the expression of N-cadherin, Snail, vimentin and Slug, whilst downregulating that of E-cadherin (21). However, to the best of our knowledge, studies on the relationship between other members of the *SPOCK* family and EMT remain elusive, which warrants further study.

SPOCK1 in gastric cancer (GC). Tumor cell metastasis is a major obstacle to the treatment of various cancers (56). *SPOCK1* has been previously shown to facilitate cancer metastasis in various cancer types. Chen *et al* (57) revealed an association between elevated *SPOCK1* expression and that of EMT-related markers in GC tissues, metastasis and poor GC prognosis. In addition, downregulating *SPOCK1* expression was found to significantly inhibit GC-cell invasion and metastasis, whereas upregulating *SPOCK1* expression resulted in the opposite effect. An investigation into the mechanism of *SPOCK1*-induced aggressive metastasis of GC cells demonstrated that *SPOCK1*-induced EMT could promote aggressive metastasis in GC cells, suggesting *SPOCK1* to be a novel therapeutic target for GC.

SPOCK1 in prostate cancer. Prostate cancer is a representative non-skin cancer and the second major cause of cancer-related mortality among men in the US. Metastasis is a major cause of mortality (58). Several studies (22,59-61) have previously evaluated the role of *SPOCK1* in prostate cancer progression. Prostate cancer tissues express substantially higher quantities of *SPOCK1* compared with those in non-cancerous tissues. In addition, metastatic cells showed significantly higher *SPOCK1* expression compared with that in non-metastatic cells. *SPOCK1* knockdown resulted in cell cycle arrest at the G_0/G_1 phase in prostate cancer cells. Conversely, overexpression of *SPOCK1* resulted in cell cycle arrest in the S phase of human prostate epithelial cells (RWPE-1). This suggests that *SPOCK1* may be involved in the aberrant division of prostate cancer cells, providing a novel target for the treatment of prostate cancer and further study in prostate cancer stem cells. In addition, apoptosis was previously found to be increased after *SPOCK1* expression was downregulated in PC3 cells, whilst cell migration and invasion were increased *in vitro* when *SPOCK1* was overexpressed. Furthermore, metastatic lung nodules in mice were significantly reduced when *SPOCK1*-null PC3 cells were injected. These findings suggest that *SPOCK1* is a mediator of prostate cancer metastasis and malignant growth. Ma *et al* (62) previously found that the mRNA expression of *SPOCK1* is significantly higher in cancer tissue compared with that in adjacent lesions. In addition, a positive association was found between *SPOCK1* and Cyclin D1, c-Myc, vimentin, MMP2 cycle and proliferation indicators. This indicates that the high expression of *SPOCK1* in cancer is associated with changes in proliferation and the expression of genes linked to invasion.

SPOCK1 in breast cancer. Fan *et al* (63) previously revealed that immortalized breast cancer cells exhibit enhanced invasive abilities after overexpressing *SPOCK1*. In addition, another previous study also assessed the association between *SPOCK1* expression and the clinicopathological features of invasive ductal carcinoma (64). High *SPOCK1* expression was found to be associated with the pathological tumor size (50). Therefore, *SPOCK1* expression may serve as an independent predictive marker of poor survival outcomes. The study of *SPOCK1* expression in non-mammary invasive ductal carcinoma subtypes has shown that *SPOCK1* expression is abundant in intraductal carcinoma *in situ* in the breast and is associated with EMT. In summary, *SPOCK1*-targeted therapy is promising and offers novel ideas for clinical application in patients with breast cancer.

Effect of SPOCK1 on MMPs. The ECM is a non-cellular matrix that incorporates various cellular components, including elastin, collagen, non-collagen, proteoglycans and aminoglycans. Previously, the ECM was considered to be a static scaffold for cells and tissues. However, in the proceeding decades, the ECM has been gradually found to also regulate cell survival, proliferation, polarity, shape, migration and metabolism, making it an important component in the tumorigenesis of cancers (65,66). Matricellular proteins are non-structural ECM molecules that mainly regulate the interaction between

the ECM and cells. They typically contain different domains that can interact with the ECM, cell surface receptors, growth factors and cytokines to regulate the transmission of molecular signals and communication among cells. In addition, matricellular proteins can also bind a number of intrinsic enzymes, thereby modulating ECM component synthesis and degradation. During tissue damage, changes in the matrix composition can affect tissue regeneration. Therefore, the alteration of MCPs can influence the occurrence and development of specific diseases.

MMPs form a family of endopeptidases that degrade the ECM. They are known to regulate a myriad of physiological activities and the progression of different diseases, such as cancer and inflammation, rendering them potential targets for disease therapy. Previous studies have demonstrated that the overexpression of *SPOCK1* can increase MMP2 and MMP9 expression and activity, which in turn promotes ECM degradation (67,68).

Activation of the PI3K/Akt signaling pathway. The PI3K/Akt signaling pathway is of significant importance in cancer research. This pathway has been demonstrated to be abnormally activated in various tumors, thereby promoting occurrence and progression. In addition, PI3K/Akt has been shown to serve a key role in the regulation of a diverse array of intracellular processes, including cell cycle regulation, cell proliferation, apoptosis and migration. In addition, it has been implicated in the maintenance of tumor stem cells and the development of drug resistance. In recent years, numerous inhibitors targeting this pathway have been developed, which revealed considerable anti-tumor activity in clinical trials. In particular, the *SPOCK1* gene has been demonstrated to induce tumorigenesis in a variety of cancers by activating the PI3K/Akt signaling pathway.

SPOCK1 in colorectal cancer. Zhang *et al* (69) previously discovered that colorectal cancer tissues exhibit elevated *SPOCK1* expression compared with that in adjacent normal tissues. In addition, high expression levels of *SPOCK1* in patients with colorectal cancer were found to be associated with tumor size and lymphatic system metastasis (55). Furthermore, knocking down *SPOCK1* expression was found to restrict cell proliferation, decrease tumorigenicity and increase cell apoptosis. *SPOCK1* can also promote malignant proliferation by regulating the PI3K/Akt signaling pathway, suggesting that *SPOCK1* may serve as a potential therapeutic and preventive target for colorectal cancer.

SPOCK1 in glioblastoma multiforme. Glioblastoma multiforme is one of the most aggressive forms of human brain malignancies with a dismal prognosis, owing to its invasive nature and high recurrence rate. Treatment of recurrent glioblastoma multiforme (RGS) is particularly challenging due to its resistance to chemotherapy. *SPOCK1* expression was observed to be significantly upregulated in RGSs, where it can regulate the migration, invasion and EMT processes. In addition, downregulation of *SPOCK1* significantly sensitized these cells to temozolomide, resulting in a substantial reduction in their aggressiveness and malignancy. *SPOCK1* was also observed to mediate drug resistance in glioblastoma multiforme by regulating the PI3K/Akt signaling pathway (27), contributing to a novel direction for genetic testing for the treatment of advanced glioblastoma.

SPOCK1 in gallbladder cancer (GBC). GBC is one of the leading causes of cancer-associated mortality worldwide, with a poor prognosis and a 5-year overall survival (OS) rate of only 5% (70). *SPOCK1* expression has been reported to be increased in GBC. Shu *et al* (71) previously investigated the impact of *SPOCK1* on the progression and prognosis of patients with GBC and found that *SPOCK1* activated the PI3K/Akt signaling pathway, which inhibited cell apoptosis whilst promoting proliferation and metastasis. Therefore, patients with GBC may benefit from targeting *SPOCK1* as a prognostic or therapeutic marker (27).

SPOCK1 in pancreatic cancer. Pancreatic cancer is well-known for its high mortality rate (72,73). The most significant characteristic of pancreatic ductal adenocarcinoma is the presence of massive stromal deposits. Given the complexity of tumor-stromal interactions in pancreatic ductal adenocarcinoma, identifying stromal proteins that can promote tumors is essential. *SPOCK1* was discovered as a possible candidate protein. In addition, *SPOCK1* expression is predominantly stromal, where its high expression can result in poor disease outcomes. Functional assessment in co-culture assays revealed that *SPOCK1* can potently influence extracellular collagen matrix composition and promote pancreatic ductal adenocarcinoma proliferation (64,74). This suggests that elevated *SPOCK1* expression is a contributing factor in pancreatic cancer progression, providing novel ideas for future drug research and development.

SPOCK1 in osteosarcoma. In osteosarcoma (75,76), high levels of *SPOCK1* expression have been associated with tumor size, metastasis, staging and pathology, where its downregulation was found to inhibit osteosarcoma cell proliferation *in vitro* and weaken tumorigenicity in nude mice. In addition, *SPOCK1* may promote osteosarcoma cell development through the mTOR/S6 kinase signaling pathway, suggesting a novel therapeutic strategy for treating osteosarcoma.

SPOCK1 in hepatocellular carcinoma (HCC). Li *et al* (77) previously examined the *SPOCK1* expression profile in 135 pairs of HCC and adjacent paraneoplastic tissues, revealing that 60% of the HCC samples had increased expression levels of *SPOCK1* mRNA and protein compared with those in paraneoplastic tissues. In addition, OS and disease-free survival (DFS) rates decreased significantly when *SPOCK1* expression was increased. In terms of the mechanism, *SPOCK1* was found to prevent cytochrome *c* release, and to inhibit HCC cell apoptosis by activating Akt and enhance MMP9 expression and activity, rendering HCC cells more invasive (Fig. 2).

SPOCK1 is regulated by numerous miRNAs. SPOCK1 can serve as a potential target for numerous miRNAs, where its elevation leads to cancer deterioration.

SPOCK1 in non-small cell lung cancer (NSCLC). Yu *et al* (78) and Xu *et al* (79) previously identified *SPOCK1* in three cell types using TargetScan and miRDB, two typical online target gene prediction programs. This previous study investigated the role of miR-130a-3p and its target gene *SPOCK1* in the etiopathogenesis of tobacco smoke-induced human lung cancer (78). The results showed that *SPOCK1* expression was high in BEAS-2B cells with unexposed bronchial

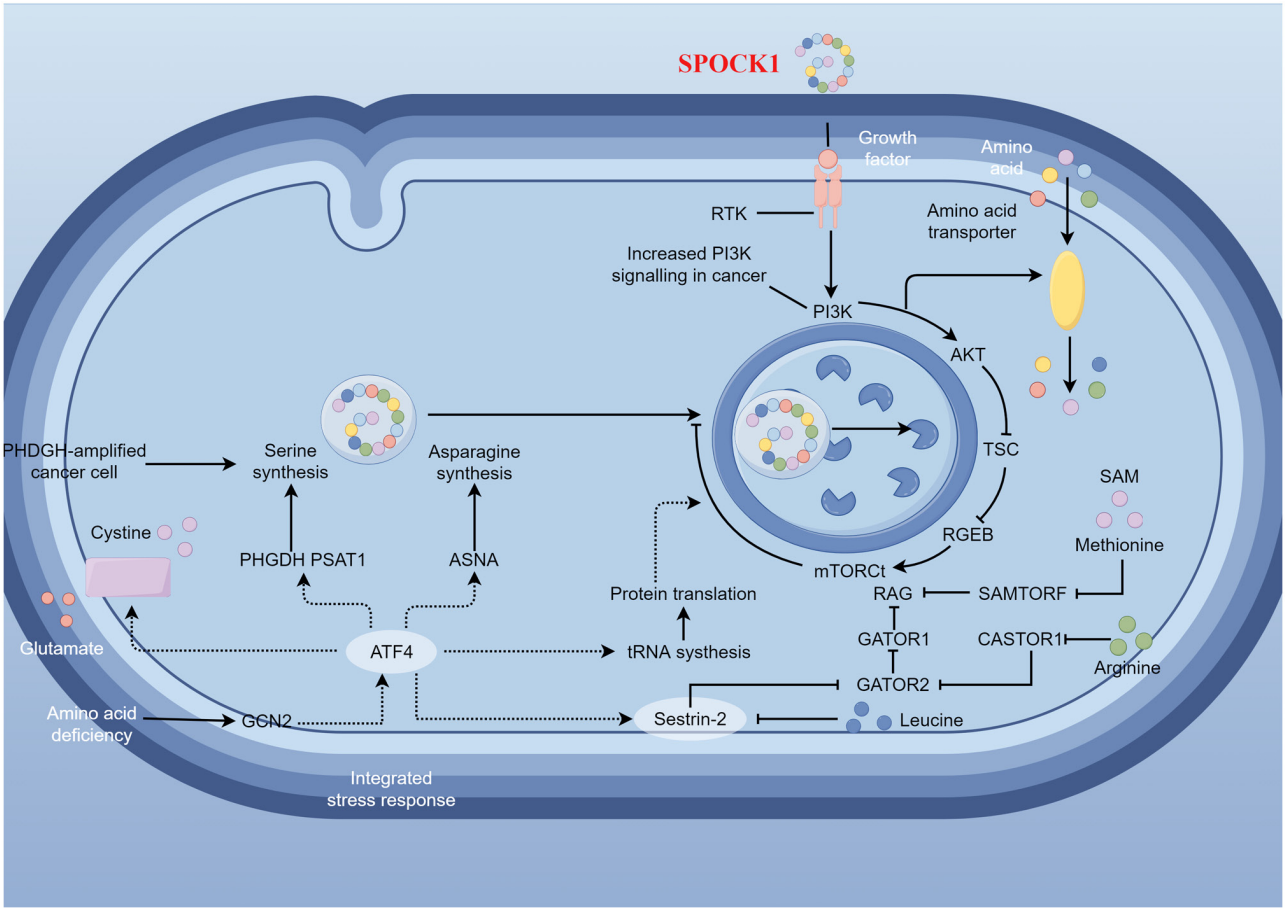

Figure 2. SPOCK1 promotes cancer occurrence by inducing the PI3K/Akt signaling pathway (by Figdraw). SPOCK, SPARC/osteonectin CWCV and Kazal-like domain protein subfamily.

epithelium. In addition, significant inhibition of cell migration was observed in transformed S30 cells exposed to cigarette smoke, regardless of *SPOCK1* silencing. In lung cancer A549 and H1299 cells transformed using S30, *SPOCK1* expression was reduced following miR-130a-3p upregulation, suggesting that cigarette-transformed cells exhibit a behavior comparable to lung cancer cells and have a reduced ability to migrate. In addition, *SPOCK1* was previously found to be overexpressed in human NSCLC cells and tissues. NSCLC cell proliferation, colony formation, migration and invasion were also reported to be significantly inhibited by the downregulation of *SPOCK1* expression *in vitro*. In addition, silencing *SPOCK1* may inhibit Wnt/β-catenin pathway activation (12,80).

SPOCK1 in head and neck squamous cell carcinoma (HNSCC). In HNSCC cells, Koshizuka *et al* (81) found that *SPOCK1* expression was controlled by miR-150-5p and miR-150-3p. In particular, when *SPOCK1* expression is knocked down in HNSCC cells, they tended to exhibit a more aggressive behavior. High *SPOCK1* expression was then subsequently confirmed in clinical specimens of HNSCC. According to The Cancer Genome Atlas database, patients expressing higher *SPOCK1* exhibited a substantially shorter OS rate.

SPOCK1 in esophageal squamous cell carcinoma (ESCC). Osako *et al* (82) previously found significantly diminished pre-miR-150-5p and miR-150-3p expression levels in ESCC tissues analyzed using RNA sequencing-based methods. Further studies into miRNA target genes using a combination of genome-wide gene expression analysis and database searches subsequently revealed that *SPOCK1* may serve as a candidate target for miR-150-5p and miR-150-3p in ESCC cells. Luciferase reporter gene assays provided further evidence demonstrating the direct modulation of *SPOCK1* by these aforementioned miRNAs. Silencing *SPOCK1* expression was then found to suppress cancer cell migration and invasion. In summary, downregulation of the pre-miR-150 chain, leading to *SPOCK1* overexpression in ESCC, may be a pathogenic mechanism of this cancer (82,83).

Regulation of SPOCK1 by miRNAs in HCC. Li *et al* (77) and Zhu *et al* (84) detected differentially expressed miRNAs in HCC to assess the impact of their expression on the proliferation, invasion and apoptosis in HCC cells, which found miR-139-5p, miR-940 and miR-193a-5p. The overexpression of *SPOCK1*, a common target gene of all three aforementioned genes in HCC, contributed to a further increase in HCC cell malignancy, proliferation and invasion, whilst suppressing apoptosis. Therefore, miR-139-5p, miR-940 and miR-193a-5p may target *SPOCK1* and inhibit HCC development. (Fig. 3).

Role of SPOCK2 in tumor development. SPOCK2 holds particular promise and it is becoming a subject of intense

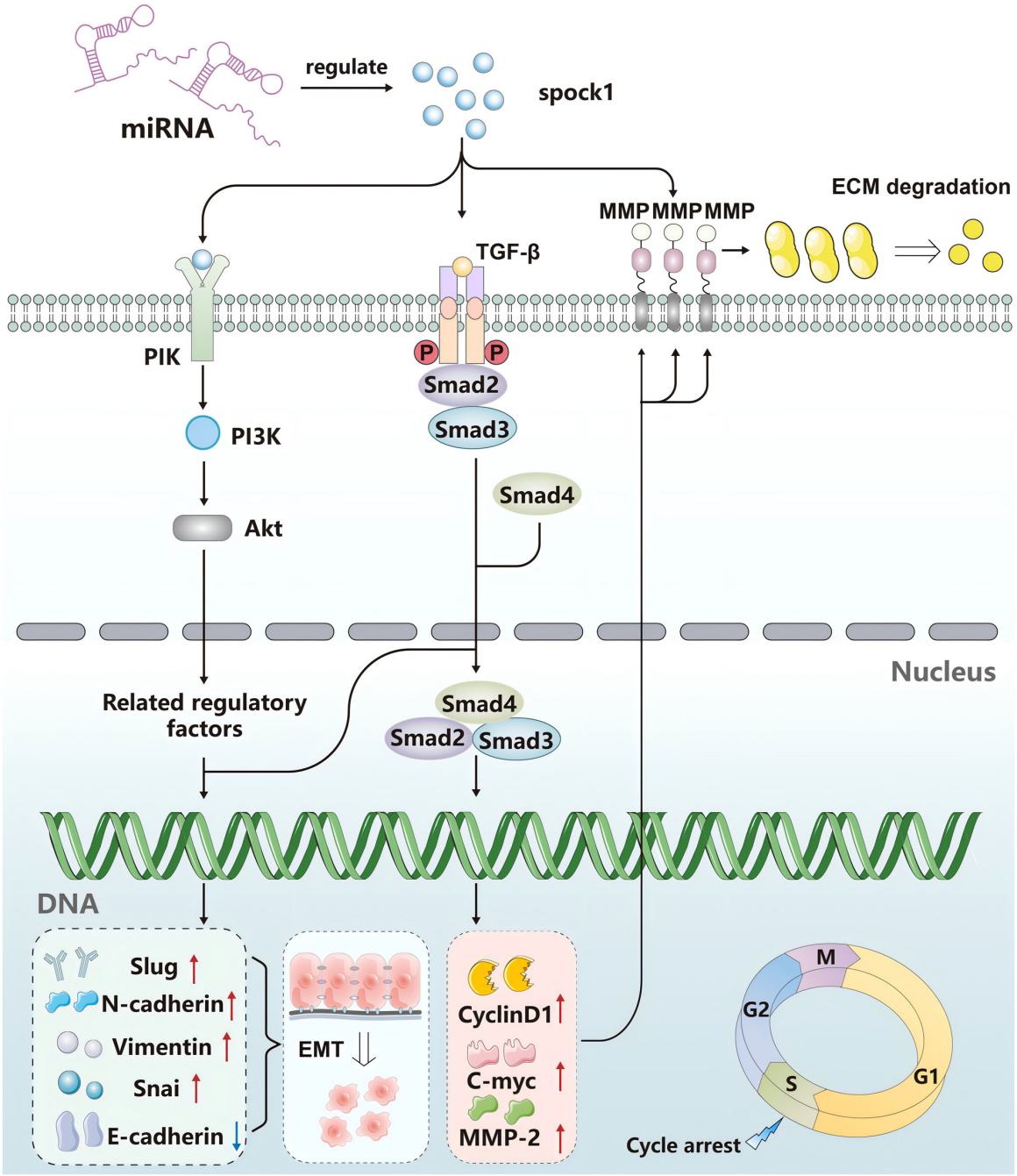

Figure 3. Regulatory mechanisms involving SPOCK1. SPOCK, SPARC/osteonectin CWCV and Kazal-like domain protein subfamily.

research. Initially, it was considered to serve as a tumor suppressor that is closely associated with methylation. *SPOCK2* does not inhibit membrane-type matrix metalloproteinases (MT-MMPs) but instead antagonizes the inhibition of MT-MMPs by other SPOCK family members. Furthermore, an imbalance in *SPOCK2* has been frequently reported to result in the expression of oncogenes.

SPOCK2 in prostate cancer. Prostate cancer is a common malignancy in men, where genetic factors and exposure to interventions increase its risk. According to a previous study by Verma *et al* (85), *SPOCK2* and non-structural maintenance of chromosomes element 1 homolog (*NSE1*) can regulate methylation in prostate cancer. Combining *NSE1* and *SPOCK2* hypermethylation was found to increase the ability to distinguish tumors from normal tissues with 80% sensitivity and 95% specificity (86). Prostate cancer tissues were also found to exhibit lower *SPOCK2* expression levels compared with benign prostatic hyperplasia tissues. In addition, *SPOCK2* was observed to suppress the expression of MT1-MMPs and MMP2s, whilst inhibiting MMP2 activation in DU145 and LNCaP cells. Upregulation of *SPOCK2* can inhibit prostate cancer cell (DU145 and LNCaP) invasion and migration (87). Another previous study (88) proposed that *SPOCK2* methylation may be a candidate biomarker for prostate, colon, ovarian (89) and breast cancers compared to normal paracancerous tissues. Further research should focus on characterizing *SPOCK2* as a potential biomarker or a treatment target for prostate cancer.

Table I. Summary of information on the distribution of the SPOCK family, signaling pathways and associated diseases.

Item	*SPOCK1*	*SPOCK2*	*SPOCK3*	(Refs.)
Main expression locations	Brain, prostate, testis	Brain, lungs, testicles, kidneys	Brain, ovaries, prostate	(35-38)
Related signaling pathways	PI3K/Akt signaling pathway, Wnt/β-catenin signaling pathways, Akt/mTOR signaling pathway, mTOR-S6K signaling pathway	Unknown	Unknown	(19,28,68,70,79, 103,105-107)
Upregulated expression-related diseases	Liver fibrosis, liver cancer, stomach cancer, oesophageal cancer, pancreatic cancer, gallbladder cancer, colorectal cancer, lung cancer, prostate cancer, urothelial cancer, ovarian cancer, AD brain tumour, breast cancer, oral submucosal fibrosis, squamous cell carcinoma of the head, osteosarcoma	BPD, ovarian cancer	ADHD, BPD	(19,28,35-38,79,103,104,106, 107)
Downregulated expression-related diseases	Unknown	Pancreatic cancer, lung cancer, prostate cancer, endometrial cancer, brain tumor	Prostate cancer	(19,30,89,108)

AD, Alzheimer's disease; BPD, bronchopulmonary dysplasia; ADHD, attention deficit hyperactivity disorder; SPOCK, SPARC/osteonectin CWCV and Kazal-like domain protein subfamily.

SPOCK2 in astrocytoma. Astrocytoma is the most common primary brain malignancy. It typically occurs in neuroectodermal tumor tissues and grows rapidly with high degrees of malignancy (90). The most common histological type is glioblastoma, a highly heterogeneous and invasive malignancy with a median survival period of 12-15 months after surgery and chemotherapy (91). Tobey *et al* previously analyzed a microarray dataset containing pediatric and adult astrocytoma samples to screen for differentially expressed genes. *SPOCK2* expression was found to be downregulated in all tests. Therefore, dysregulation of *SPOCK2* may serve a role in the development of malignant astrocytomas and represent a novel therapeutic target. However, further studies are necessary (92).

SPOCK2 in endometrial cancer. Endometrial cancer is a representative type of uterine cancer. Owing to insufficient early symptoms and signs, lack of testing methods and poor survival rate after late-stage detection, finding early biomarkers of endometrial cancer is crucial. Ren *et al* (93) have previously found that the *SPOCK2* gene may serve as a biomarker for endometrial cancer. Specifically, these aforementioned studies concluded that patients with endometrial cancer have lower *SPOCK2* expression compared with that in healthy individuals. Lower *SPOCK2* protein expression levels were also found to be associated with distant metastasis and myometrial infiltration. By upregulating SPOCK2, endometrial cancer cells were observed to exhibit stunted proliferation, invasion and adhesion, whereas apoptosis was increased. Mechanistically, it restricted MT1-MMP and MMP2 expression, whilst also inhibiting MMP2 activation (Fig. 4).

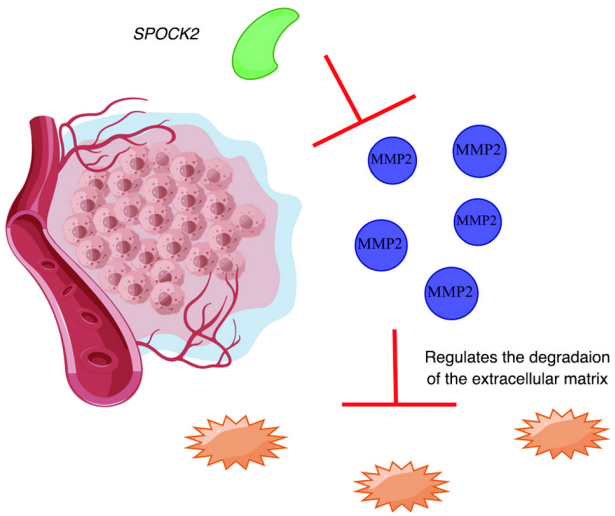

Figure 4. SPOCK2 inhibits MMP2 expressions and activation, leading to an imbalance in the degradation that regulates the extracellular matrix. SPOCK, SPARC/osteonectin CWCV and Kazal-like domain protein subfamily.

SPOCK2 in ovarian cancer. Lou *et al* (19) previously reported significantly elevated expression levels of *SPOCK2*

in ovarian cancer and suggested that the dysregulation of hsa-miR-363-3p/*SPOCK2* may worsen the progression of this cancer. The hsa-miR-363-3p/*SPOCK2* axis is involved in regulating the actin cytoskeleton. Mechanistically, *SPOCK2* may regulate the actin cytoskeleton, thereby affecting cell adhesion, invasion, migration and ultimately ovarian cancer progression (94). These extensive studies suggest that the *SPOCK2* gene is not merely a suppressor gene but can also serve as an oncogene in ovarian cancer, which requires further exploration.

Role of SPOCK3 in tumor development. The *SPOCK3* gene has not been explored in depth compared with *SPOCK2* and *SPOCK1*. This may be due to its similarity with *SPOCK1* in terms of structure and function. By contrast, studies on *SPOCK1* have been more comprehensive, which may partially reflect some of the possible functions of *SPOCK3*. The N-Tes splice variant of *SPOCK3* (7,95) was previously found to inhibit MT1-MMP- and MT3-MMP-mediated MMP2 activation, with the key sequence located at amino acid residues 33-84 after the N-terminal signal peptide (96).

Luo *et al* (30) previously compared tumor and control tissues of patients with prostate cancer and found a clear positive association between *SPOCK3* expression and DFS of patients with prostate cancer. In addition, patients with low *SPOCK3* expression levels had inferior DFS compared with those with high *SPOCK3* expression levels (44,97). This suggests that *SPOCK3* is a potential prognostic marker for treatment or prognosis (98). Similarly, in previous studies on glioma, *SPOCK3* downregulation was found to be associated with the inhibition of glioma cell migration and invasion. *SPOCK3* was previously found to serve an important role in regulating the physiology of glioma cells (95,99). However, to the best of our knowledge, relatively few studies have been conducted on the function of *SPOCK3* and the regulatory relationship between *SPOCK3* and malignancies.

4. Outlook

In summary, the present review summarized existing research on the expression profile of the most important members of the SPOCK family, the signaling pathways they were associated with and their possible roles in a variety of malignancies (Table I). Cancers typically develop in multiple steps, covering multiple stages, including the activation of multiple proto-oncogenes and inactivation of tumor suppressor genes. The specific mechanism underlying the role of the SPOCK family in the apoptosis, invasion and metastasis of tumor cells require further investigation. It is hypothesized that the *SPOCK* family of genes may regulate apoptosis through the PI3K/Akt and Wnt/β-catenin signaling pathways in malignant tumors. This may in turn regulate the EMT process, causing the tumor cells to either gain or lose their metastatic and invasive abilities on the one hand, whereas by regulating the activity of Bax and Bad (pro-apoptotic proteins) on the other hand. However, the SPOCK subfamily may yet have specific relevance to certain cancers, such that large-scale fundamental and clinical studies are required before the SPOCK family can be exploited as a biomarker for clinical applications. In non-malignant diseases, overexpression of *SPOCK1* may promote stellate cell activation, proliferation and migration through activation of the integrin α5β1/PI3K/Akt signaling pathway, thereby enhancing liver fibrosis (100). In bronchopulmonary dysplasia, the expression of *SPOCK2* is gradually upregulated in a time-dependent manner during the transition from AT2 to AT1 cells, which can be used as one of the key markers for this transition (101). Further research on the SPOCK family may provide a breakthrough in diagnosing and treating specific malignant diseases in the near future.

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Availability of data and materials

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Authors' contributions

MX contributed to the study design, literature search and selection and analysis of the literature/information. JX and EJ were involved in the writing process, including manuscript drafting, editing and reviewing, as well as the creation of figures and tables. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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