Role of SPAG6 in regulating physiological functions and tumorigenesis (Review)

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Abstract. Sperm-associated antigen 6 (SPAG6) belongs to the cancer/testis antigen family. It is a microtubule-binding protein located on chromosome 10p12.2 and it plays an important role in various physiological processes, including ciliary movement, immune synapse formation and neurodevelopment. Abnormal SPAG6 expression occurs in multiple malignancies and developmental disorders; however, its underlying molecular mechanisms in tumorigenesis, tumor progression, clinical outcomes and therapeutic response have not been presented. This review provides a comprehensive overview of the physiological functions of SPAG6 and its mechanisms in disease, with a focus on its expression profile, function and association with disease progression and treatment response in hematologic malignancies (e.g., myelodysplastic syndrome, acute myeloid leukemia and B-cell acute lymphoblastic leukemia) and solid tumors (e.g., breast cancer, lung cancer and osteosarcoma). SPAG6 promotes tumor progression and drug resistance by attenuating the cell cycle and through epigenetic modifications and remodeling of the tumor immune microenvironment. In addition, it may serve as a diagnostic and prognostic marker for various diseases as well as a therapeutic target.

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Abbreviations: AK, adenylate kinase; AKT, AKT serine/threonine kinase; ALDH1, aldehyde dehydrogenase 1 family member A1; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AMPK, AMP-activated protein kinase; B-ALL, B-cell acute lymphoblastic leukemia; BCR::ABL1, BCR (BCR activator of RhoGEF and GTPase)::ABL1 (ABL proto-oncogene 1 non-receptor tyrosine kinase); BL, burkitt lymphoma; circMYH9, circular RNA myosin heavy chain 9; DNA, deoxyribonucleic acid; DUSP1, dual specificity phosphatase 1; EIF4A3, eukaryotic translation initiation factor 4A3; ERK, extracellular signal-regulated kinase; G1 phase, Gap 1 phase of the cell cycle; IFN-α, interferon alpha; JAK, Janus kinase; LUSC, lung squamous cell carcinoma; MAP1, microtubule-associated protein 1; MAPK, mitogen-activated protein kinase; MDS, myelodysplastic syndromes; MM, multiple myeloma; MPN, myeloproliferative neoplasm; mRNA, messenger RNA; mTOR, mechanistic target of rapamycin; MYC, MYC proto-oncogene; MYO1D, myosin ID; Nanog, Nanog homeobox; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; Smad, SMA- and MAD-related protein; Sox2, SRY-box transcription factor 2; SPAG6, sperm associated antigen 6; STAT1, signal transducer and activator of transcription 1; TGF-β, transforming growth factor beta; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; ULK1, Unc-51 like autophagy activating kinase 1

Contents

1. Introduction

Sperm-associated antigen 6 (SPAG6), also known as Repro-SA-1, is a homolog of *Paramecium falciforme* paralytic

Key words: SPAG6, cancer/testicular antigen, prognostic marker, molecular targeted therapy, DNA methylation

flagellum 16 (PF16). It is located in the axonemal center and is a microtubule-associated protein (1). SPAG6 has several functions, including sperm acrosome formation; ciliary/flagellar movement; immune synapse formation and function; neuronal proliferation and differentiation; fibroblast morphology, growth and migration; and middle ear and Eustachian tube epithelial cell function. It belongs to the cancer/testis antigen (CTA) family and its expression is associated with various cancers. It may also represent a tumor prognostic marker and therapeutic target (2).

This review involved a systematic literature search to comprehensively gather publications focused on the physiological functions of SPAG6 and its role in oncology. The databases searched included PubMed, Web of Science and other knowledge service platforms, covering the time period from each database's inception to June 2025. The retrieved literature was screened and data were extracted based on clearly defined inclusion and exclusion criteria. This systematic approach aimed to elucidate the molecular functions of SPAG6 and its mechanisms in tumor initiation and progression and provide a foundation for subsequent comprehensive analyses.

2. Molecular structure and function of SPAG6

SPAG6 was first identified by Neilson *et al* (3) in 1999. They screened a cDNA library from the testes of infertile men exhibiting high-titer, anti-sperm autoantibodies in the serum and discovered that *SPAG6* encodes a new antigen. This gene was previously known by several names, including *Repro-SA-1*, *CT141* and *RP11301N24.4*; however, the HUGO Gene Nomenclature Committee (https://www.genenames.org/data/gene-symbol-report/#!/hgnc_id/HGNC:11215) approved the official symbol *SPAG6*. It is located on the 10p12.2 region of human chromosome 10 and encodes four alternative splicing isoforms. The full-length transcript consists of 10 exons and the translated protein has 16 domains, including 8 conserved armadillo repeats, which play a role in mediating protein-protein interactions (4,5). SPAG6 is primarily expressed in tissues containing ciliated cells, such as testicular germ cells, lung tissue, nervous system and inner ear; however, it is not present in the prostate, spleen, thymus, small intestine, colon, peripheral blood leukocytes, heart, placenta, liver, muscles, kidneys and pancreas. Thus, it is considered a member of the CTA family and may serve as a tumor prognostic biomarker and therapeutic target (2,6). *SPAG6* encodes a microtubule-associated protein that attenuates cell growth, differentiation, migration and cell polarity regulation (7,8). It may also represent a novel tumor serum biomarker, which supports its inclusion as a member of the CTA family and suggests that its transcripts are targets for immunotherapy. Abnormal *SPAG6* expression or function is associated with the development of various solid tumors, including breast and lung cancers (2,9). The role of SPAG6 has also been reported in hematological malignancies. Patients with acute myeloid leukemia (AML), lymphoma, myeloproliferative neoplasms (MPNs) or myelodysplastic syndromes (MDSs) often exhibit high and sustained *SPAG6* expression, which is significantly associated with poor outcomes (10-13).

SPAG6 regulates various physiological functions. SPAG6 plays a role in constructing and maintaining the cytoskeleton (2,14,15). The amino acid sequence derived from the full-length human cDNA is highly homologous to the product of the PF16 site in *Chlamydomonas reinhardtii*. The PF16 protein is localized to the central pair structure of the flagellar axoneme, which consists of a pair of central microtubules, nine sets of peripheral dyads and kinesin arms attached (16-18). As a CTA, PF16 exhibits tissue-specific expression. It is frequently expressed in immune-privileged tissues, such as the testis; however, it is abnormally activated in tumor tissues. SPAG6 can induce spontaneous humoral and cellular immune responses and is relatively safe in normal tissues, indicating that it is a suitable candidate for tumor immunotherapy (19,20). Under physiological conditions, SPAG6 is frequently expressed in cell types with ciliary structures, such as sperm cells, neural tissue, the inner ear and respiratory epithelial cells. It regulates microtubule/cytoskeletal dynamics and their resulting cell function (e.g., sperm maturation and neural system development) through binding to microtubule proteins (21-23). Notably, SPAG6 function is not limited to ciliated cells, as it also plays an important role in nonciliated cells. A study revealed its novel role in the mouse vestibular system. SPAG6 deficiency leads to vestibular dysfunction, abnormal ultrastructure of vestibular hair cells and accelerated apoptosis (24). Furthermore, it is involved in regulating neuronal migration, developmental differentiation and neurogenesis, which emphasizes its role in the nervous system. Because of their functional similarities, including signal transduction and polarity maintenance, immune synapses and cilia are closely related. Because of the central role of SPAG6 in ciliary movement, it may also be involved in immune regulatory processes (25,26).

SPAG6 regulates ciliary/flagellar movement, ciliary formation and axoneme orientation and establishes polarity in tracheal epithelial cells. SPAG6 exhibits functions similar to those of homologous proteins in *Chlamydomonas*, such as regulating ciliary/flagellar movement (27-29). Hu *et al* (30) reported that SPAG6 ameliorates damage caused by brain edema following cerebral ischemic stroke reperfusion by maintaining the structure and function of motile cilia, attenuating the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)-mechanistic target of rapamycin (mTOR) signaling pathway, and inhibiting inflammatory and autophagic responses. Mice lacking *SPAG6* do not survive to adulthood because of hydrocephalus. Male survivors lose their reproductive capacity because of impaired ciliary/flagellar motility and ultrastructural abnormalities in the sperm axonemes. Compared with wild-type mice, *SPAG6* knockout mice show bronchial epithelial ciliary dysfunction. In addition, ciliary formation defects occur in the ventricular ependymal, middle ear and tracheal epithelial tissues (17). Teves *et al* (7) demonstrated that *SPAG6* knockout mice display a disorganized ciliary arrangement and reduced density in tracheal epithelial cells, along with decreased ciliary beating frequency and irregular rhythms. Furthermore, the number of axonemes in epithelial cells is significantly reduced, whereas the orientation of the central microtubule pairs is random. These abnormal changes in ciliary structure and function may be associated with disrupted microtubule distribution, which results in misdirected axoneme/basal body orientation and disrupted epithelial cell polarity.

SPAG6 regulates the formation and function of immune synapses. In immune cells without cilia, SPAG6 acts through an alternative mechanism because the formation of immune synapses and cilia involves the same processes. The microtubule-organizing center is a subcellular organelle responsible for forming and organizing microtubules. In eukaryotic cells, it usually refers to the centrosome, which is comprised of a pair of orthogonally arranged centrioles (i.e., parent and daughter centrioles) (31). When antigen-presenting and effector cells undergo homologous recognition to initiate an immune response, the centrosomes, actin cytoskeleton, Golgi apparatus and secretory vesicles within the effector cells relocate and aggregate at the immune synapse site. This relocation of subcellular organelles promotes receptor-ligand interactions and results in the release of cytokines to their target sites (26). Similarly, during targeted killing by effector cells, the centrosome becomes re-oriented and docks with the synapse membrane to form a synapse gap and releases lytic enzymes that destroy the target cells (21,24). Cooley *et al* (25) found that SPAG6 regulates the function of lymphocyte centrosomes and is expressed in primary and secondary lymphoid tissues. *SPAG6* defects in mice result in a dysregulated synapse cleft, centrosome polarization and actin clearance. The abnormal synapse formation observed in *SPAG6*-deficient mice may be associated with impaired cytotoxic T-cell function and humoral immune responses. This manifests as weakened germinal center responses, fewer follicular CD4+ T cells, defects in antibody class switching and abnormal B1B cell proliferation.

SPAG6 plays a role in the functional regulation of epithelial cells in the middle ear and Eustachian tube. The epithelial tissue associated with the middle ear and Eustachian tube consists of multiciliated cells (MCCs) and non-MCCs. It is associated with otitis media with effusion (32). MCCs contain hundreds of cilia on their surface, and their coordinated beating facilitates the transport of secretions from the middle ear cavity to the nasopharynx through the Eustachian tube. Effective mucociliary clearance requires consistent ciliary orientation within and between cells and along the tissue axis (32). Abnormal ciliary function occurs in primary ciliary dyskinesia or Kartagener syndrome and results in middle ear effusion and inflammatory responses (33,34). Studies have demonstrated that *SPAG6* deletion causes hearing loss in mice. potentially by regulating prestin expression (35). *SPAG6* may affect hearing by regulating prestin expression, whereas its deletion causes otitis media in mice. Additionally, *SPAG6* is expressed in the cilia of the middle ear epithelium in mice, and its targeted mutation can lead to pathological changes in the middle ear, which are attributed to ciliary dysfunction (36,37). *SPAG6* mutations disrupt polarity maintenance in the middle ear epithelial cells, which results in abnormal ciliary movement and reduced fluid and mucus transport efficiency. This disrupts the balance between mucus secretion and clearance, which ultimately causes middle ear effusion and otitis media (38). With respect to auditory function, cylindrical outer hair cells (OHCs) in the organ of Corti of the mammalian cochlear detect receptors (39). Wang *et al* (39) observed SPAG6 expression in OHCs and found that it was bound to microtubule-associated protein 1, which jointly stabilizes the dynein structure. This suggests that SPAG6 is indispensable for maintaining the normal physiological function of OHCs. Furthermore, vestibular and auditory functions collaborate through shared ciliary structures and neural pathways (the vestibulocochlear nerve) within the hair cells of the inner ear to enable spatial localization and perception. Li *et al* (24) generated SPAG6-deficient mice and showed that its mutants exhibit vestibular disorders associated with abnormal ultrastructural changes in the vestibular hair and Scarpa ganglion cells of the inner ear. The changes included swollen microvilli and reduced mitochondrial cristae. This suggests that microtubule stability is regulated by *SPAG6* and is essential for vestibular function.

SPAG6 regulates neuronal proliferation and differentiation processes. Normal development of the mammalian brain relies on the coordination of the proliferative and differentiation activities of neural progenitor cells (NPCs) (40,41). Disruption of this process results in an abnormal number of neurons, which can lead to neurological disorders, such as epilepsy, autism spectrum disorders and intellectual developmental delays (42-44). Studies on chicken embryo development indicate that SPAG6 is primarily expressed in the ventral ventricular zone of the spinal cord (adjacent to the basal plate region) (45). *SPAG6* knockout mice have enlarged brains and reduced body size and experience premature death because of severe hydrocephalus (17). Thus, SPAG6 may be involved in regulating cell proliferation and division. Furthermore, ventricular enlargement accompanied by cortical plate thinning (46), along with the aforementioned cranial volume abnormalities and hydrocephalus phenotype, suggests that SPAG6 plays an important regulatory role in the ciliary movement function of the ependymal layer. Armadillo repeat domain-containing proteins contribute to neural cell division processes and related pathological mechanisms by regulating microtubule assembly and spindle formation (38,47,48). SPAG6 is expressed in the microtubules of COS-1 cells and plays a role in neural development and differentiation (16). Hu *et al* (49) showed that SPAG6-overexpressing cells preferentially differentiate into neurons. SPAG6 overexpression inhibits the proliferative activity of NPCs, promoting their differentiation toward a neuronal lineage while suppressing astrocyte generation. Yan *et al* (22) demonstrated that SPAG6 overexpression reduces neuronal migration rates and inhibits axonal branching and extension, suggesting that it regulates neurogenesis by stabilizing microtubule structures and inhibiting excessive remodeling. The expansion capacity and differentiation orientation of NPCs together determine the number of neurons produced during brain development, which ultimately influences brain volume and cortical thickness (50). Cortical plate thinning observed in SPAG6-deficient mice may result from the disruption of the balance between NPC proliferation and differentiation. Mitchell *et al* (51) proposed that SPAG6 regulates neuronal migration by targeting microtubule regulation and primarily controls centrosome localization and somatic movement, which further indicates the role of SPAG6 in neural development (52). Using SPAG6-deficient mice and mammalian spiral ganglion neuron (SGN) explants, studies have demonstrated that the absence of SPAG6 affects neurite and growth cone growth (1). Furthermore, SPAG6 deficiency decreased synaptic density in SGN explants and increased the sensitivity of SPAG6-mutant SGNs to the microtubule stabilizer paclitaxel. These results suggest that SPAG6 contributes to the development and function of SGNs. SPAG6 promoter

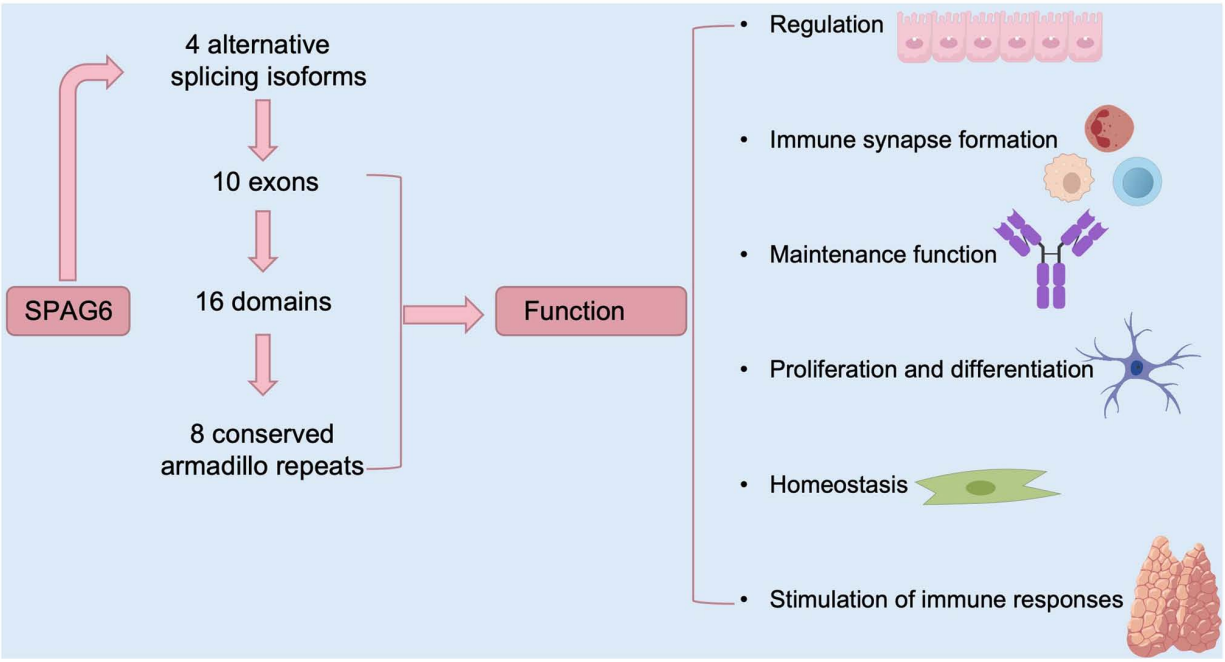

Figure 1. Structural and functional characteristics of SPAG6. SPAG6 undergoes alternative splicing to generate four distinct isoforms. Its full-length transcript comprises 10 exons, and the encoded protein contains 16 domains, including 8 conserved armadillo repeat sequences. The SPAG6 protein plays an important role in multiple biological processes, including immune synapse formation, maintenance of cellular function, stimulation of immune responses, cell proliferation and differentiation, and maintenance of homeostasis. SPAG6, sperm-associated antigen 6.

methylation is increased during the *in vitro* differentiation of human embryonic stem cells into NPCs or stem cells, suggesting that its expression is subject to stage-specific epigenetic regulation during neurogenesis (53).

SPAG6 regulates the morphology, growth, migration and cilia formation of fibroblasts. Primary mouse embryonic fibroblasts (MEFs) were isolated and cultured from *SPAG6* knockout and wild-type mouse embryos (8). Compared with wild-type MEFs, *SPAG6*-deficient MEFs showed various morphological abnormalities, including generalized enlargement of cell volume, nuclear enlargement and aggregation of vesicles in the cytoplasm. Re-introducing *SPAG6* reversed these abnormalities. In addition, the deficient cells had slower growth rates and reduced motility. Microtubule acetylation, which is an important post-translational modification of microtubules (54), is significantly reduced in *SPAG6*-deficient MEFs. The reduction in acetylation disrupts the functional integrity of microtubules, yielding phenotypic changes, including the inhibition of cell proliferation, defects in migration, adhesion abnormalities, mitotic defects and impairment of cilia formation. This mechanism may explain the increase in cytoplasmic vesicles and reduced transfection efficiency observed in *SPAG6*-deficient MEFs (8). A previous study confirmed that the degree of microtubule acetylation is positively associated with transfection efficiency (55). The physiological function of SPAG6 is illustrated in Fig. 1 and the molecular mechanisms are presented in Table I.

3. Role of SPAG6 in tumors

CTAs are a family of antigens that are only expressed in testicular and placental tissues. They are abnormally activated in various tumor tissues while maintaining tissue-specific expression (56). CTAs are present in human immune-privileged tissues and specific tumor lesions. They elicit spontaneous humoral and cellular immune responses without harming normal tissues; thus, they are ideal candidates for tumor immunotherapy. CTAs are closely associated with tumor cell proliferation, metastasis, invasion, disease recurrence and poor prognosis (57-59). SPAG6 encodes a microtubule-associated protein involved in cell growth, differentiation, migration and polarity regulation. It is a novel CTA with potential as a tumor serum biomarker, which confirms its inclusion in the CTA family, and it is a promising candidate for tumor immunotherapy. SPAG6 abnormalities are closely associated with hematological malignancies and various solid tumors, including breast and lung cancer (2,9).

Role of SPAG6 in hematological malignancies. Studies have confirmed that SPAG6 has the potential to serve as a prognostic marker and therapeutic target for hematological malignancies.

MDS. MDS is a clonal hematopoietic stem cell disorder characterized by significant heterogeneity (60). This disease involves ineffective hematopoiesis, cytopenias, morphological developmental abnormalities and transformation to AML (61). Li *et al* (62) used SPAG6-short hairpin RNA lentiviral vectors to knock down *SPAG6* in SKM-1 cells, which resulted in the activation of the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) signaling pathway, indicating that *SPAG6* attenuates apoptosis by modulating the TRAIL pathway. Yin *et al* (63) found that *SPAG6* silencing in SKM-1 cells increases phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*) expression, which induces apoptosis through the PI3K/AKT pathway. Jiang *et al* (12) showed that *SPAG6* mRNA levels were significantly higher in bone marrow cells from patients with MDS and MDS-AML than in

Table I. Physiological functions and molecular mechanisms of sperm-associated antigen 6.

Physiological function	Molecular mechanisms	(Refs.)
Tumor immunogenicity	Cancer/testis antigen that stimulates immune responses Abnormally high expression in tumor tissues, silent in normal tissues	(10-13,19,20)
Regulation of ciliary/flagellar movement	Maintains the integrity of the axoneme microtubule structure Mediates ciliary-directed beating and coordination Regulates ciliogenesis	(7,17,27-29)
Immune synapse formation	Regulates microtubule-organizing center (centriole) relocation to synapses Maintains actin clearance Mediates the targeted release of cytotoxic granules	(21,24,26)
Middle ear mucus clearance	Establishes polarity of multi-ciliated cells Ensures ciliary-directed transport function	(32,36,38)
Auditory and vestibular function	Stabilizes dynein by binding to MAP1 Maintains the ultrastructure of outer hair cells Inhibition of ciliated vestibular cells Ciliated cell apoptosis	(24,39)
Neurodevelopmental regulation	Stabilization of microtubule structure (inhibition of excessive remodeling) Regulation of neural progenitor cell, proliferation/differentiation balance Coordination of neuronal migration (centriole-guided)	(22,46,49,50)
Fibroblast homeostasis	Promotion of microtubule protein acetylation Maintenance of cell morphology/motility Regulation of ciliogenesis and vesicle transport	(8,54,55)

those from healthy controls using reverse transcription-quantitative PCR (RT-qPCR). *In vitro* experiments revealed that *SPAG6* knockdown inhibits SKM-1 cell proliferation, causes cell cycle arrests at the G1/S phase and disrupts cell differentiation. Zhang *et al* (64) showed that *SPAG6* silencing induces autophagy through the AMP-activated protein kinase (AMPK)/mTOR/unc-51 like autophagy activating kinase 1 (ULK1) signaling pathway, thereby enhancing SKM-1 cell apoptosis. Collectively, these studies suggest that SPAG6 contributes to MDS pathogenesis and development, indicating its potential as a novel therapeutic target. Luo *et al* (65) expanded on this understanding from an epigenetic regulation perspective by showing that *SPAG6* knockout, combined with the demethylating agent decitabine (DAC), reduces the expression of DNA methyltransferases and methyl-CpG-binding domain proteins. This combination also enhances apoptosis induced by DAC and the histone deacetylase inhibitor LBH589. A study from the same group, by Luo *et al* (66), demonstrated that suppressing *SPAG6* expression in SKM-1 cells enhances DAC-induced apoptosis and promotes *PTEN* demethylation. Overall, these results support SPAG6 as a target for demethylation therapy in MDS.

AML. AML is an aggressive malignancy involving white blood cells (67,68). It primarily manifests as symptoms associated with bone marrow failure and organ infiltration (69). Luo *et al* (70) reported that *SPAG6* expression in patients with AML positively correlates with risk stratification. Patients with high SPAG6 expression had shorter overall survival than those with low *SPAG6* expression. Furthermore, *SPAG6* knockdown in the HL60 AML cell line promotes apoptosis and arrests the cell cycle at the G1 phase, thus confirming it as a protumor factor in AML. Steinbach *et al* (10) found that *SPAG6* and six other genes are highly overexpressed in pediatric patients with AML but were normal in patients with sustained complete remission. This suggests that *SPAG6* promotes disease progression. *SPAG6* was considered a potential indicator for evaluating treatment efficacy and predicting prognosis in pediatric AML (71). Skou *et al* (72) found that peripheral blood levels of *SPAG6* and two other genes predict relapse in pediatric patients with AML and are useful for minimal residual disease (MRD) monitoring in patients lacking leukemia-specific targets. Mu *et al* (73) identified *SPAG6* as a significantly upregulated gene in AML. Its overexpression was negatively correlated with disease prognosis. *SPAG6* interacts with and relocates myosin ID (*MYO1D*) from the cytoplasm to the cell membrane. This activates the PI3K/AKT signaling pathway and extracellular signal-regulated kinase (ERK) pathway, thereby regulating AML growth and prognosis. Thus, *SPAG6* may represent a novel therapeutic target for AML.

Adult B-cell acute lymphoblastic leukemia (B-ALL). B-ALL is a genetically heterogeneous malignancy (74) that may be classified into distinct molecular subtypes based on recurrent gene rearrangements, chromosomal abnormalities or specific gene mutations (75). Zhao *et al* (76) initially reported *SPAG6* overexpression in the bone marrow of adult patients with B-ALL, which markedly decreased after treatment and complete remission. Studies using lentiviral transfection to knock down *SPAG6* in the human B-ALL cell lines B-ALL-1 and NALM-6 showed significant inhibition of cell proliferation and apoptosis. These results indicate that *SPAG6* downregulation attenuates cell proliferation and apoptosis by

modulating the transforming growth factor-β (TGF-β)/Smad signaling pathway.

Multiple myeloma (MM). MM is a hematologic malignancy characterized by the malignant clonal proliferation of plasma cells (77). It accounts for >10% of all hematopoietic malignancies (78). Li *et al* (79) conducted a bioinformatics analysis for plasma cell tumor tissues and bone marrow samples from patients with MM. Significant *SPAG6* expression was observed in MM cell lines, plasma cell tumor tissues and patient bone marrow. Increased *SPAG6* mRNA levels were positively correlated with elevated hypercalcemia, increased plasma cell proportion and the severity of skeletal infiltration. Functional experiments further revealed that *SPAG6* overexpression enhances MM cell proliferation, migration and antiapoptotic capacity *in vitro*, whereas its downregulation showed inhibitory effects. A direct interaction was confirmed between *SPAG6* and dual-specificity phosphatase 1 (DUSP1), which attenuates the expression of downstream molecules in the mitogen-activated protein kinase (MAPK)/ERK signaling pathway. These results suggest that *SPAG6* plays an important role in MM development by attenuating the DUSP1-MAPK/ERK axis and may be an effective therapeutic target.

BCR activator of RhoGEF and GTPase (BCR)::ABL proto-oncogene 1, non-receptor tyrosine kinase (ABL1)-negative MPNs. MPN is a malignant clonal disorder caused by somatic mutations in hematopoietic stem/progenitor cells (80). It is characterized by the abnormal increase in peripheral blood cell counts and bone marrow fibrosis (81). Xia *et al* (82) showed that *SPAG6* is expressed by MPN cells at the mRNA and protein level, with the highest expression observed in nucleated erythroid precursor cells and megakaryocytes. They hypothesized that abnormal *SPAG6* expression contributes to the development of MPN and suggested that it may serve as a novel tumor marker for BCR::ABL1-negative MPN. Conversely, Ding *et al* (83) observed the significant upregulation of *SPAG6* mRNA in primary MPN cells and MPN-derived leukemia cell lines. *In vitro* studies revealed that forced *SPAG6* expression enhances clonogenic potential and accelerates the G1-to-S phase transition. Conversely, downregulating *SPAG6* enhances interferon-α (IFN-α)-mediated apoptosis promotion and cycle arrest through the signal transducer and transcription activator 1 (STAT1) pathway. Furthermore, the expression of SPAG6 protein decreased concomitantly following the inhibition of STAT1 signaling.

Burkitt lymphoma (BL). BL is a highly aggressive malignancy originating from mature B cells (84). It is characterized by distinct clinical and morphological features, a germinal center B-cell immunophenotype, high proliferative activity and *MYC* rearrangements involving the immunoglobulin gene loci (85). Zhang *et al* (11) demonstrated that suppressing *SPAG6* expression reduced the viability of Daudi and Raji cells. Conversely, PTEN inhibition using small inhibitory RNA or the specific PTEN inhibitor SF1670 restored proliferation and promoted apoptosis induced by SPAG6 deficiency *in vitro* and *in vivo*. These results suggest that SPAG6 promotes proliferation and suppresses apoptosis in BL cells through the PTEN/PI3K/AKT signaling pathway and further indicate that SPAG6 contributes to BL progression and may serve as a prognostic biomarker for these patients. The mechanisms of action and clinical significance of SPAG6 in various hematological tumor types are summarized in Table II.

Role of SPAG6 in solid tumors. SPAG6 is differentially expressed at various tumor stages and grades (86). In osteosarcoma and lung squamous cell carcinoma (LUSC), its expression correlates with prognosis. SPAG6 also plays a significant role as an oncogene and serves as a prognostic biomarker. In addition, SPAG6 influences tumor immune infiltration and the tumor microenvironment, which indicates that it is a promising immunotherapy target for treatment.

Breast cancer. Breast cancer is a highly heterogeneous malignant tumor (87). Its occurrence and development are driven by genetic and environmental factors, making it one of the leading causes of cancer-related death in women (88). Circular RNAs (circRNAs) play an important regulatory role in tumor progression (89). Fan *et al* (90) showed that circMYH9 enhances *SPAG6* mRNA stability by recruiting the EIF4A3 protein, thus promoting its expression. SPAG6 overexpression reverses the inhibitory effect of circMYH9 knockdown on the malignant phenotype of breast cancer cells. Furthermore, circMYH9 knockout inhibits PI3K/AKT signaling by upregulating PTEN expression, which is similarly antagonized by SPAG6 overexpression. In addition, circMYH9 modulates the PTEN/PI3K/AKT signaling pathway through the EIF4A3-SPAG6 axis, thereby promoting the malignant progression of breast cancer cells.

Although mammography remains the standard imaging modality for early breast cancer screening, it has certain limitations. Mijnes *et al* (91) developed an epigenetic analysis method based on cell-free DNA in blood. This minimally invasive technique detects the methylation status of tumor suppressor genes and serves as a liquid biopsy to complement traditional imaging techniques. The combined detection of SPAG6, period circadian regulator 1 (PER1) and inter-alpha-trypsin inhibitor heavy chain 5 (ITIH5) achieved 64% sensitivity for breast cancer detection. Although liquid biopsy has technical challenges, the 'SNiPER' panel, which includes SPAG6, NK2 homeobox 6, ITIH5 and PER1, holds promise (91). Manoochehri *et al* (92) demonstrated that a methylation scoring model established from the first three differentially methylated regions in the *SPAG6*, *LINC10606* and *TBCD/ZNF750* regions yields high sensitivity and specificity for detecting triple-negative breast cancer (TNBC). *LINC10606* and *TBCD/ZNF750* showed strong discriminatory power in patients with TNBC compared with healthy controls [area under curve (AUC)=0.78 in the test set, AUC=0.74 in the validation set]. Therefore, noninvasive DNA methylation detection may provide novel biomarkers for the early diagnosis of TNBC.

Nasopharyngeal carcinoma. Nasopharyngeal carcinoma is a malignant tumor originating from the mucosal epithelium of the nasopharynx (93), with a predilection for the pharyngeal recess (Rosenmüller's fossa) (94). Zhang *et al* (95) used machine learning to identify genes associated with nasopharyngeal carcinoma and determined their correlation with the immune microenvironment. Of note, four genes, including *SPAG6*, exhibited high predictive efficacy (AUC >0.9) in the training and validation sets. The expression of these genes was significantly correlated with the degree of immune cell

Table II. Mechanism of action and clinical significance of SPAG6 in various hematological tumor types.

Tumor type	Core functions	Signaling pathway/ molecular mechanism	Clinical significance	(Refs.)
Myelodysplastic syndrome	Inhibits apoptosis	Negative regulation of the TRAIL apoptosis pathway Silencing activates the PTEN/ PI3K/AKT pro-apoptotic pathway Silencing activates the AMPK/ mTOR/ULK1 autophagy pathway	Enhances the pro-apoptotic effect of decitabine; potential target for demethylation therapy	(12,62-65)
Acute myeloid leukemia	Promotes proliferation/ inhibits apoptosis	Combines with *MYO1D* to activate PI3K/AKT and ERK pathways Knockdown blocks the G1 phase and promotes apoptosis	Independent prognostic marker; predictive factor for childhood AML recurrence	(10,71-73)
Adult B-cell acute lymphoblastic leukemia	Drives proliferation/ inhibits apoptosis	Regulation of proliferation and apoptosis through the TGF-β/ Smad pathway	Treatment response monitoring biomarker; gene silencing inhibits tumor growth	(76)
Multiple myeloma	Promotes proliferation and migration and inhibits apoptosis	Combines with *DUSP1* to activate the MAPK/ERK pathway	Associated with hypercalcemia and plasma cell proportion; potential therapeutic target	(79)
BCR::ABL1-negative myeloproliferative neoplasms	Promotes clone formation, cell cycle progression	Inhibition of STAT1-mediated IFN-α-induced apoptosis SPAG6 downregulation when STAT1 signaling is blocked	Disease phenotype biomarker associated with sensitivity to interferon therapy	(82,83)
Burkitt lymphoma	Promotes proliferation/ inhibits apoptosis	Regulated by the PTEN/PI3K/ AKT pathway (PTEN inhibition reverses the silencing effect of SPAG6)	Potential prognostic biomarker; therapeutic target	(11)

AKT, AKT serine/threonine kinase; ALL, acute lymphoblastic leukemia; AMPK, AMP-activated protein kinase; BCR, BCR activator of RhoGEF and GTPase; ABL1, ABL proto-oncogene 1, non-receptor tyrosine kinase; DUSP1, dual specificity phosphatase 1; ERK, extracellular signal-regulated kinase; G1 phase, Gap 1 phase of the cell cycle; IFN-α, interferon alpha; MAP1, microtubule-associated protein 1; MAPK, mitogen-activated protein kinase; mTOR, mechanistic target of rapamycin; MYO1D, myosin ID; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; Smad, SMA- and MAD-related protein; SPAG6, sperm-associated antigen 6; STAT1, signal transducer and activator of transcription 1; TGF-β, transforming growth factor beta; TRAIL, TNF-related apoptosis-inducing ligand; ULK1, Unc-51 like autophagy activating kinase 1.

infiltration, with *SPAG6* showing a particularly strong association with the immune infiltration phenotype.

Thyroid cancer. Thyroid cancer is a malignant tumor originating in the thyroid gland (96), and it is the most commonly diagnosed endocrine malignancy worldwide (97). Located in the anterior neck region, the thyroid gland secretes hormones that regulate metabolism (98). Wang (99) proposed that SPAG6 exerts tumor-suppressing effects in thyroid cancer. SPAG6 overexpression inhibited tumor cell invasion and proliferation. Li *et al* (86) used immunofluorescence techniques to show that SPAG6 expression positively correlates with immune checkpoint molecules in thyroid carcinoma *in vitro*. SPAG6 overexpression suppresses malignant cell behavior, including reduced proliferation and migration, and affects the functional phenotypes associated with DNA repair, MYC signaling, peroxidase activity and the G2/M checkpoint.

Squamous cell carcinoma of the skin. Squamous cell carcinoma is the second most common nonmelanoma skin tumor, which accounts for ~20% of all skin cancers (100). It has strong metastatic potential and is capable of metastasizing to multiple organs in the body, thereby posing a high risk of mortality. An in-depth study of its molecular mechanisms is necessary to establish prevention and treatment strategies (101). Gim *et al* (102) selected early-stage squamous cell

carcinoma tissue samples representing invasive and precancerous regions. Using the NanoString GeoMx Digital Spatial Profiler for spatial transcriptomics analysis, they identified *SPAG6* as having the highest absolute log2-fold change in expression among other cancer-associated genes in fibroblasts. *SPAG6* was associated with fibroblast development and function. In addition, significant alterations in its expression were evident during the progression of actinic keratosis to squamous cell carcinoma.

Osteosarcoma. Osteosarcoma is the most common primary malignant bone tumor (103). It primarily affects adolescents and young adults and is highly invasive and prone to metastasis. Although surgery combined with chemotherapy significantly improves patient survival, the prognosis for metastatic or recurrent osteosarcoma is poor (104). Bao *et al* (105) examined SPAG6 expression in tumor tissues from 42 patients with osteosarcoma and 12 osteochondroma control tissues using immunohistochemistry, RT-qPCR and western blot analysis. The SPAG6 protein positivity rate in the osteosarcoma tissues (71.43%) was significantly higher than that in the control tissues (33.33%; $P<0.05$). Both mRNA and protein levels were markedly increased compared with the levels in adjacent normal tissue. High SPAG6 expression positively correlated with higher pathological grade, metastasis and advanced Enneking stage ($P<0.05$). SPAG6-positive patients experienced significantly shorter overall survival. These results indicate that SPAG6 overexpression is associated with malignant progression and poor prognosis in osteosarcoma, thus suggesting its potential as a prognostic biomarker.

Lung cancer. Lung cancer is one of the most common and deadliest malignant tumors (106). Based on its histology, it may be broadly classified into non-small cell lung cancer (NSCLC; accounting for ~85% of all cases) and SCLC (accounting for ~15% of cases) (107,108). Early detection is important for improving survival outcomes. DNA methylation is an important epigenetic regulatory mechanism that contributes to the development of various malignancies, including lung cancer, by modulating transcriptional activity. It demonstrates significant potential for predicting the early diagnosis, prognosis and treatment response of lung cancer (109).

NSCLC originates from lung tissues, such as the bronchial mucosa, glandular epithelium or pulmonary alveoli. It is the most prevalent type of lung cancer (110) and has a poor prognosis (111). Altenberger *et al* (112) reported that *SPAG6* and *LINE-1* type transposase domain containing 1 (*L1TD1*) mRNA expression is significantly lower in tumor tissues from patients with NSCLC than in normal lung tissues from the same patients. In NSCLC cell lines exhibiting downregulated mRNA expression, treatment with epigenetic modifiers reactivated the expression of these genes. Tumor-specific hypermethylation of *SPAG6* and *L1TD1* in NSCLC tissues with this methylation pattern effectively distinguished tumors from normal tissues. These results indicate that SPAG6 and L1TD1 undergo tumor-specific methylation in NSCLC, which regulates SPAG6 expression at the transcriptional level through DNA methylation (112).

LUSC is a type of NSCLC characterized by tumor heterogeneity, genetic mutations, cancer stem cells, immune resistance and chemotherapy resistance. Because it is usually diagnosed at an advanced stage, it has a poor prognosis (113). Epigenetic modifications, primarily DNA methylation (112), are associated with genomic instability in LUSC. Wu *et al* (114) found an effect of SPAG6 DNA methylation on its expression in LUSC. They identified contributors to SPAG6 DNA hypermethylation. For example, DNA methyltransferase 3 b (DNMT3b)-mediated hypermethylation of the *SPAG6* promoter in LUSC resulted in *SPAG6* downregulation, whereas *SPAG6* reversed the malignant phenotype of LUSC cells. Mechanistically, SPAG6 negatively attenuates the JAK/STAT signaling pathway by suppressing the transcriptional activity of STAT1 and STAT3. In addition, SPAG6 expression was found to be positively correlated with immune cell infiltration in LUSC tissues, whereas it was negatively correlated with the expression of immunosuppressive genes, such as cytotoxic T-lymphocyte associated protein 4 and programmed cell death 1. Furthermore, SPAG6 suppressed tumor stem cell properties by downregulating the stemness Nanog homeobox (Nanog), aldehyde dehydrogenase 1 family, member A1 (ALDH1) and Sox2.

High-grade serous epithelial ovarian cancer (HGSOC). HGSOC is the most common and aggressive epithelial ovarian cancer subtype (115). It displays high invasiveness and a poor patient prognosis. A deeper understanding of HGSOC tumorigenesis may provide insights for the development of new therapeutics (116). Coan *et al* (117) observed SPAG6 expression in ciliated cells of the fallopian tube. Impaired ciliary motility disrupts laminar fluid flow over the tubal epithelium, which may reduce the management of oxidative stress induced by follicular fluid and contribute to tumorigenesis.

Bladder cancer. Bladder cancer originating from the bladder mucosa (118) is a common malignant tumor of the urinary system (119). Kitchen *et al* (120) observed frequent methylation in the *SPAG6* promoter in bladder cancer tissues. They also observed significantly increased *SPAG6* methylation levels in recurrent and advanced bladder cancers, suggesting that *SPAG6* functions as a tumor suppressor gene in these tissues. *SPAG6* methylation may represent an independent predictor of bladder cancer recurrence and progression (120).

All of these findings indicate that SPAG6 shows diverse regulatory functions across different tumor microenvironments; however, its pathogenic mechanism is primarily attributed to dysregulated expression caused by epigenetic and post-transcriptional regulation, rather than frequent mutations in the gene itself. This characteristic is similar to that of other cancer-testis antigens. Its biological functions within tumor cells and the underlying molecular mechanisms remain to be fully elucidated. Further in-depth studies are needed to elucidate these mechanisms. The mechanisms of action and clinical significance of SPAG6 in different types of solid tumors are summarized in Table III and Fig. 2. Furthermore, Fig. 3 provides a schematic of SPAG6's role and regulation across various cancers.

Other syndromes. As a gene initially identified from a human testicular cDNA expression library (3), the encoded product of *SPAG6* is a component of the '9+2' microtubule-based centriole complex, which plays an important role in maintaining the structural integrity of sperm tail microtubules and ensuring proper flagellar motility (3,16,17). Abnormal *SPAG6* expression is associated with male infertility, particularly with phenotypes, such as asthenozoospermia, teratozoospermia

Table III. Mechanism of action and clinical significance of SPAG6 in different types of solid tumor.

Tumor type	Increase or decrease in molecular expression/ activity and Core functions	Signaling pathway/molecular mechanism	Clinical significance	(Refs.)
Breast cancer	↑Promotes cancer	circMYH9/EIF4A3→↑SPAG6→↓PTEN→activation of PI3K/AKT Drives cell proliferation and metastasis	Liquid biopsy markers (SNiPER combination sensitivity 64%)	(90-92)
Nasopharyngeal carcinoma	↑Promotes cancer	Significantly positively correlated with immune cell infiltration Machine learning screening of key genes	Potential targets for immunotherapy	(95)
Thyroid cancer	↑Anti-cancer	Inhibits cell proliferation/migration Positively correlated with immune checkpoint genes Regulates DNA repair/MYC target pathways	Protective factor for differentiated thyroid cancer	(86,99)
Squamous cell carcinoma of the skin	↑Promotes cancer	Regulation of fibroblast development and function Significant changes in AK expression during progression	Potential markers for early diagnosis	(102)
Osteosarcoma	↑Promotes cancer	Expression is positively correlated with pathological grading/ metastasis/staging Significant increase in mRNA and protein levels	Independent prognostic marker (high expression=low survival rate)	(105)
Lung squamous cell carcinoma	↓Anti-cancer	DNA hypermethylation silences expression Inhibits STAT1/STAT3 → blocks JAK/STAT Negatively regulates stemness markers (Nanog/ALDH1/Sox2) Negatively correlated with immunosuppressive genes	Positively correlates with immune infiltration markers Reverses malignant phenotype	(114)
Non-small cell lung cancer	↓Anti-cancer	Tumor-specific DNA methylation Epigenetic drugs can re-express	Methylation detection distinguishes between tumor and normal tissue	(112)
High-grade serous epithelial ovarian cancer	No conclusion	Ciliary dysfunction → reduced oxidative stress clearance May be involved in tumor initiation	Early onset mechanism hypothesis	(117)
Bladder cancer	↓Anti-cancer	Tumor-specific DNA methylation	Independent predictors of tumor relapse and progression	(119,120)

AK, adenylate kinase; AKT, AKT serine/threonine kinase; ALDH1, aldehyde dehydrogenase 1 family member A1; circMYH9, circular RNA myosin heavy chain 9; DNA, deoxyribonucleic acid; EIF4A3, eukaryotic translation initiation factor 4A3; JAK, Janus kinase; LUSC, lung squamous cell carcinoma; mRNA, messenger RNA; MYC, MYC proto-oncogene; Nanog, Nanog homeobox; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; Sox2, SRY-box transcription factor 2; SPAG6, sperm associated antigen 6; STAT1/3, signal transducer and activator of transcription 1/3.

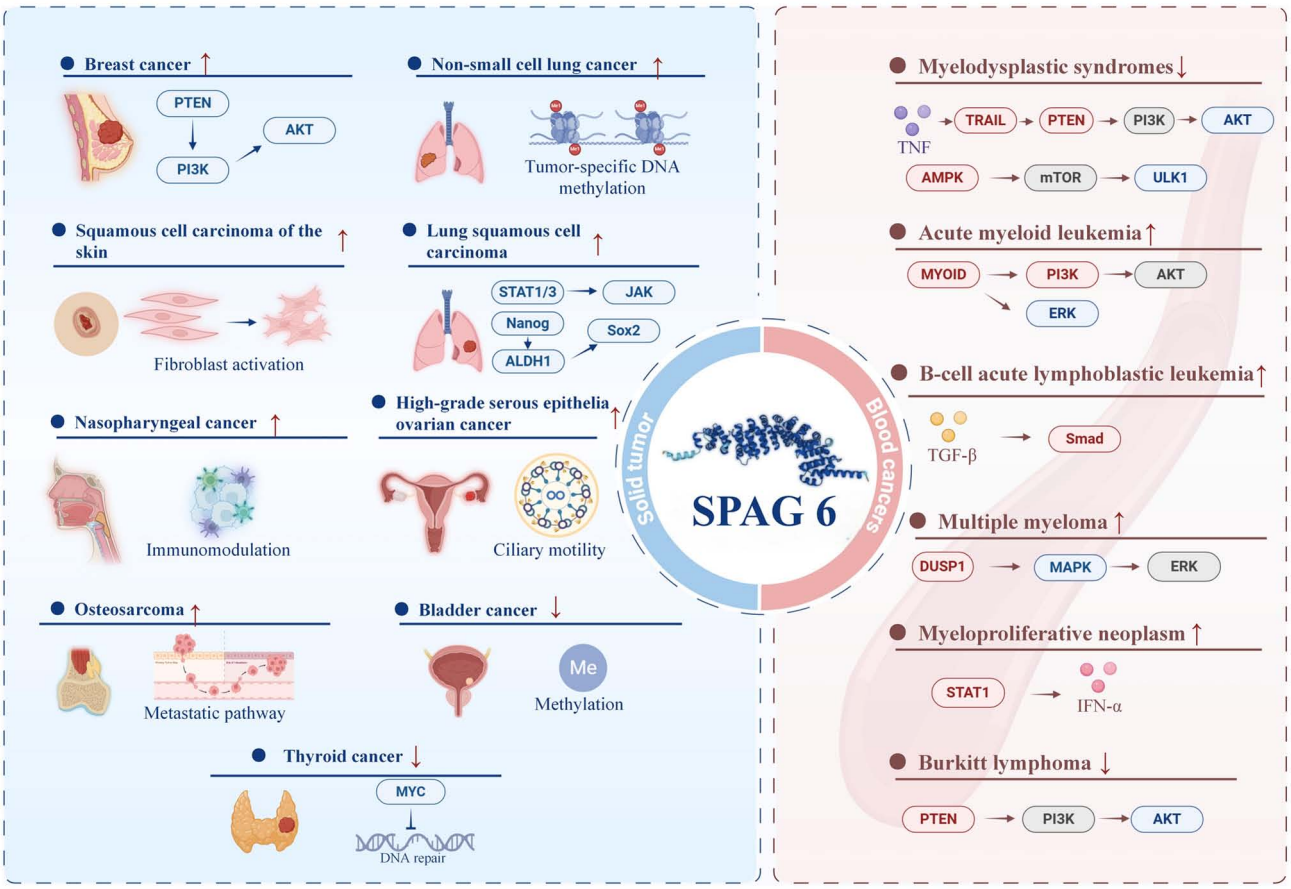

Figure 2. Alterations in SPAG6 among various cancers as well as the associated signaling pathways and molecular mechanisms. Arrows (↑/↓) indicate increased or decreased SPAG6 expression/activity in various cancers. The right panel depicts the role of SPAG6 in hematological malignancies, whereas the left panel illustrates its function in solid tumors. The regulatory pathways annotated for each tumor type include PTEN-PI3K-AKT, JAK-STAT, TGF-β-Smad, MAPK/ERK and AMPK-mTOR. These pathways are involved in various biological processes, including cell proliferation, immune regulation, metabolism and epigenetic modifications (e.g., methylation), as well as stem cell characteristics (e.g., Nanog, Sox2 and ALDH1). SPAG6, sperm-associated antigen 6; AKT, AKT serine/threonine kinase; ALDH1, aldehyde dehydrogenase 1 family member A1; AMPK, AMP-activated protein kinase; DUSP1, dual specificity phosphatase 1; ERK, extracellular signal-regulated kinase; IFN-α, interferon alpha; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; mTOR, mechanistic target of rapamycin; MYC, MYC proto-oncogene; Nanog, Nanog homeobox; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; Smad, SMA- and MAD-related protein; Sox2, SRY-box transcription factor 2; SPAG6, sperm associated antigen 6; STAT1/3, signal transducer and activator of transcription 1/3; TGF-β, transforming growth factor beta; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; ULK1, Unc-51 like autophagy activating kinase 1.

and azoospermia, which are linked to multiple morphological abnormalities of the sperm flagella (MMAF). Significantly reduced SPAG6 mRNA and protein expression was observed in patients with idiopathic asthenospermia (121). Whole-exome sequencing and ultrastructural analysis of patients with teratospermia consistently reveal downregulated *SPAG6* expression (122). SPAG6 protein levels are also markedly decreased in patients with MMAF (123). Proteomic analysis of spermatogenic efferent duct mutation carriers revealed differential SPAG6 expression associated with flagellar assembly processes (124). In addition, compound heterozygous *SPAG6* mutations were identified in patients with primary ciliary dyskinesia (125), and biallelic dynein heavy chain domain 1 variants were observed in patients with azoospermia, which was associated with reduced sperm *SPAG6* levels (126). SPAG6 mutations may influence pregnancy outcomes following intracytoplasmic sperm injection (127). Functional studies indicate that targeted disruption of *SPAG6* expression results in decreased sperm motility, increased apoptosis (128) and markedly reduced levels of axonemal proteins (15,129,130). Studies using animal models confirm that *SPAG6* deficiency causes sperm motility defects and abnormal microtubule architecture, which results in infertility (15,17). It also disrupts centrosome polarization and immunological synapse formation, thereby compromising lymphocyte function (25,131). Furthermore, significant downregulation of *SPAG6* was observed in cryptic testicular tissue (131), further indicating a role in normal testicular physiology. Overall, *SPAG6* is an important factor in sperm flagellar development and motility. Its abnormal expression or function constitutes a genetic basis for various male reproductive disorders and provides a foundation for the development of related gene therapies.

4. Clinical significance of SPAG6

In hematologic malignancies, high SPAG6 expression in MDS promotes disease progression by inhibiting the TRAIL apoptosis pathway (reducing FAS-Associated Via Death Domain binding to death receptors), activating the PI3K/AKT signaling pathway (downregulating PTEN) (63), and attenuating the

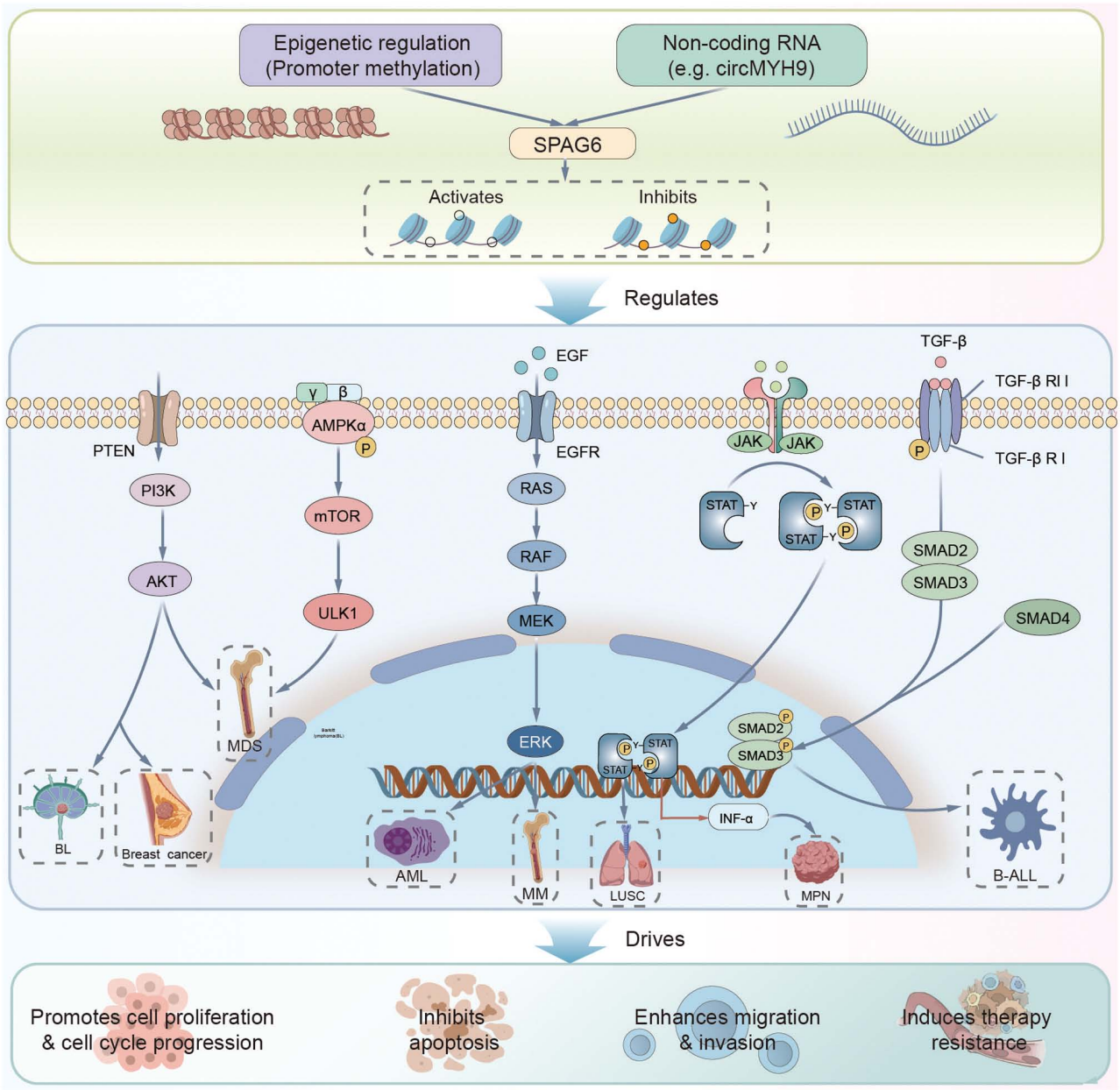

Figure 3. Schematic diagram illustrating the central role of the SPAG6 gene in oncogenesis: Its expression is regulated by upstream mechanisms such as promoter methylation and non-coding RNAs (e.g., circMYH9); subsequently, SPAG6 activates or participates in key signaling pathways, including PTEN/PI3K/AKT/mTOR, ERK, and JAK/STAT, playing a core functional role in various hematological malignancies (such as multiple myeloma and acute myeloid leukemia) and solid tumors (such as breast cancer and lung squamous cell carcinoma); ultimately, it drives malignant progression by promoting cell proliferation, inhibiting apoptosis, enhancing migration and invasion and inducing therapy resistance. SPAG6, sperm-associated antigen 6. AKT, AKT serine/threonine kinase; ALDH1, aldehyde dehydrogenase 1 family member A1; AML, acute myeloid leukemia; AMPK, AMP-activated protein kinase; BL, Burkitt lymphoma; B-ALL, B-cell acute lymphoblastic leukemia; circMYH9, circular RNA myosin heavy chain 9; ERK, extracellular signal-regulated kinase; LUSC, lung squamous cell carcinoma; MDS, myelodysplastic syndromes; MM, multiple myeloma; MPN, myeloproliferative neoplasm; mTOR, mechanistic target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; Smad, SMA- and MAD-related protein; Sox2, SRY-box transcription factor 2; SPAG6, sperm associated antigen 6; STAT1/3, signal transducer and activator of transcription 1/3; TGF-β, transforming growth factor beta; ULK1, Unc-51 like autophagy activating kinase 1.

G1/S transition of the cell cycle (12). SPAG6 silencing induces AMPK/mTOR/ULK1-mediated autophagic apoptosis and enhances the DNA demethylation efficacy of decitabine (64). In AML, SPAG6 serves as an independent prognostic marker (71), with high expression activating the PI3K/AKT and ERK pathways through the formation of a *SPAG6-MYO1D* complex that drives leukemia growth and is associated with MRD monitoring (73). SPAG6 expression is significantly reduced in adult patients with B-ALL during remission and it maintains tumor cell proliferation through the TGF-β/Smad signaling pathway (76). In MM, *SPAG6* binds to *DUSP1* and activates the MAPK/ERK pathway, thereby promoting the malignant phenotype and exhibiting an association with bone infiltration (79). In BCR-ABL1-negative MPN, SPAG6 forms a positive feedback loop with STAT1 (83). Its silencing enhances the proapoptotic effect of IFN-α. In BL, *SPAG6* activates the PI3K/AKT pathway by inhibiting PTEN, and its downregulation inhibits tumor growth *in vivo* and *in vitro* (11).

In solid tumors, circMYH9 activates the PTEN/PI3K/AKT pathway in breast cancer by stabilizing *SPAG6* mRNA (90). SPAG6 methylation patterns serve as liquid biopsy markers. In nasopharyngeal carcinoma, *SPAG6* is downregulated because of high promoter methylation (95). It is associated with immune infiltration and considered an important diagnostic gene. In thyroid cancer, *SPAG6* overexpression inhibits proliferation and migration and regulates the DNA repair/MYC target pathway (86). In osteosarcoma, the SPAG6 positivity rate (71.43%) is significantly increased and associated with disease grade, metastasis and poor prognosis (105). In LUSC, DNMT3b-mediated hypermethylation of *SPAG6* results in its silencing, which activates the JAK/STAT pathway and enhances cancer stemness (114). In NSCLC, *SPAG6* expression is downregulated by tumor-specific methylation (112). Ovarian cancer studies have reported that SPAG6-related ciliary dysfunction contributes to a tumor initiation microenvironment (117). In bladder cancer, frequent methylation occurs in the *SPAG6* promoter region, and *SPAG6* methylation levels are significantly increased in recurrent and advanced bladder cancer (120).

Although the protumor/antitumor mechanisms of SPAG6 in various tumor types have become gradually clearer, several unresolved issues persist in this field. For instance, although SPAG6 acts as an oncogene in most tumors, such as hematological malignancies and osteosarcoma, it exhibits tumor-suppressing effects in a minority of cancers, including nasopharyngeal carcinoma and LUSC. The reasons for this contrasting role warrant further investigation. Second, studies have predominantly focused on SPAG6's regulation of a few classical pathways, such as PTEN/PI3K/AKT and MAPK/ERK; however, the precise regulatory factors upstream (e.g., transcription factors or noncoding RNAs governing its expression) and its broader downstream effector networks remain elusive. In particular, its role in modulating the tumor immune microenvironment and DNA damage response is unknown. This lack of mechanistic insight hinders its clinical translation, while the development of specific inhibitors targeting SPAG6 remains in its infancy. To date, there have been no reports of small-molecule inhibitors or traditional Chinese medicine-derived inhibitors of SPAG6, as research is primarily focused on identifying the underlying mechanism. Based on the latest findings from these mechanistic studies, the development of targeted strategies for attenuating SPAG6 has significant clinical implications. Because of the differential expression of SPAG6 in various tumors, the following targeted treatment strategies should be considered: First, for tumors with high *SPAG6* expression, such as hematological tumors and osteosarcoma, developing specific inhibitors to block key oncogenic pathways mediated by *SPAG6*, such as the PTEN/PI3K/AKT signaling pathway, may be fruitful. Second, for tumor types with silenced *SPAG6* expression, such as nasopharyngeal carcinoma and LUSC, identifying epigenetic regulatory approaches, such as demethylation drugs, to restore its expression is necessary. Finally, because *SPAG6* silencing enhances the efficacy of drugs, such as decitabine and IFN-α, in MDS and MPN models, SPAG6 inhibitors combined with traditional chemotherapy/targeted drugs offer synergistic therapeutic potential. Such multifaceted treatment strategies will provide personalized intervention for patients with cancer harboring various SPAG6 expression profiles.

5. Summary and outlook

In summary, SPAG6 is a tubulin protein with multiple physiological functions, including regulation of ciliary/flagellar movement, mediation of the formation and function of immune synapses, neuronal proliferation and differentiation. It regulates the morphology, growth and migration of fibroblasts and attenuates the function of middle ear and Eustachian tube epithelial cells. In addition, SPAG6 acts as an oncogene in most tumors, promoting tumorigenesis through its high expression and regulating signaling pathways, such as PTEN/PI3K/AKT and MAPK/ERK, to promote tumor proliferation, migration and drug resistance (e.g., hematological tumors, osteosarcoma and breast cancer). It also exhibits tumor-suppressing effects in bladder cancer (methylation silencing) and thyroid cancer (overexpression inhibits the malignant phenotype). It exhibits significant tissue specificity in hematological tumors (AML, MDS and lymphoma) and serves as a prognostic marker and therapeutic target. SPAG6 silencing enhances tumor cell sensitivity to chemotherapy. In solid tumors, SPAG6 is closely associated with immune infiltration, cancer stemness and epigenetic regulation (e.g., breast cancer circRNA stabilizes *SPAG6* mRNA), and pancancer analyses suggest that its expression is associated with immune microenvironment remodeling. Overall, SPAG6 is a potential biomarker for tumor classification, prognosis assessment and targeted intervention. It may be used to guide treatment selection and assess disease prognosis. Nevertheless, SPAG6-related studies have several limitations. *In vitro* and *in vivo* studies are needed to evaluate its potential as a therapeutic target and prognostic marker in tumors. In addition, it is necessary to examine its upstream and downstream regulatory pathways and identify proteins that interact with SPAG6 through protein-protein interactions to elucidate the mechanisms underlying its role in tumorigenesis and tumor progression.

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Authors' contributions

YL and YW conceptualized the structure and main ideas of this paper and provided guidance for the writing process. YL and PZ performed the literature search. QY, PZ and HX drafted the main body of the paper. RZ and RW revised and polished the paper. All authors have read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, the authors used Grammarly (https://www.grammarly.com/) for grammar checking and language enhancement. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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