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Review

The Emerging Role of Sperm-Associated Antigen 6 Gene in the Microtubule Function of Cells and Cancer

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Accumulated evidence shows that sperm-associated antigen 6 (SPAG6) gene has multiple biological functions. It maintains the normal function of a variety of cells including ciliary/flagellar biogenesis and polarization, neurogenesis, and neuronal migration. Moreover, SPAG6 is found to be critically involved in auditory transduction and the fibroblast life cycle. Furthermore, SPAG6 plays an essential role in immuno-regulation. Notably, SPAG6 has been demonstrated to participate in the occurrence and progression of a variety of human cancers. New evidence shows that SPAG6 gene regulates tumor cell proliferation, apoptosis, invasion, and metastasis. Therefore, in this review, we describe the physiological function and mechanism of SPAG6 in human normal cells and cancer cells. We also highlight that SPAG6 gene may be an effective biomarker for the diagnosis of human cancer. Taken together, targeting SPAG6 could be a novel strategy for the treatment of human diseases including cancer.

The sperm-associated antigen 6 (SPAG6), also known as CT141, repro-sa-1, RP11-301N24.4, and pf16, was identified as a cancer-testis antigen (CTA) and is encoded by a gene located at chromosome 10p12.3.[1,2] Human SPAG6 has nine splicing variants: SPAG6-201, SPAG6-202, SPAG6-203, SPAG6-204, SPAG6-205, SPAG6-206, SPAG6-207, SPAG6-208, and SPAG6-209 (Figure 1). Murine SPAG6 also exists in the form of two isoforms, the parental SPAG6-BC061194 and its derivative, that are encoded by two homologous genes.[3] The SPAG6 mRNA consists of 10 exons and 16 domains, whereas the protein contains eight consecutive WD repeats, which are known to mediate protein-protein interactions during brain development (Figure 1; Figure S1).[4] Studies indicate that SPAG6 is a microtubule-associated protein that is essential for cytoskeleton formation. For instance, SPAG6 is present in the central organ protein of algae C1 microtubules and is a component of the central organ of the "9+2" axon,[5] which is essential for ciliary and flagellar movement. In addition, it has also been detected in the inner ear hair cells, lung bronchial cells, ovarian epithelial cells, lymphocytes, and fibroblasts,[6,7] as well as in testicular germ cell tumors, hematological malignancies, breast and lung cancer, and bladder cancer.[1,8,9] In this review, we focus on the function of SPAG6 in various cells and the molecular mechanism involved in SPAG6-mediated tumorigenesis and progression. In addition, we also discuss its potential role in tumor diagnosis and treatment.

Physiological Functions of SPAG6

Ciliary/Flagellar Biogenesis and Polarization

As a microtubule-binding protein, SPAG6 plays an important role in the biogenesis of motility structures like cilia and flagella,[10] although the mechanisms are likely different.[10] The motile cilia are formed after the assembly of nine microtubules with two linear centripetal microtubules,[11] and *spag6* knockout mice ciliary drift because of the loss of ciliary pair of central tubulin.[12] Zhang et al.[13] showed that *spag6*$^{-/-}$ mice had intact cilia on their trachea epithelial cells with the "9+2" axoneme structure, whereas another study found that the bronchial epithelial cell ciliary function was defective in these mice.[7] Furthermore, the spermatozoan flagella of the *spag6*$^{-/-}$ mice have the "9+1" or "9+0" axoneme arrangement.[5] SPAG6 can affect the function of these motility structures by altering the apical microtubule network,[7] which lies upstream of the planar cell polarity (PCP) signaling cascade[14,15] and controls the tissue-level polarity of tracheal epithelial cells.[16] The PCP proteins Prickle2, Dishevelled1, Dvl2, Vangl1, and Vangl2 localize asymmetrically to the tracheal epithelial cell cortex,[17] and disruption of these proteins obliterates rotational polarity of these cells.[18–20] The distribution of the PCP proteins is also disrupted in the *spag6*$^{-/-}$ mice, which impairs the sub-apical

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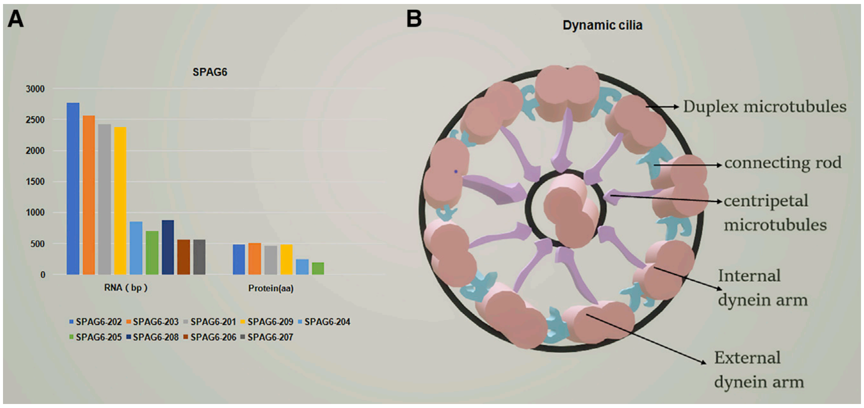

Figure 1. The Expression Level of SPAG6 and Its Location in the Centripetal Microtubules

(A) The information about the RNA and the protein expressed by the SPAG6 gene, which has nine transcripts and only six proteins translated. (B) The cross section of the cilia, where SPAG6 expresses the centripetal microtubules.

microtubule stability and polarization of basal bodies in the relevant cells.[7] Furthermore, Teves et al.[6] detected arrhythmic and reduced ciliary beat in the tracheal epithelial cells of *spag6*$^{-/-}$ mice, along with disarrayed and significantly sparse cilia. In addition, the orientation of the basal feet that determine axoneme orientation was random,[7] and the levels of mucin, α-tubulin, and Vangl2 were significantly decreased.[7] Taken together, SPAG6 regulates ciliary/flagellar motility, and ciliogenesis, axoneme orientation, and tracheal epithelial cell polarity are adversely affected in its absence.[7]

Neurogenesis and Neuronal Migration

Studies have increasingly shown a critical role of SPAG6 in neurological functions.[19] Co-localization of SPAG6 and microtubules has been observed in various cells lines,[4,21,22] and SPAG6 is overexpressed in the chicken embryonic spinal cord.[23] The human SPAG6 and the *Chlamydomonas* pf16 can bind to the centrosome proteins Wdr62 and LIS1 through their contiguous WD repeats,[24,25] indicating their involvement in Wdr62-mediated mitotic spindle regulation and neurogenesis.[19] Mitchell et al.[16] proposed that SPAG6 likely affects neuronal migration by targeting microtubules, which control centrosome movement and soma during neuronal migration,[26,27] indicating that SPAG6 also plays a role in neural development.[28] The two modes of neuronal migration, known as soma translocation and radial glia-dependent locomotion, need coordinated cytoskeletal remodeling.[29] Yan et al.[28] showed that overexpression of SPAG6 delayed the rate of neuronal migration, branching, and elongation, indicating that it can stabilize the microtubules and prevent remodeling.[28] Another study found fewer microtubules and actin filaments, in addition to other abnormal membranous structures, in the spiral ganglion neurons (SGNs) of *spag6*$^{-/-}$ mice.[30] SPAG6 is localized in the perinuclear cytoplasm of wild-type SGNs and decreased in the *spag6*$^{-/-}$ mice by perinatal day 30.[30] Furthermore, RNAi-mediated inhibition of *spag6* in multi-ciliated mouse ependymal cells completely abolished the central pair microtubules and ciliary localization of SPAG6, resulting in rotational ciliary movement.[15] Finally, the *spag6* promoter is hypermethylated during the differentiation of human embryonic stem cells into neural progenitor/stem cells (NPCs) *in vitro*, indicating that SPAG6 levels are modulated during neurogenesis.[31]

Auditory Transduction

SPAG6 is also essential for the mechano-sensory function of the cylindrical outer hair cells (OHCs) in the organ of Corti, which is the receptor organ for hearing in the mammalian cochlea.[32] Evidence suggests that patients with primary ciliated hair cells of Corti often have hearing impairment simultaneously.[33] The normal physiological function of the hair cells on the Corti is to maintain hearing production by changing their length and stiffness by a recognized molecular click that drives from a putative molecular motor designated prestin,[34,35] and dyskinesia happens to the cilia of the hair cells missing spag6.[36] Wang et al.[37] were the first to detect SPAG6 in the OHCs, and they found that SPAG6 and the microtubule-associated protein MAP1S bound to and stabilized prestin,[38] which is essential for maintaining the normal function of the OHCs.[37] In their study, immunofluorescent staining of SPAG6 and prestin shows expression of SPAG6 in the lateral wall of OHCs, and in the cuticular plate in OHCs, while prestin located in the cuticular plate of OHCs.[37] Prestin protein and mRNA levels were decreased in SPAG6 defect mice.[37] Li et al.[39] studied the relationship between SPAG6 and otitis media, and they have proved that SPAG6 regulates cilia/basal body polarity through the PCP-dependent mechanisms that not only regulate cilium location or orientation, but also regulate basal body docking to the apical surface during ciliogenesis in the middle ear and Eustachian tubes.[40,41] Li et al.'s[39] study showed that the orientation of the ciliary basal feet was random in the middle ear epithelial cells of SPAG6-deficient mice. Frizzled class receptor 6 (FZD6), a core planar cell polarity (PCP) protein, is a mediator of the Wnt pathway and plays a critical role in differentiation and organism development.[42] FZD6 does not have a polarized distribution in the mutant tympanic ciliary epithelium,[39] indicating that FZD6 protein location was altered by inactivation of SPAG6 in the middle ear, and that planar polarity of the middle ear was affected,[39] which is in keeping with the report that FZD6 knockout mice have developmental disorders in the planar polarity of the OHC and neural tube closure,[43] and "9+2" axonemes of cilia were conserved; also, the orientation of central microtubules was not uniform,[39] indicating that as in the brain and lungs, SPAG6 mutation affects the polarity of the middle ear epithelium, with the gross structure of the "9+2" axonemes being conserved.[7,13] It is interesting to note that other research has shown that the SPAG6 gene has not been associated with hair cell differentiation.[44] There are also some experimental observations worth exploring. In one study, the researchers found that SPAG6-deficient mice exhibited abnormal behaviors, such as logy motion and continuous head tossing, and fur loss occurred in comparison with their corresponding SPAG6 overexpression and SPAG6 normal expression littermates.[37]

Immuno-regulation

The immune synapse has the same centrosome nucleation mechanism as the cilia and, therefore, similar functions.[5] Intraflagellar transport protein 20 (IFT20), a protein involved in ciliary formation like SPAG6, also plays an important role in the immune synapse between the T cell receptor (TCR) and antigen-presenting cells (APCs) or target cells.[45,46] This led to the speculation that SPAG6 may also be involved in the lymphatic system. de la Roche et al.[47] showed that the TCR immune synapses function as cilia at the junction of T cells and APCs/target cells. In addition, SPAG6 is expressed in both primary and secondary lymphoid tissues, and its deletion ruptured immune synapses because of the absence of centrosome polarization and actin clearance in the synaptic cleft. During the homologous recognition between APCs and effector cells, the centrosomes, actin, Golgi bodies, and secretory vesicles are reoriented at the immune synapse, allowing receptor/ligand interactions and targeted release of cytokines.[47] In addition, during targeted killing of effector cells, the docking between centrosomes and the effector cells' synaptic membrane also occurs in the same direction, thereby effectively forming the synaptic gap for targeted lysozyme release.[46,48] Based on these findings, it is reasonable to surmise that SPAG6-mediated polarization of synaptic centrosomes and the removal of actin are essential for T cell cytotoxicity.[5]

Fibroblast Life Cycle

SPAG6 has been implicated in fibroblast growth, morphology, migration, and ciliary movement.[7] Li et al.[7] found that mouse embryonic fibroblasts (MEFs) lacking the *Spag6* gene had greater surface area, abnormal morphology, slower proliferation rates, and less motility compared with the wild-type MEFs, and these defects were alleviated upon forced expression of exogenous SPAG6. In addition, the *Spag6*$^{-/-}$ MEFs also showed impaired surface adhesion, significant reduction in primary cilia, and even presence of multiple cilia in some cells because of non-polarized F-actin. Multiple centrosomes were also observed in the cytoplasm of the *Spag6*$^{-/-}$ MEFs,[7] along with increased levels of the microtubule stability marker acetylated tubulin in *spag6*$^{+/+}$ MEFS.[7] Consistent with this, these defective MEFs were highly sensitive to paclitaxel, a known microtubule stabilizer.[7] Also, microtubule acetylation is positively correlated with transfection efficiency[49] and was low in the *Spag6*$^{-/-}$ MEFs.[7] Therefore, Li et al.[7] also proposed the functional mechanism of SPAG6 regulating MEF cells; that is, the function of SPAG6 itself may be a tubulin acetyltransferase or deacetylase inhibitor. Re-expression of spag6 with adenovirus vector rescued abnormal morphology and reduced levels of acetylated tubulin.[7] However, the loss of MEFs acetylated tubulin expression in spag6 can damage the function of microtubules and cause the phenotype changes of cell growth, migration, adhesion, division, and ciliogonium.[7] In one study, Glu-tubulin is released from C-terminal tyrosine of alpha tubulin by tubulin carboxypeptidase,[50] and its expression level was modestly increased when SPAG6 expression level was increased. Given the fact that SPAG6 is also upregulated in cancers.[51] Li et al.[7] proposed that high SPAG6 expression in these cancer cells may also increase Glu-tubulin expression.

Regulation of SPAG6

Spef1, an evolutionary conserved molecular binding protein, binds microtubules (MTs) through its amino-terminal calponin-homologous domain, and forms homologous dimer and stabilizes microtubules through its C-terminal curling helix region; Spef1 also enables mammalian ciliary central apparatus formation.[15] Cilia signal of Spef1 was weakened in multiciliated ependymal cells (mEPCs) treated with cisplatin, and cilia signal of Spag6 was also weakened.[15] One study showed that the PF6, SPAG6, and PF20 proteins form an interactive network, which presumably links the central apparatus microtubules and their projections into a functional unit, and their gene promoters share a common transcription factor binding site.[4,52] Although these proteins do not regulate the primary ciliary function of Madin-Darby canine kidney (MDCK) cells, they modulate the expression of nine peri-ciliary and cilia-associated proteins.[52] SOX5 is a transcription factor, which has a homology to the region of the testis-determining factor, sex-determining region of the Y (SRY).[53] The SRY-related high mobility group box or SOX family of transcription factors regulates the development of retinal, muscle, endothelial, epidermal, intestinal, lymphoid, and cartilaginous tissues,[53] and a 48-kDa SOX5 protein (S-SOX5) that is encoded by a SOX5 gene in particular has been associated with SPAG6 regulation.[54] Forkhead box J1 (FOXJ1) is a transcriptional factor that participates in several cellular processes including immune homeostasis and tumorigenesis.[55] S-SOX5 and FOXJ1 synergistically upregulate *spag6* promoter activity,[56] and therefore may mediate cilia/flagella biogenesis by globally regulating the expression of axon protein-encoding genes.[52] Pf20 is co-expressed with SPAG6 in microtubules during polymerization, and transient scrotal heat can affect SPAG6 expression.[57]

Pathological Function of SPAG6

Studies have increasingly shown a critical role of SPAG6 in tumor progression, especially in hematological malignancies that harbor higher SPAG6 levels compared with solid tumors.[58] As a testicular-specific and cytoskeletal protein,[59] genetic deficiency of SPAG6 is associated with inherited diseases such as immotile-cilia syndrome, situs inversus totalis, hydrocephalus, anosmia, and retinitis pigmentosa, as well as male and female infertility.[60] The CGI promoters of *spag6* and several other genes were methylated in a neuroblastoma (NBLs) cell line, resulting in the silencing of downstream genes.[61] In addition, *spag6* and *c20orf85* were the only differentially expressed genes between normal fallopian tube epithelia (FTE) and high-grade serous ovarian cancer (HGSOC) samples.[62] Kitchen et al.[21] also found frequent methylation of the *spag6* promoter in bladder cancer tissues. Global gene expression analysis of the bronchial epithelium showed high levels of ciliogenesis-associated genes such as *dnai2*, *spag6*, *asp*, and *foxj1*,[22] of which *spag6* is involved in the transcriptional regulation of DNA methylation in non-small-cell lung cancer.[63] In addition, *spag6* and *l1td1* were methylated in non-small-cell lung cancer (NSCLC) primary tumors as opposed to the non-malignant lung tissues, which regulated the transcription of different genes.[63] The

Table 1. Roles of spag6 in Various Types of Cells and Organs

Cell Types	Functions	Target	Reference
Tracheal epithelial cells	cilia density, tissue-level polarity, axoneme orientation, beat frequency	core PCP genes	7,13–16
Neurons	migration neurogenesis and differentiation	cytoskeletal hypermethylation of the SPAG6 promoter CpG	19,24,27,28,30,73
Outer hair cells	mechanosensory function, hearing generation	prestin; PCP proteins; FZD6	34,35,38,39
Immune system	synapse disruption maintains T cell cytotoxicity, antigen presentation	centrosome	13,33,46,47
Fibroblast cell	growth, morphology, migration, and ciliogenesis	acetylated tubulin microtubule	7,51
Neuroblastoma	diagnosis	genes were methylated	61
MRD/ALL/MDS	apoptosis	p53 caspase PTEN/PI3K/AKT TRAIL	21,22,25,29,61,62,64,68
Bladder cancer	diagnosis	promoter-related CpG island methylation	21
HGSOC/FTE	diagnosis	not detected	62
NSCLC	diagnosis	DNA methylation of SPAG6	63

MDS, myelodysplastic syndrome.

recurrent t(10;11) (p12;Q14) translocation in acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and malignant lymphoma results in the fusion of the genes encoding the putative zinc-finger transcription factor AF10 and the clathrin assembly lymphoid myeloid leukemia protein (CALM).[64] Transcriptional analysis of 20 CALM/AF10 fusion-positive leukemia samples showed a significant upregulation of *spag6* near the fusion site,[25] which was, however, absent in leukemic cells isolated from CALM/AF10 transplanted mouse models that lacked the specific translocation.[25]

Monitoring of minimal residual disease (MRD) has become a powerful diagnostic tool for AML.[9] Steinbach et al.[9] identified SPAG6, along with chemokine C-C motif ligand 23 (CCL23), mesothelin (MSLN), suppression of tumorigenicity 18 (ST18), G antigen family D2 (GAGED2), Wilms tumor gene (WT1), and preferentially expression antigen in melanoma 9 (PRAME9), as an MRD marker in AML monitoring. In a follow-up sample analysis of 145 cases, these seven genes were overexpressed in the leukemia cells of 52 children with AML and 25 recovered patients and were found in the test results of patients in complete remission.[9] Thus, SPAG6 is a potential marker for predicting MRD in children with acute myelopathies.[9] Yang et al.[65] showed that SPAG6 silencing inhibited the growth of malignant myeloid SKM-1 and K562 cell lines by activating p53,

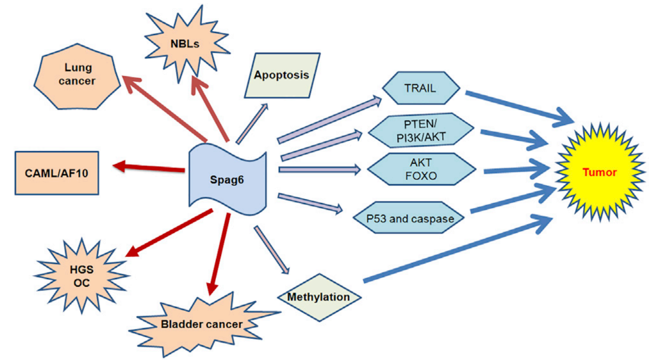

Figure 2. SPAG6 Regulates Multiple Functions in Various Cell Types
SPAG6 controls numerous cellular signaling pathways to participate in tumorigenesis including lung cancer and bladder cancer.

phosphatase and tensin homolog (PTEN), and caspase-3, -8, and -9, and triggered the caspase-dependent apoptotic cascade. Therefore, SPAG6 is a potential prognostic factor in hematological malignancies. One study showed that SPAG6 may also be involved in TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis.[66] Both death receptor and mitochondria-triggered apoptosis are culminated via caspases.[26,67] Jiang et al.[68] also found that *spag6* gene silencing in SKM-1 cells activated the PTEN/phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway by increasing PTEN phosphorylation and decreasing that of AKT, leading to apoptosis and differentiation of the leukemia cells. DNA methyltransferase 1 (DNMT1) is a cytosine methylase that is important for normal mammalian development, and DNMT1 mutations are correlated with human cancer.[69] In addition, *spag6* knockdown was also associated with downregulation of DNMT1, suggesting that SPAG6 may indirectly control the expression of PTEN through DNA methylation.[70] The anti-proliferative effects of *spag6* knockdown were mediated via upregulation of the cyclin-dependent kinase inhibitor $p27^{Kip1}$ and the AKT/forkhead box protein O (FOXO) pathway.[68] The tumor suppressor $p27^{Kip1}$ induces G1-S phase arrest by inhibiting the cyclin E-CDK2 and cyclin A-CDK2 complexes, which are abnormally downregulated in multiple tumors.[29,71] The *spag6* knockdown in SKM-1 cells exhibited G1 phase arrest, $p27^{Kip1}$ upregulation, and cyclin E1 and CDK2 downregulation (Table 1).[68]

Conclusions

SPAG6 regulates multiple functions in various cell types, and its loss can lead to pathophysiological conditions via different molecular mechanisms (Figures 2 and 3). The differential expression of SPAG6 in tumor tissues indicates its diagnostic potential. Furthermore, SPAG6 is also a promising anti-cancer therapeutic target because its absence can stabilize the microtubules,[30,45] enhance the effects of apoptosis-inducing drugs,[65] and induce cell-cycle arrest.[64] SPAG6 plays a major role in regulating the microtubule/cytoskeletal system,[7] as well as the associated cellular functions, by binding to microtubules.[5] Studies also show a positive correlation between gluten-tubulin and SPAG6 overexpression in malignant tumors.[51]

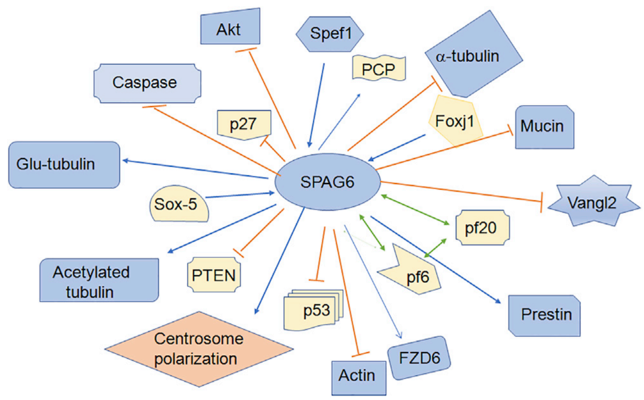

Figure 3. SPAG6 Exerts Its Multiple Functions via Different Molecular Mechanisms

SPAG6 targets numerous genes to participate in its biological function. SPAG6 is regulated by SOX5, Spef1, and Foxj1.

Microtubule-actin interactions are critical for cell movement and morphogenesis,[65] although it remains to be determined whether SPAG6 affects the actin filaments directly or indirectly through the microtubules. SPAG6 has also been implicated in immune deficiencies,[5] because its absence disrupts the effector-target cell synapses that are necessary for an effective immune response. Recent studies show a possible role of SPAG6 in microtubule assembly and cell migration. Various factors are involved in SPAG6 regulation, including the newly ascertained transcription factors GATA-3 and Pou4f3.[36,72] There are several key aspects of SPAG6 biology that need to be studied further. For example, more data are required to support the diagnostic and therapeutic possibilities of SPAG6 in cancer, in addition to identifying the pathologically relevant *spag6* mutations in cancer patients. Furthermore, the upstream regulatory factors and downstream targets of SPAG6, and the pro-tumorigenic factors that synergize with SPAG6 need to be identified. Tissue-specific knockout or transgenic mouse models can greatly elucidate the role of SPAG6 in cancer development.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.omto.2019.08.011.

AUTHOR CONTRIBUTIONS

D.-F.Z., Q.W., J.-P.W., Z.-Q.B., S.-W.W., and L.M. searched the literature and made the figures and tables. D.-F.Z. and D.-M.C. wrote the manuscript. Z.P.W. and Y.-S.T. edited the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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