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REVIEW

Tumor suppressor role of protein 4.1B/DAL-1

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Abstract Protein 4.1B/DAL-1 is a membrane skeletal protein that belongs to the protein 4.1 family. Protein 4.1B/DAL-1 is localized to sites of cell–cell contact and functions as an adapter protein, linking the plasma membrane to the cytoskeleton or associated cytoplasmic signaling effectors and facilitating their activities in various pathways. Protein 4.1B/DAL-1 is involved in various cytoskeleton-associated processes, such as cell motility and adhesion. Moreover, protein 4.1B/DAL-1 also plays a regulatory role in cell growth, differentiation, and the establishment of epithelial-like cell structures. Protein 4.1B/DAL-1 is normally expressed in multiple human tissues, but loss of its expression or prominent down-regulation of its expression is frequently observed in corresponding tumor tissues and tumor cell lines, suggesting that protein 4.1B/DAL-1 is involved in the molecular pathogenesis of these tumors and acts as a potential tumor suppressor. This review will focus on the structure of protein 4.1B/DAL-1, 4.1B/DAL-1-interacting molecules, *4.1B/DAL-1* inactivation and tumor progression, and anti-tumor activity of the 4.1B/DAL-1.

Keywords Protein 4.1B/DAL-1 · Tumor suppressor · Interacting molecules · Solid tumor

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Introduction

Protein 4.1B/DAL-1 (differentially expressed in adenocarcinoma of the lung) is an important membrane skeletal protein that belongs to the protein 4.1 family, and the protein 4.1 family belongs to the protein 4.1 superfamily. Protein 4.1B/DAL-1 links the plasma membrane to the cytoskeleton or associated cytoplasmic signaling effectors and facilitating their activities in various pathways. It has been validated that protein 4.1B/DAL-1 served as a broad-spectrum tumor suppressor in a variety of cancers. Promoter methylation and loss of heterozygosity (LOH) at the *4.1B/DAL-1* locus on 18p11.3 are responsible for the inactivation of *4.1B/DAL-1* gene expression. Abnormal expression of protein 4.1B/DAL-1 may lead to tumorigenesis and/or promote tumor progression. Protein 4.1B/DAL-1 can be involved in different mechanisms that modulate cell growth, motility, adhesion, and cytoskeleton organization. Therefore, protein 4.1B/DAL-1 is likely to

become an important prognostic indicator of human cancers and a potential target molecule for antitumor therapies.

Overview of subfamilies of protein 4.1 superfamily in tumorigenesis and tumor progression

The protein 4.1 superfamily comprises a group of proteins that share a conserved FERM (4.1/ezrin/radixin/moesin) domain at the N-terminal. On the basis of protein sequence similarity, this superfamily can be divided into five subfamilies: protein 4.1 family, merlin/ERM (ezrin/radixin/moesin) protein family, talin-related molecules, PTPH (protein tyrosine phosphatase) proteins and NBL4 (novel band 4.1-like 4) proteins. This protein superfamily serves as membrane cytoskeleton linkers between transmembrane proteins and participates in a wide variety of cellular events, such as proliferation, survival, motility, and cell–cell/cell–substrate adhesion and cell shape [1, 2]. Moreover, the protein 4.1 family and merlin/ERM family have been shown to be involved in tumorigenesis and tumor progression. The protein 4.1 family and merlin are negative growth regulators (tumor suppressors), however, high expression of ERM proteins has been observed in a variety of epithelial cancers to promote tumor progression [3].

Protein 4.1 family: structure, modification, and functions

The protein 4.1 family contains four homologous proteins in mammalian non-erythroid cells: 4.1R (erythrocyte type), 4.1N (neuron type), 4.1G (general type) and 4.1B (brain type). The protein 4.1 family members are encoded by distinct paralogous genes, and a variety of tissue- and development-specific protein isoforms are generated by pre-mRNA alternative splicing and alternative first exons [4–6]. Despite the enormous variety of splice variants, each member of the protein 4.1 family is characterized by three highly conserved structural and functional domains (Fig. 1a). The unique domains act as modulators of protein 4.1 interactions mediated by the conserved domains and that the tissue- and cell-specific splicing patterns of these domains confer the unique characteristics upon each 4.1 proteins [7, 8]. Through three highly conserved domains, 4.1 proteins can bind to membrane molecules, thereby playing important roles in membrane organization and signal transduction. The binding capacity of 4.1 proteins is regulated by calmodulin and/or phosphorylation. Both calmodulin/Ca^{2+} and phosphorylation can result in the down-regulation of the membrane- and/or spectrin-actin binding activities of 4.1 proteins [9–11]. Interestingly, the U2 domain contains a Ser^{312} and an Arg^{309} residue that are the primary substrates for PKC (protein kinase C). These two residues are conserved in four protein

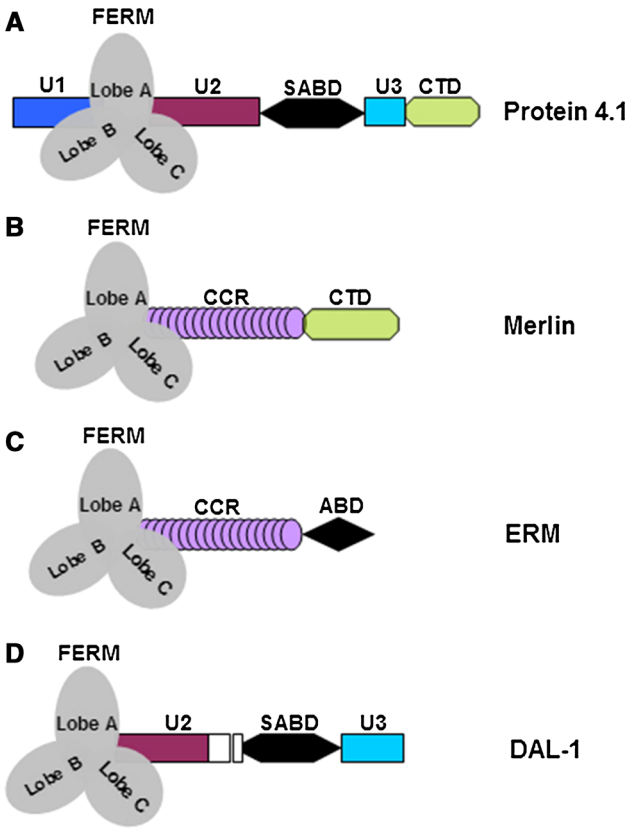

Fig. 1 Structural domains of protein 4.1, merlin/ERM, and DAL-1. The protein 4.1 family (4.1R, 4.1N, 4.1G, and 4.1B) contains three conserved structural domains (FERM, SABD, and CTD) and three unique domains (U1, U2, and U3). The U1 domain is an N-terminal headpiece (HP), the U2 domain is situated between the FERM and the SABD, and the U3 domain lies between the SABD and the CTD. As compared with protein 4.1R, the sequence identity of protein 4.1B in the FERM and SAB domains is 74 and 50 %, respectively. The CTD is highly homologous to the NuMA binding domain of 4.1R (93 % identity). Merlin and ERM members display a similar structural organization: merlin is composed of an N-terminal FERM domain, a coiled-coil region (CCR), and a carboxy-terminal domain. Unlike the merlin proteins, ERM members lack the CTD, while containing an actin-binding domain (ABD) in its C-terminal domain. DAL-1 is a protein fragment of 4.1B. The *white box* denotes internal sequences that are absent in DAL-1

4.1 members. It is possible that PKC-dependent phosphorylation plays a key regulatory role in the function of 4.1 proteins [12]. The protein 4.1 family is an important component of the membrane skeleton. It can stabilize the membrane skeleton and assemble protein complexes. This family of proteins is necessary to maintain cellular morphology, cell polarity, and the mechanical properties of the cell membrane [13]. The protein 4.1 family also plays important roles in membrane organization and signal transduction in tumorigenesis and tumor progression. 4.1R and 4.1N are negative regulators in the tumorigenesis and progression [13]. Moreover, 4.1B/DAL-1 serves as a broad-spectrum tumor suppressor in a variety of cancers [14–16].

Merlin/ERM family: differences with the protein 4.1 family

Similar to the protein 4.1 family, merlin/ERM proteins have been shown to be involved in tumorigenesis and tumor progression [17, 18]. Merlin (moesin, ezrin, radixin-like protein) has a relatively broad tumor suppressor function, however, ERM proteins have been indicated to confer oncogenic activity. Merlin and ERM display a similar structural organization: merlin is composed of an N-terminal FERM domain, a coiled-coil region (CCR), and a carboxy-terminal domain (Fig. 1b). Unlike the merlin proteins, ERM members lack the CTD while containing an actin-binding domain (ABD) in its C-terminal domain (Fig. 1c). It is possible that conserved functions of the protein 4.1 and merlin/ERM family are correlated with the FERM domain, whereas their specific functions reside within less conserved domains. The reverse effects on tumor cells between ERM family and protein 4.1 family may be attributable to the different effects of post-transcriptional modifications. In this regard, phosphorylation of protein 4.1 negatively regulates its interaction with membrane proteins and the cytoskeleton. While ERM proteins upon are phosphorylated, they are activated and interact with transmembrane proteins and F-actin [17, 19]. Furthermore, the distinct subcellular localization in polarized epithelial cells (ERMs/the apical domain versus protein 4.1/the basolateral domain) may also be responsible for their reverse effects [12, 20, 21].

Protein 4.1B/DAL-1: gene, structure, and localization

Protein 4.1B, or type II brain 4.1 (genes KIAA0987, *EPB41L3*), is a neuronal-enriched protein 4.1 homolog [22]. *EPB41L3* is located on human chromosome 18p11.3, and the large *EPB41L3* gene is approximately 240 kb in length. *EPB41L3* contains exons encoding full-length FERM (exons 4–11), full-length CTD (exons 18–21), U1 (exon 2 and part of exon 4), U2 (exons 13–14) and U3 (exon 17C) [23]. With regard to the SAB domain, while exon 17 is present, exon 16 is missing [24]. Among the conserved domains of protein 4.1B, only exon 16 in the SABD and exon 21 in the CTD exhibit tissue-specific alternative splicing [8, 22]. DAL-1 is a splice variant of protein 4.1B. Significantly reduced expression (>50 %) of DAL-1 was detected in primary non-small cell lung cancer (NSCLC) tissues as compared with patient-matched normal lung tissues [25]. DAL-1 contains the amino acid residues spanning from Met^{110} to Ser^{853} in protein 4.1B, and it lacks a U1 structural domain at the N-terminal, a CTD domain at the C-terminal, a partial region of the U2 and SABD domains [25, 26] (Fig. 1d). Although DAL-1 lacks a partial actin-binding domain, the necessary residues remain to function as a tumor suppressor, and may also associate with the actin skeleton indirectly by binding to all known 4.1B-binding proteins [27]. In addition, a study identified a Golgi-specific 200-kDa protein 4.1B variant and found that it is required for both the structural integrity of the Golgi complex and for the assembly of a subset of plasma membrane proteins. Depletion of this variant in HBE cells led to disruption of the Golgi structure and impaired membrane trafficking of Na^+/K^+-ATPase, ZO-1, and ZO-2 [28].

4.1B/DAL-1 is located beneath the plasma membrane and is distributed to cell–cell junctions [29] (Fig. 2), as well as cell-basement membrane contacts [25]. In the PNS and CNS, 4.1B is concentrated at internodes, paranodes, and juxtaparanodes in myelinated axons. This protein contributes to the stabilization of membrane proteins at paranodes, the clustering of juxtaparanodal proteins, and the regulation of the internodal axon caliber [30–33]. In addition to the nervous system, protein 4.1B is also expressed in the lungs [25], kidneys [34], intestines [35],

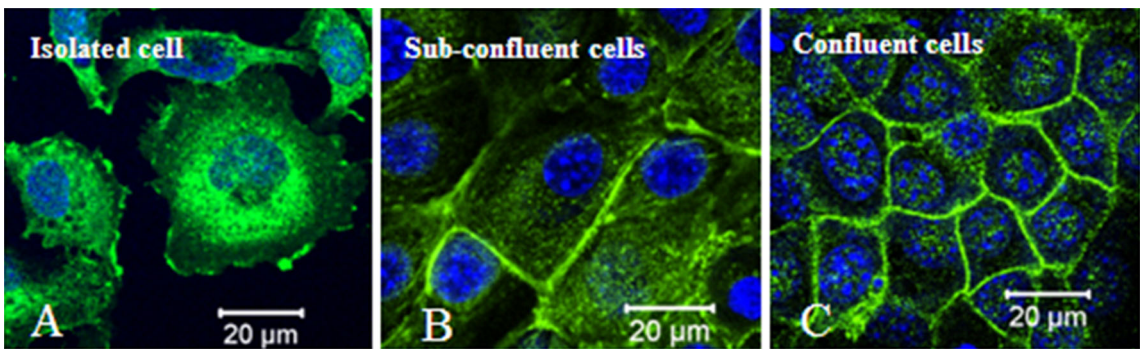

Fig. 2 Sub-cellular localization of protein 4.1B in primary mouse embryonic fibroblasts (MEF). **a** In isolated MEF cells, protein 4.1B is predominantly located at peri-nuclear region and also distributed in cytoplasm and membrane ruffling edges. **b** Protein 4.1B is recruited to areas of cell–cell contact in sub-confluent conditions. **c** Protein 4.1B is very strongly enriched at cell–cell contacts in completely confluent cells [29]

pancreas [36], testes [37], prostate [38], ovaries [39], and breasts [40]. In these tissues, 4.1B/DAL-1 maintains cell–cell and cell–matrix interactions and stabilizes the organization of the cytoskeleton by linking transmembrane proteins to the cytoskeleton [41].

Protein 4.1B/DAL-1-interacting molecules

To elucidate the tumor suppressor functions of protein 4.1B/DAL-1, the binding ligands that directly interact with protein 4.1B/DAL-1 in various tumors must be identified (Fig. 3). To date, a series of potential protein 4.1B/DAL-1-interacting molecules have been described specifically (Table 1). Furthermore, protein 4.1B/DAL-1 is also known to interact with ERM proteins, as well as the amino terminus of merlin in vitro and two known merlin interactor proteins, CD44 and βII-spectrin [27].

Spectrin and actin

The SAB domain of protein 4.1B mediates the formation of spectrin-4.1B-actin ternary complexes. It is less efficient than the 4.1R SABD due to a decrease in the actin-binding activity, while its binding affinity for spectrin is the same as that of the 4.1R SAB domain [42]. The fully functional SABD is encoded by the alternatively spliced exon 16 plus the constitutively expressed exon 17. Exon 16 encodes one spectrin-binding motif, while exon 17 encodes the actin-binding motif and the second spectrin-binding motif [24]. The majority of the nonerythroid protein 4.1 expressed in mammals lacks a high affinity SAB domain (no exon 16 paralog in 4.1N; splicing out of exon 16 from 4.1R, 4.1G, and 4.1B in many tissues) [23]. Although 4.1B does not retain exon 16, exon 17 alone could confer physiologically relevant cytoskeleton-binding activity. Protein 4.1B serves as a key membrane-cytoskeleton linker that likely tethers transmembrane proteins in the plasma membrane and functions in organizing the cytoskeleton. It has been shown that a 4.1B/spectrin II cytoskeletal complex stabilized the expression of multiple FERM-binding adhesion molecules in axonal membrane. While the loss of 4.1B resulted in a significant reduction of spectrin II, Nectin-like (Necl)-1 and -2 expression along the internodal membrane of the axon [33]. In addition, a recent report showed that 4.1B-deficient mouse embryo fibroblasts (MEF) cells failed to form actin stress fibers [29].

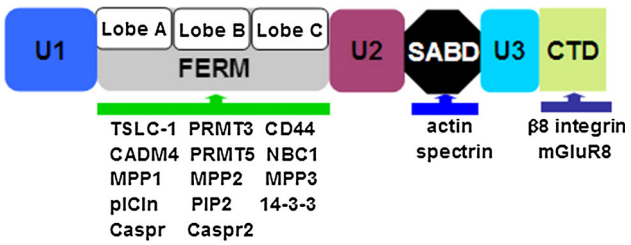

Fig. 3 Schematic representation of protein 4.1B and interacting molecules. Protein 4.1B binds to interacting molecules through FERM, SABD, and CTD. Binding ligands: *TSLC-1* tumor suppressor in lung cancer 1, *CADM* cell adhesion molecule, *PRMT* protein arginine methyltransferase, *NBC1* Na^+ bicarbonate cotransporter 1, *MPP* membrane palmitoylated protein, *pICln* chloride channel, *PIP2* phosphatidylinositol 4,5-bisphosphate, *14-3-3* tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, *Caspr* contactin-associated protein, and *mGluR8*, metabotropic glutamate receptor isoform 8

14-3-3 family members

The 14-3-3 proteins are a family of acidic regulatory proteins that consist of seven isoforms: β, γ, ε, ζ, η, τ and θ. The 14-3-3 proteins serve as adapter proteins that regulate protein–protein interactions and the subcellular localization of proteins. This protein family is also involved in regulating many biologically important processes, including the cell growth cycle, apoptosis, signal transduction, polarity, and skeletal structure [54]. Due to its unique binding interaction with 4.1B/DAL-1, but not merlin, ezrin, or radixin, 14-3-3 is believed to be involved in the control of 4.1B/DAL-1-mediated cell growth. Disruption of the interaction of 14-3-3 with the 4.1B FERM domain did not impair the growth-inhibitory effects of 4.1B/DAL-1 in meningioma cells, suggesting that 14-3-3 may not be an indispensable molecule for 4.1B/DAL-1-mediated growth inhibition [26, 43].

Caspr/paranodin and Caspr2

Several 4.1 proteins are expressed in the nervous system. However, only 4.1B is enriched at paranodes and juxtaparanodes in myelinated axons. 4.1B associates with the neural cell adhesion molecules, Caspr/paranodin and Caspr2 (a protein closely related to Caspr/paranodin), at paranodes and juxtaparanodes, respectively. Both Caspr and Caspr2 bind to the N-terminal FERM domain of protein 4.1B through their GNP motifs [55]. Caspr is required for the generation of a membrane barrier at the paranodal junction (PNJ), which prevents juxtaparanodal components from entering the paranodal space. The interaction of Caspr with protein 4.1B is necessary for its stabilization at the PNJ and the generation of an efficient membrane barrier at this site. Loss of 4.1B in the PNS leads to mislocalization of Caspr at the paranodes and destabilization of paranodal axo-glial septate junctions. Caspr2 is necessary for clustering Kv1 channels at the juxtaparanodal region (JXP) [32, 56]. As for Caspr2, 4.1B is necessary for the accumulation of Caspr2 and Kv1 channels at juxtaparanodal axonal

Table 1 Protein 4.1B/DAL-1-interacting molecules

Molecules	Characteristics	Binding domain	Functions of binding protein-4.1B/DAL-1 complex	Note	References
Spectrin, actin	The cytoskeleton	SABD	Provides cytoskeletal linkage and modulates important cytoskeletal functions in a wide variety of cells and tissues	1, 2	[42]
14-3-3 proteins	Adapter proteins	FERM	Not determined	3, 4, 5, 6	[43]
Caspr/paranodin, Caspr2	Neuronal transmembrane glycoprotein	FERM	At paranodes and juxtaparanodes, 4.1B associates with Caspr/paranodin and Caspr2, respectively, and anchors them to the axonal cytoskeleton	5, 6, 7	[30]
pICln	Swelling-activated anion channel	FERM	Participating in the regulation of cell volume and cellular RNA splicing processes	3, 5	[44]
NBC1	Na^+–HCO_3^- cotransporter	FERM	Maintaining normal intracellular homeostasis	5, 6	[45]
PRMT3, PRMT5	Protein arginine methyltransferase	FERM	4.1B/DAL-1 is not itself a substrate for PRMT3/5, but rather modulates PRMT3/5-mediated methylation activity	3, 4, 5, 6	[44, 46]
β8-integrin	Cell adhesion receptor	CTD	4.1B/DAL-1 involves in β8-integrin adhesion and signaling pathways	6, 7	[47, 48]
CD44	Transmembrane receptor and stem cell marker	FERM	Binding of 4.1B/DAL-1 to CD44 is likely implicated in 4.1B/DAL-1 growth suppression	8	[14, 26]
PIP2	Membrane phospholipids	FERM	PIP2 has been suggested to play a regulatory role in the association of 4.1B/DAL-1 with membrane proteins	9	[49]
TSLC-1, CADM4	Immunoglobulin superfamily cell adhesion molecules	FERM	Organization of the actin cytoskeleton; construction of epithelial-like cell structure	5, 6, 7	[50, 51]
MPP1/2/3	Membrane-associated guanylate kinase family members	FERM	Construction of epithelial-like cell structure	5, 6, 7	[52]
MGluR8	Metabotropic glutamate receptors	CTD	Proper targeting and/or stabilization of mGluR8 at the plasma membrane and inhibition of mGluR8-mediated reductions in intracellular cAMP concentrations	3, 6	[53]

Supported by *1* co-sedimentation assay, *2* resonant mirror detection, *3* yeast two-hybrid interaction, *4* in vitro binding assays, *5* co-immunoprecipitation, *6* GST fusion protein pull-down, *7* subcellular co-localization, *8* in vivo interaction studies, *9* PIP2-binding motifs were obtained from the UniProt database

membrane. In 4.1B mutant mice, Caspr2 and Kv1 channels are not clustered at the JXP [30, 31]. Thus, 4.1B is a critical component of the paranodal and juxtaparanodal domains and play an important role in the organization of myelinated axons.

pICln and NBC1

pICln, a cell-swelling activated chloride channel that is primarily present in the cytosol of resting cells, is targeted to and inserted into the plasma membrane in response to cell swelling. Once inserted into the membrane, pICln forms a channel-like structure, resulting in Cl^- efflux and regulatory volume decrease [57–59]. pICln has been found to bind to the FERM domain of 4.1B and 4.1R, both of which may anchor pICln to the cell membrane and link pICln to the spectrin/actin skeleton to regulate cell volume [12, 60]. A high metabolic rate is a prominent characteristic of the majority of malignant tumors. Tumor cells have very efficient acid extrusion mechanisms that help dispose of excess acid to the point of actually decreasing cytosolic $[H^+]$ levels below those of normal cells. Membrane acid–base transport represents a key pathway for the disposal of cellular acid and can be mediated by a number of ion transport proteins, such as NHE1 (Na^+/H^+ exchanger 1) and NBC1. NHE1, a 4.1R-binding molecule, is significantly activated in 4.1R-deficient erythrocytes [61]. Interestingly, over-activation of NHE1 can cause cytoplasmic alkalization in breast cancer cells and generate an acidic hypoxic tumor microenvironment, thereby promoting tumor cell proliferation and metastasis [21]. 4.1B can interact with NBC1 in the basolateral membrane of the renal proximal tubules [45]. NBC1 has been found to be expressed in renal cell carcinomas and may be involved in the establishment of an acidic microenvironment [62]. Evidence suggests that an increase in cell volume and intracellular alkalinization occurs during cell proliferative processes [63, 64]. Therefore, 4.1 proteins may maintain normal cell volume and intracellular homeostasis by stabilizing the cytoskeleton and regulating their binding proteins, such as pICln, NHE1, and NBC1. Loss of 4.1 proteins may lead to dysregulation of critical ion transport proteins and thus promote tumor development.

PRMT family

PRMT family members can catalyze the sequential transfer of methyl groups from *S*-adenosyl-L-methionine to the guanidino nitrogens of arginine residues in proteins. Protein methylation by PRMTs has been implicated in the regulation of various processes, including signal transduction, transcriptional regulation, and RNA splicing [65]. 4.1B/DAL-1 is not only a substrate for PRMT3/5, but it can also suppress PRMT3-mediated protein methylation and enhance or inhibit PRMT5-mediated protein methylation in a substrate-specific manner [44, 46]. 4.1B/DAL-1 may regulate the intracellular mRNA splicing process by inhibiting PRMT5-mediated spliceosomal Sm proteins, including B/B′, D1 and D3, methylation. Both PRMT5 and pICln are components of the 20S methyl transfectase enzyme complex (methylosome). In the presence of pICln, pICln interacts with PRMT5 and stimulates PRMT5 methylation of Sm proteins [66]. Methylation of Sm proteins by the methylosome is necessary for directing Sm proteins from methylosome to the SMN (survival of motor neuron) complex for assembly into snRNP (small nuclear ribonucleoprotein) core particles in the cytoplasm [67, 68]. Subsequently, snRNPs transport into the nucleus and participate in pre-mRNA splicing events. It is possible that a 4.1B/DAL-1-PRMT5-pICln complex may either interfere with PRMT5 protein and pICln protein assembly on methylosome or prevent PRMT5 from methylating the spliceosomal Sm B/B′, D1, and D3 proteins. Abnormal functions of 4.1B/DAL-1 may lead to a disruption of cells' ability to form functional SMN complexes, thereby causing abnormal pre-mRNA splicing [44].

Integrins

Integrins are heterodimeric transmembrane receptors that consist of α and β glycoprotein subunits. Integrins are involved in mediating cellular interactions with extracellular matrix (ECM) and cell–cell interactions. Studies have identified that 4.1B associated with αvβ8 integrin through direct interaction between cytoplasmic tail of β8 and CTD of 4.1B. 4.1B-αvβ8 integrin-mediated adhesion and signaling pathways are important for the normal formation and function of the heart and the proper development of the CNS [47, 48]. It has been reported that αvβ8 integrin expression was generally absent in human lung cancers, suggesting that dysregulation of 4.1B-αvβ8 integrin signaling pathways may be involved in the lung tumor progression [69]. Moreover, although 4.1B has been found to not directly interact with β1 integrin, β1 integrin and its heterodimer partner α5 integrin also showed decreased surface expression in 4.1B-deficient MEF cells. Fluorescence pulse-chase studies found that after 4 h of chase, β1 integrin in 4.1B WT-MEF cells could undergo continuous internalization and recycling. However, in 4.1B-deficient MEF cells, the internalized β1 integrin vesicles were mainly accumulated at the perinuclear region and could not recycle to the membrane, suggesting that 4.1B can control cell surface expression of β1 integrin by mediating its trafficking [29].

CD44

CD44 is a transmembrane glycoprotein that binds to hyaluronic acid (HA) in the extracellular matrix. It is involved in cell adhesion, migration, and signaling. Merlin, ERM proteins, and 4.1R and 4.1B have all been shown to bind to CD44. Merlin and ERM proteins maybe have different or even opposite effects on CD44 signaling events. CD44 has been proposed to be involved in the formation and elongation of microvilli in various cell types by concentrating activated ERM proteins at the plasma membrane [70]. However, Merlin may compete with ERM proteins for binding to CD44, which depends on the cell proliferative status. At high cell density, hypophosphorylated/activated merlin displaces ERM proteins from membrane components and mediates contact inhibition signals of growth from the extracellular matrix through its interaction with CD44. Whereas at low cell density phosphorylated/activated ERM proteins associate with CD44 and phosphorylated/inactivated merlin, this complex might serve to prevent merlin activation and facilitate cell proliferation [17, 71]. Protein 4.1B/DAL-1 and 4.1R also interact with CD44 via their FERM domain. It has been demonstrated that incremental expression of both protein 4.1B and 4.1R was similar to merlin in the cell membrane fractions under growth arrest conditions [27, 72]. It is tempting to speculate that under growth arrest conditions, protein 4.1B and 4.1R are redistributed to the cell membrane where they may interact with critical effector molecules like CD44 to negatively regulate cell growth, as has been described for merlin.

PIP2

Phosphatidylinositol 4,5-biphosphate (PIP2) is a low-abundance phospholipid in the plasma membrane inner leaflet. PIP2 interacts with the FERM domain and regulates the activities of protein 4.1 superfamily proteins, especially their membrane and actin binding activities. For example, PIP2 plays a regulatory role in the association of the ERM proteins with membrane proteins and with the actin cytoskeleton. PIP2 binding triggers conformational changes that unmask the membrane protein-binding sites in the FERM domain. Such conformational changes are critical for the association of ERM proteins with cytoplasmic tail of CD44 and the recruitment of ERM proteins to the plasma membrane in vivo and in vitro [73, 74]. In addition, the sequence of events consisting of binding to PIP2 followed by the phosphorylation is critical for the proper activation and correct apical localization of ezrin in epithelial cells [75]. Binding of PIP2 to 4.1R selectively modulated the ability of 4.1R to interact with its different binding partners. It has been reported that 4.1R bound to PIP2-containing liposomes through its FERM domain. PIP2 binding induced a conformational change in this domain accompanied by an increase in glycophorin C (GPC) binding and a decrease in band3 binding. The potential PIP2-binding motifs are highly conserved in the protein 4.1 family. It is likely that the functions of 4.1B are similarly regulated by PIP2 in many different cell types [49].

TSLC-1

TSLC-1 is an immunoglobulin superfamily cell-adhesion molecule and also termed CADM1/Necl-2/SgIGSF/RA175/IGSF4/SynCAM1. TSLC-1 can maintain the phenotype of epithelial cells and inhibit EMT (epithelial–mesenchymal transition) through the formation of mature cell–cell adhesion [52, 76]. TSLC-1 is frequently absent in many types of epithelial tumors, particularly in some invasive or metastatic tumors [77, 78]. In culture, NSCLC cells lacking TSLC-1 display a typical transformed morphology with immature cell adhesions [79]. The cytoplasmic transmembrane domain of TSLC-1 contains a 4.1-binding motif, which is homologous to neurexin IV, paranodin, syndecan-2, and GPC. TSLC-1 can bind to the lobe C in the FERM domain of 4.1B/DAL-1 via the 4.1-binding motif (Fig. 4) [50]. The fact has been confirmed that TSLC-1 interacted with the actin cytoskeleton and participated in organizing the actin cytoskeleton through 4.1B/DAL-1 [50, 80, 81]. The PDZ (PSD-95/Dlg/ZO-1) binding motif at the C-terminus of TSCL-1 is linked to MAGUK family members, including MPP1/2/3, CASK, Pals2, and syntenin [52, 79]. Both the 4.1- and PDZ-binding motifs may play important roles in the lateral localization of TSLC-1 in human epithelia [76]. Moreover, the 4.1- and PDZ-binding motifs are likely involved in the

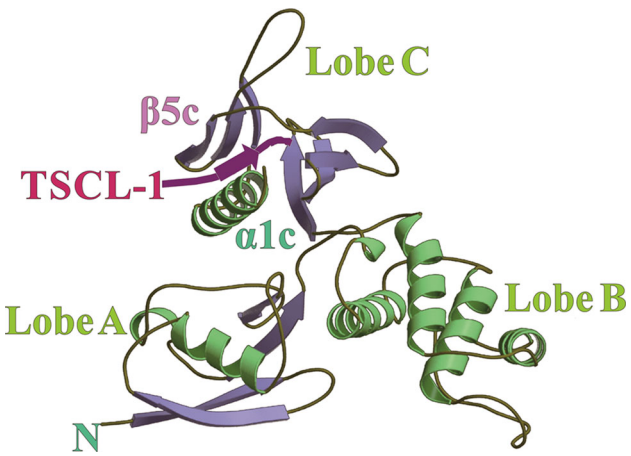

Fig. 4 Molecular graphic illustrating the interaction of the DAL-1 FERM domain with TSLC-1.The DAL-1 FERM domain consists of three lobes (*Lobe A*, *B*, and *C*), interacting with the TSLC-1 peptide (*pink*) in a cleft between helix α1C and β-strand β5C [50]

anti-tumor activity of TSLC-1. Since deletion of the 4.1- or PDZ-binding motifs significantly enhanced the tumorigenicity of the A549 NSCLC cell line in a nude mouse model [82]. In addition, the presence of the 4.1- and PDZ-binding motifs is essential for the ability of TSLC-1 to inhibit cell proliferation and induce apoptosis in A549 cells [83]. NSCLC and breast cancers frequently display a loss of TSLC-1 and/or 4.1B/DAL-1 [81, 84, 85]. Interestingly, no significant difference in clinicopathological features was observed between the tumors with low expression of either 4.1B/DAL-1 or TSLC-1 versus both 4.1B/DAL-1 and TSLC-1 in primary breast cancer [78]. This evidence strongly suggests that dysfunction of the TSLC-1-4.1B/DAL-1 cascade is involved in the pathogenesis of breast cancers.

Membrane-associated guanylate kinases (MAGUKs)

MAGUKs are a large family of scaffold proteins that are located at sites of epithelial cell–cell contact and at the synaptic junctions of neuronal cells [86]. Three MAGUK proteins, including MPP1/p55 [87], CASK (calcium/calmodulin-dependent serine protein kinase) [88], and hDlg (human discs large)/SAP97 [89], share a conserved protein 4.1-binding site in their HOOK domain. 4.1 proteins can form a 4.1-transmembrane protein-MAGUK ternary complex. This ternary complex provides a functional unit serving as a clustering apparatus at the plasma membrane. MPP3, *Drosophila* discs large tumor suppressor homologue, has been demonstrated to form a ternary complex with TSLC-1 and 4.1B/DAL-1 at epithelial cell–cell attachment sites. However, MPP3 expression has been detected in all NSCLC and SCLC (small-cell lung cancer) cell lines. Whether MPP3 is implicated in the tumorigenesis of lung cancer still needs further studies [81, 90].

4.1B/DAL-1 inactivation and tumor progression

Mechanisms of *4.1B/DAL-1* inactivation

Many studies have confirmed that protein 4.1B/DAL-1 is lost or down-regulated in a variety of malignant and benign tumors (Table 2), such as NSCLC [25, 85, 91], breast cancer [78, 84, 92], meningiomas [93, 94], ependymomas [95, 96], renal cell carcinomas [34], prostate cancer [38, 97], ovarian cancer [39], liver cancer [98], nasal NK/T cell lymphomas [99], and colon cancer [35]. Biallelic promoter methylation and loss of heterozygosity (LOH) at the *4.1B/DAL-1* locus on 18p11.3 are the main mechanisms responsible for the inactivation of *4.1B/DAL-1* gene expression (Tables 3, 4). An approximately 400-bp fragment around the promoter and exon 1 of the *4.1B/DAL-1* gene contains 38 CpG sites with a GC content of 75 %, matching the criteria for a CpG island. Among them, hypermethylation of the 14 CpG sites (CpG sites 19-32) within the 92-bp fragment around the *4.1B/DAL-1* promoter (Fig. 5) played a critical role in *4.1B/DAL-1* gene silencing [34, 85]. Monosomy of chromosome 18 or large deletions on chromosome 18p are the main mechanisms for LOH of *4.1B/DAL-1* gene [92, 94]. In addition, point mutations have not been demonstrated to be the main mechanism responsible for *4.1B/DAL-1* inactivation [100]. There are additional mechanisms leading to the silencing of 4.1B/DAL-1 that do exist, such as histone deacetylation and loss of transcription factor, which require further investigation.

The relationship between *4.1B/DAL-1* and tumor progression

In squamous cell lung carcinomas, *DAL-1* methylation seemed to be a relatively early event; the incidence was 90 % in stage I tumors, but it was not altered with tumor progression to advanced stages. In contrast, *DAL-1* methylation may be a late event in lung adenocarcinomas, since the incidence increased significantly as the tumor stage advanced from stage I to stage IV. Preferential *DAL-1* methylation was also observed in invasive, metastatic, and pleural lung adenocarcinomas. Furthermore, a significant correlation between *DAL-1* methylation in tumors and shorter disease-free and overall survival was observed for patients with lung adenocarcinomas. Therefore, *DAL-1* methylation may be used as a potential prognostic indicator for NSCLC, particularly for lung adenocarcinomas [85]. In breast cancer, the incidence of *TSLC-1* and/or *DAL-1* methylation in patients with grade 3 tumors was more frequent than in patients with grade 1 and 2 tumors [84]. Yuka et al. confirmed that as compared to non-invasive lesions, invasive lesions generally display low and abnormal 4.1B expression in breast cancer tissue samples. Additionally, low or abnormal 4.1B expression in tumors was significantly correlated with lymph node metastasis, advanced pT stages of pT2 and pT3, and advanced pathological stages of II and III [78]. In kidney, 4.1B is located in the basolateral membrane of the renal proximal tubules, while RCCC primarily originate from the proximal tubule cells [45, 51]. Abnormal *4.1B* methylation likely occurs during the early stages of RCCC. Since *4.1B* hypermethylation occurred in 42 % of tumors with pathological stage I and 47 % of tumors with stage pT1a, while the incidence of *4.1B* hypermethylation did not increase significantly in tumors with more advanced stages. In addition, *4.1B* hypermethylation was significantly associated with higher nuclear grade (an indicator of abnormal nucleus of tumor cells) and shorter recurrence-free survival [34]. In meningiomas, LOH of

Table PS2 *4.1B/DAL-1* deficiency in tumors

Tumor type	Number of cases/cell lines	Number of cases/cell lines with *4.1B/DAL-1* deficiency (%)	References
NSCLC cell lines	16	14 (87)	[81, 91]
Adenocarcinoma	11	9 (82)	
Squamous cell carcinoma	1	1 (100)	
Large cell carcinoma	3	3 (100)	
Nonspecified NSCLCs	1	1 (100)	
Pleural fluid NSCLC cells	10	9 (90)	
SCLC cell lines	11	4 (36)	
Primary NSCLC tumors	39	21 (54)	[25]
Adenocarcinoma	15	6 (40)	
Squamous cell carcinoma	16	11 (69)	
Large cell carcinoma	1	1 (100)	
Nonspecified NSCLCs	7	3 (43)	
Breast cancer			
Breast cancer cell lines	8	6 (75)	[84]
Primary breast cancers	67	49 (73)	[78]
Lymph node metastasis			
0	46	29 (63)	
1–3	17	16 (94)	
≥4	4	4 (100)	
Lymphovascular invasion			
−	50	33 (66)	
+	17	16 (94)	
pT stage			
1	31	18 (58)	
2	33	28 (85)	
3	3	3 (100)	
Pathological stage			
I	23	11 (48)	
II	40	34 (85)	
III	4	4 (100)	
Renal clear cell carcinoma			
RCC cell lines	19	10 (53)	[34]
Primary RCCC	19	12 (63)	
Meningioma			
Benign, non-recurring (grade I)	20	12 (60)	[77]
Benign, recurring (grade I)	16	10 (63)	
Atypical (grade II)			
High proliferative	23	15 (65)	
Brain invasive, low proliferative	22	17 (77)	
Anaplastic (grade III)	7	6 (86)	
Ovarian cancer			
Ovarian cancer cell lines	19	15 (78)	[39]
Benign ovarian tumors	33	8 (24)	
Invasive ovarian tumors	794	524 (66)	
Clear cell	61	42 (69)	
Serous	454	300 (66)	
Mucinous	70	43 (61)	
Endometrioid	150	86 (57)	
Undifferentiated	38	10 (26)	
Papillary	21	8 (38)	

DAL-1 at chromosome 18p11.3 was originally reported in 71 % (12/17) of sporadic meningiomas, regardless of histological grade, suggesting that loss of *DAL-1* was an early event in the tumorigenesis of meningiomas [93]. However, subsequently, Nunes et al. reported that LOH of *4.1B/DAL-1* occurred only in 19 % (12/62) of meningiomas. LOH of *4.1B/DAL-1* was more common in malignant meningiomas (three of five tumors, 60 %) than that in benign meningiomas (1 of 31, 3.2 %), indicating that loss of *4.1B/DAL-1* may be involved in the development of meningiomas rather than an initiating event [94]. In ovarian cancers, the expression of 4.1B was found in normal ovarian epithelia tissues. However, there is absent expression of 4.1B in 24 % of benign ovarian tumors and in 66 % of invasive ovarian tumors. This suggests that 4.1B loss is a late rather than an initiating event during the ovarian cancer development process [39]. In prostate cancer, Wong et al. [38] established an orthotopic xenotransplant model of prostate cancer by using PC3 cells, a human prostate adenocarcinoma cell line, and separated the highly, moderately, and poorly metastatic cell sublines. This study found that 4.1B expression was significantly down-regulated in highly metastatic cell sublines as compared to parental PC3 cells and the poorly metastatic cell sublines, and showed an intermediate level in moderately metastatic cell sublines. Down-regulation of 4.1B level in poorly metastatic cell sublines enhanced their metastatic propensity in an orthotopic model of prostate cancer, suggesting that down-regulation of *4.1B* promotes prostate cancer progression to metastatic phenotype. Furthermore, *4.1B*-deficient mice displayed increased predisposition to developing aggressive, undifferentiated carcinomas relative to *4.1B*-heterozygous mice. In both models, tumorigenic cells lacking 4.1B displayed reduced rates of apoptosis.

Table 3 *4.1B/DAL-1* methylation in tumors

Tumor type	Number of cases/ cell lines	Number of cases/cell lines with *4.1B/DAL-1* methylation (%)	References
Lung cancer			
NSCLC cell lines	39	17 (44)	[81, 91]
Adenocarcinoma	28	12 (43)	
Squamous cell carcinoma	5	3 (60)	
Large cell carcinoma	6	2 (33)	
Pleural fluid NSCLC cells	9	6 (67)	
SCLC cell lines	11	3 (27)	
Primary lung tumors			[81, 85]
NSCLC			
Adenocarcinoma	133	75 (56)	
Squamous cell carcinoma	109	60 (55)	
Large cell carcinoma	8	4 (50)	
Adenosquamous cells carcinoma	18	8 (44)	
SCLC	4	0 (0)	
Corresponding nonmalignant lung tissue samples	30	3 (10)	
Pleural fluid NSCLC	103	59 (57)	
Breast cancer			
Breast cancer cell lines	8	6 (75)	[84]
Primary breast cancers	95	26 (27)	
Ductal carcinoma	81	21 (26)	
Lobular carcinoma	13	4 (31)	
Other	1	1 (100)	
Renal clear cell carcinoma			
RCC cell lines	19	9 (47)	[34]
Primary RCCC	55	25 (45)	
Prostate cancer			
Prostate carcinoma cell lines	4	3 (75)	[97]
Primary tumor tissues	113	89 (79)	

Table 4 *4.1B/DAL-1* LOH in tumors

Tumor type	Number of cases/cell lines	Number of cases/cell lines with *4.1B/DAL-1* LOH (%)	References
Lung cancer			
Primary NSCLC tumors	100	39 (39)	[85]
Adenocarcinoma	66	26 (39)	
Squamous cell carcinoma	25	8 (32)	
Large cell carcinoma	7	3 (43)	
Adenosquamous cells Carcinoma	2	2 (100)	
Pleural fluid NSCLC	100	39 (39)	
Breast cancer			
Breast cancer tissues	43	27 (63)	[92]
Ductal carcinoma	16	9 (56)	
Invasive ductal carcinoma	21	14 (67)	
Metastatic disease	6	4 (67)	
Renal clear cell carcinoma			
RCC cell lines	19	9 (47)	[34]
Primary RCCC	54	4 (7.4)	
Sporadic meningioma			
Sporadic meningioma	62	12 (19)	[94]
Benign	31	1 (3)	
Malignant	5	3 (60)	

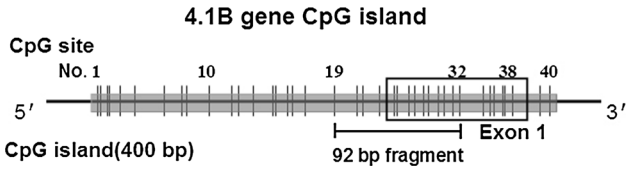

Fig. 5 Results of the methylation analysis of the 5′ upstream region of the 4.1B promoter and schematic. *Vertical bars* indicate CpG sites numbered 1–40. The *box* indicates exon1 of the 4.1B/DAL-1 gene. The sequence traces correspond to the 92-bp fragment containing the 14 CpG sites numbered 19 through 32 within the upstream region of the transcription initiation site and the beginning of exon 1

Antitumor function of protein 4.1B/DAL-1

Metastasis is a complex multistep process. Tumor cells require a series of molecular changes to assist them to progress through every step of the metastatic cascade, including cytoskeletal rearrangements and alteration in adhesiveness, increased motility and invasion, and enhanced survival and proliferation. Recent studies have shown that 4.1B/DAL-1 can maintain cell adhesion and F-actin cytoskeleton organization, participate in regulating cells differentiation, growth, motility, and apoptosis, suggesting that 4.1B/DAL-1 might act as an important tumor suppressor in tumor progression.

Protein 4.1B/DAL-1 and cell differentiation

4.1B/DAL-1 plays a crucial regulatory role in cell growth and differentiation. In normal epithelia, 4.1B/DAL-1 is related to cell proliferation and differentiation and its high expression could be a marker for the maturity of epithelial cells [101]. In the intestinal epithelium, 4.1B/DAL-1 strongly expresses in the basolateral membrane of the simple columnar epithelium in villi [35, 101]. Intestinal epithelial cells originate from the stem cells in the crypts and subsequently undergo functional and morphological differentiation as they migrate along the crypt–villus axis. Interestingly, 4.1B/DAL-1 expression gradually increased during the epithelial cells' migration from the crypt to the top of villi, with the crypt showing no expression of 4.1B [35, 101]. However, the membranous expression of protein 4.1B/DAL-1 was reduced during the malignant transformation of the intestinal epithelium [35]. In addition, two studies reported a concomitant loss of 4.1B and E-cadherin at cell–cell contact regions upon the phenotypic transition from adenoma (benign tumor) to carcinoma (invasive malignant tumor) in the pancreatic and colonic epithelia [36, 101]. This evidence suggests that 4.1B/DAL-1 may function in maintaining an epithelial and differentiated organization, and normal cellular proliferation and adhesion, thereby preventing the malignant transformation of epithelial cells.

Protein 4.1B/DAL-1 maintains cell adhesion

The desquamation of cells from the primary tumor caused by disruption of intercellular adhesion allows metastatic cells to escape from their original site and to acquire a more motile and invasion phenotype. To date, concomitant loss of both TSLC-1 and 4.1B/DAL-1 has been frequently observed in NSCLC, breast cancer, meningiomas, and nasal NK/T-cell lymphomas [81, 84, 85, 99]. Sakurai-Yageta et al. [52] reported that 4.1B-TSLC-1-MPPs complexes were distributed at cell–cell adhesion sites in HEK293 cells. Inhibition of TSLC-1 expression in HEK293 cells by siRNA caused abrogation of epithelial-like structure and displayed a flat morphology with immature cell adhesions. In addition, HEK293 cells lacking TSLC-1 showed the mislocalization of 4.1B, MPP2, E-cadherin, and ZO-1 from the membrane. 4.1B/DAL-1 is not only an adhesion molecule but the TSLC-1-4.1B/DAL-1 cascade appears to be involved in building stable adhesions between adjacent epithelial cells and organizing the actin cytoskeleton [80]. Functional loss of TSLC-1 and/or 4.1B/DAL-1 may disrupt intercellular adhesion and the association between the membrane and the cytoskeleton, which may lead tumor cells to metastasis or invasion. Furthermore, a recent study found that 4.1B/DAL-1 was associated with CADM4, a protein homology to TSLC-1, in human proximal tubules. Loss of CADM4 is involved in the tumorigenicity of human renal cell carcinoma. Interestingly, disruption of the CADM4-4.1B cascade frequently occurs in human RCC cells and RCCC tumors. The average size of tumors lacking CADM4 or 4.1B or both was significantly larger than that of the tumors expressing both CADM4 and 4.1B. In addition, no pathological changes were observed between the tumors lacking both CADM4 and 4.1B and those lacking only CADM4 or 4.1B, suggesting that CADM4-4.1B cascade participated in normal cell adhesion of the proximal tubules [51].

Protein 4.1B/DAL-1 suppresses cell motility

Metastatic tumor cells are characterized by more rapid cell migration and the presence of more pseudopodia-like protrusions than primary tumor cells [102]. The morphological and dynamic changes in these highly motile structures originate from the continuous reorganization of the actin cytoskeleton in response to extracellular signals [103]. However, 4.1B/DAL-1 can anchor F-actin to the cell membrane, inhibiting cell motility by supporting orderly arrangements of actin stress fibers. 4.1B/DAL-1 loss may promote F-actin reorganization and enhance cell motility. For example, Cavanna et al. [104] demonstrated that the depletion of 4.1B caused the loss of stress fibers in non-metastatic sarcoma cells and was accompanied by enhanced cell motility, while metastatic sarcoma cells exogenously expressing 4.1B migrated at half the speed of control metastatic cells. Furthermore, the oncogenic transcription factor E-26 related gene (ERG) is frequently overexpressed in prostate cancer, which results in the increased invasiveness and metastasis of prostate cancer cells. Significantly lower levels of 4.1B expression were exhibited by prostate tumors overexpressing ERG compared with tumors that did not overexpress ERG. It is speculated that 4.1B down-regulation may promote ERG-mediated prostate cancer progression [105].

Protein 4.1B/DAL-1 inhibits cell proliferation

The proper membrane localization of the U2 domain seems to be necessary for protein 4.1B/DAL-1-mediated growth suppression. Gerber et al. found that when the U2 domain was properly localized to the plasma membrane, 4.1B/DAL-1 suppressed meningioma cell growth by sequentially activating Src, Rac1, MLK3, and JNK, resulting in reduced expression levels of cyclinA and decreased retinoblastoma (Rb) protein hyperphosphorylation (see Fig. 6). However, deletion of the U2 domain of DAL-1 abolished the ability for suppressing meningioma cell growth [14, 15]. Kuns et al. [40] reported that 4.1B loss significantly enhanced

mammary epithelial cell proliferation of pregnant 4.1B-null mice, while 4.1B overexpression resulted in reduced cyclinA expression and Rb phosphorylation, which was accompanied by decreased tyrosine kinase receptor erbB2 phosphorylation, causing the mammary epithelial cells to arrest in G1 phase. However, the G1 cell cycle arrest-related signaling molecules JNK, MAPK, and Akt were not activated, which suggested that specific signaling pathways involved in 4.1B/DAL-1-mediated growth inhibition may exist in different types of cells. Furthermore, 4.1B/DAL-1 may mediate its growth-inhibitory effects via PRMT5, its binding partner. PRMT5 has an inhibitory effect on cyclinE1 promoter activity through methylating histone H4 on the nucleosome that surrounds the cyclinE1 regulatory element [106]. The abnormal expression of 4.1B/DAL-1 likely alters the association of cyclinE1 promoter with histone H4 by affecting PRMT5 methylation, and then results in cell cycle change.

Protein 4.1B/DAL-1 promotes apoptosis

4.1B/DAL-1 inhibits tumor cell growth by not only suppressing cell proliferation but also inducing apoptosis (see Fig. 6). 4.1B/DAL-1 induced apoptosis in MCF-7 breast cancer cells through significantly enhancing Caspase-8 activation, but without activation of downstream effecter Caspases (Caspase-3, -6, and -7), suggesting that 4.1B/DAL-1-induced apoptosis in MCF-7 may involve a Caspase-8-dependent pathway that functions independently of the major effector Caspase pathways [107, 108]. Modulation of post-translational protein methylation may also be a potential mechanism underlying 4.1B/DAL-1-induced apoptosis in MCF-7 cells. It has been observed that the apoptosis levels in the 4.1B/DAL-1-null MCF-7 cells were not influenced by the hypomethylating treatment of cells with protein methylation inhibitor adenosine dialdehyde (AdOX). However, AdOX treatment specifically increases the mortality of the 4.1B/DAL-1-induced MCF-7 cells [107]. Thus, the association of 4.1B/DAL-1 with protein methylation pathway components, such as PRMTs protein, is important for controlling tumorigenesis. In addition, Robb et al. [14] reported that in IOMM-Lee meningioma cells, JNK phosphorylation levels and apoptosis-related genes, including Caspase-1, Toll-like receptor (TLR) 4 and grb2-associated binder 1 (gab1), were up-regulated upon DAL-1 expression. JNK is the downstream mediator of TLR4 and gab1 signaling. Several studies have found that TLR4 and gab1 appeared to induce Caspase-mediated apoptosis via stimulating JNK activation [109, 110]. Therefore, it is possible that 4.1B activates JNK activation through TLR4 and/or gab1 signaling, thereby initiating Caspase-mediated apoptosis.

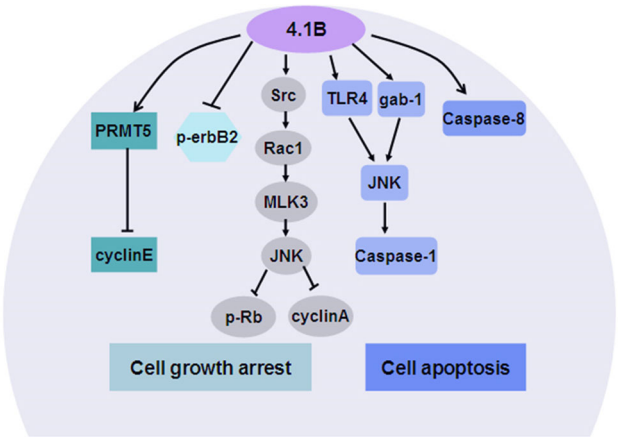

Fig. 6 Mechanism underlying the effects of protein 4.1B/DAL-1 on tumor cell growth arrest and apoptosis. In the process of cell growth arrest, 4.1B/DAL-1 can suppress cell growth by sequentially activating Src, Rac1, MLK3, and JNK, resulting in reduced cyclinA levels and decreased retinoblastoma (Rb) protein hyperphosphorylation. 4.1B can also inhibit erbB2 phosphorylation, causing cell cycle arrest in the G1 phase. Furthermore, 4.1B/DAL-1 can bind to PRMT5, thereby inhibiting cyclinE1 transcription. In the process of cell apoptosis, 4.1B/DAL-1 can enhance Caspase-8 activation levels to induce cell apoptosis. Alternatively, 4.1B/DAL-1 can activate successively activate TLR4/gab1, JNK, and Caspase-1 signaling to induce apoptosis

Summary and perspective

Down-regulation or loss of protein 4.1B/DAL-1 expression has been observed in many types of human tumors, which can promote tumor development during the relatively early or late stages. 4.1B/DAL-1 expression is gradually decreased during the development process in lung adenocarcinomas, breast cancers, meningiomas, prostate cancers, and ovarian cancers. In other words, as compared with normal tissues, 4.1B/DAL-1 expression was down-regulated during the early stages. Its expression was then further reduced or absent, promoting the progression of tumors to invasive or metastatic phenotypes. 4.1B/DAL-1 participates in the organization of transmembrane proteins and membrane–cytoskeletal interaction. Loss of it likely alters the assembly of transmembrane proteins, their activation states, and cytoskeletal organization, which may upset membrane-mediated signaling pathways or cell cycle regulation and culminate in malignant metastasis and invasion phenotype. Given that 4.1B/DAL-1 may serve as a negative modulator of tumor progression across a broad-spectrum of tumor types, demethylation of the 4.1B/DAL-1 promoter through DNA methyltransferase inhibitors or delivery of 4.1B/DAL-1 into tumor cells lacking 4.1B/DAL-1 by adenovirus/lentivirus or plasmids may be used as cancer treatment strategies.

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Conflict of interest The authors declare no conflicts of interest.

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