

Pivotal roles of protein 4.1B/DAL-1, a FERM-domain containing protein, in tumor progression (Review)

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Abstract. Protein 4.1B/DAL-1, encoded by erythrocyte membrane protein band 4.1-like 3 (EPB41L3), belongs to the protein 4.1 superfamily, a group of proteins that share a conserved four.one-ezrin-radixin-moesin (FERM) domain. Protein 4.1B/DAL-1 serves a crucial role in cytoskeletal organization and a number of processes through multiple interactions with membrane proteins via its FERM, spectrin-actin-binding and C-terminal domains. A number of studies have indicated that a loss of EPB41L3 expression is commonly observed in lung cancer, breast cancer, esophageal squamous cell carcinoma and meningiomas. DNA methylation and a loss of heterozygosity have been reported to contribute to the downregulation of EPB41L3. To date, the biological functions of protein 4.1B/DAL-1 in carcinogenesis remain unknown. The present review summarizes the current understanding of the role of protein 4.1B/DAL-1 in cancer and highlights its potential as a cancer diagnostic and prognostic biomarker in cancer therapeutics.

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1. Introduction

Protein 4.1B/DAL-1 is encoded by erythrocyte membrane protein band 4.1-like 3 (EPB41L3; also termed DAL-1/KIAA0987) and is important for cytoskeletal rearrangements, intracellular transport and signal transduction, with high levels of expression in the brain, intestine and kidney, and lower levels in the pancreas, lung and liver (1-4). Protein 4.1B/DAL-1 belongs to the 4.1 protein superfamily, which is characterized by the presence of a highly conserved four.one-ezrin-radixin-moesin (FERM) domain at the N-terminus (3-6). Compared with 4.1 superfamily members, 4.1B contains three conserved domains (FERM, SABD and CTD) and three unique domains (U1, U2 and U3) (7). DAL-1, a short form of 4.1B, is also located on human chromosome 18p11.3 and lacks the U1, parts of the U2 and SABD, and full-length CTD (Fig. 1).

Protein 4.1B is a known tumor suppressor, and inactivation contributes to the progression and development of numerous cancer types, including lung adenocarcinoma (8-11), meningiomas (12-16), breast cancer (17,18), ovarian cancer (19) and prostate cancer (20,21). A loss of protein 4.1B expression in cancer is partly associated with a loss of heterozygosity (LOH) on 18p11.3 and/or the hypermethylation of CpG islands (17). Protein 4.1B, a pivotal regulator of cytoskeletal structure, regulates various cytoskeletal-associated processes in cancer, including cell migration and invasion (22). Protein 4.1B has therefore received attention as a potential therapeutic target in cancer.

2. Structural characteristics of the protein 4.1 superfamily

Proteins isolated from human red blood cell membranes were first recognized as members of the protein 4.1 superfamily. Since their discovery, over 40 proteins have been demonstrated to belong to the protein 4.1 superfamily based on sequence homology (3,23). In addition to the protein 4.1 subfamily, the protein 4.1 superfamily consists of four additional subfamilies: i) Talin-related molecules; ii) the ezrin, radixin, moesin protein family; iii) protein tyrosine phosphatases proteins; and iv) novel band 4.1-like 4 proteins (4). Members of the protein 4.1 superfamily possess diverse biological functions and share a highly conserved FERM domain of 200-300 amino acids, which is associated with the interaction with cytoplasmic domains of

specific transmembrane proteins. In addition to the FERM domain, the protein 4.1 subfamily possesses two highly conserved spectrin-actin-binding (SAB) and C-terminal (CT) domains (7). The protein 4.1 family includes four homologues, 4.1R (erythrocyte-type), 4.1N (neuronal-type), 4.1G (general-type) and 4.1B (brain-type), which are encoded by EPB41, EPB41L1, EPB41L2 and EPB41L3, respectively (Fig. 2) (5,24).

3. Functional roles of individual domains of protein 4.1B/DAL-1

FERM domain. The FERM domain, first isolated from human erythrocyte ghosts, is a unique module in the family of peripheral membrane proteins that functions as a plasma membrane-cytoskeleton linker (6,25). Structurally, the FERM domain has a cloverleaf-like architecture with N-lobes, α -lobes and C-lobes (also termed F1, F2 and F3). The N-terminal FERM domain of 4.1B has a number of interacting partners. For example, 4.1B binds via its FERM domain to the cytoplasmic domain of cell adhesion molecule (CADM)1/tumor suppressor in lung cancer 1 (TSLC1) (11,26) and CADM4 (27). In addition, members of the membrane-associated guanylate kinase homologs, including membrane palmitoylated protein (MPP)1/p55, MPP2/discs large MAGUK scaffold protein 2 and MPP3, form a tripartite complex through their interaction with 4.1B and CADM1 (28). Protein arginine N-methyltransferase (PRMT)3 and PRMT5 also interact with 4.1B (29,30). The interactions between 4.1B and contactin associated protein (CNTNAP)1 or CNTNAP2 serve essential roles in the organization of myelinated axons (31). The FERM domain of 4.1B also mediates binding to the 14-3-3 isoforms β , γ and η (32).

SAB domain, CT domain and other unique domains. Protein 4.1B/DAL-1 maintains the mechanical integrity of cell membranes through its ability to form ternary complexes with spectrin and actin via its SAB domain (33). Functional variation exists amongst the different splice variants of SAB comprising of exons 16 and 17. Strong spectrin and actin binding affinity requires both exons, whilst exon 17 alone demonstrates weak affinity (34,35). The SAB domain is also responsible for the interaction with sarcomeric proteins, such as myosin, α -actin, and tropomyosin (36). The association between 4.1B and $\alpha\beta$ 8 integrin occurs via its CT domain (37). In addition to these domains, protein 4.1B contains three unique regions (U1, U2 and U3). Membrane localization of the U2 domain, which is located between the FERM and SAB domains, is essential for 4.1B to function as a growth suppressor in meningioma (14). Collectively, protein 4.1B/DAL-1 engages in a wide range of cellular functions through its interactions with dynamic molecules via its specific FERM, SAB and CT domains.

4. Relevance of protein 4.1B/DAL-1 in cancer

Aberrant protein 4.1B/DAL-1 expression occurs due to a LOH and/or DNA hypermethylation, and is implicated in numerous cancer types with a crucial role in tumor development and progression (Fig. 3).

Lung carcinoma. DAL-1, a short form of protein 4.1B possessing all of its functional domains, was originally

identified to be downregulated in primary lung tumors and lung cancer cell lines with a potential role in the suppression of tumor growth (8,38,39). The hypermethylation of DAL-1 strongly correlates with a loss of DAL-1, and predicts a short overall survival in patients with NSCLC (9). In addition, EPB41L3 localizes within chromosomal region 18p11.3, which is influenced by a LOH in NSCLC (13). These studies indicate that both DNA methylation and LOH promote aberrant DAL-1 expression in NSCLC.

A noticeable feature of protein 4.1B/DAL-1 in lung cancer is its close association with epithelial-mesenchymal transition (EMT). It has been reported that protein 4.1B/DAL-1 maintains the phenotype of epithelial cells and attenuates EMT by inhibiting PI3K/Akt/Mdm2/p53 signaling as a result of the suppression of heat shock protein 5 in NSCLC (40,41). A loss of protein 4.1B/DAL-1 leads to a substantial decrease in the expression of numerous EMT markers, including E-cadherin and β -catenin in lung cancer cells (42). Additionally, through interaction with the tumor suppressor gene TSLC-1, an immunoglobulin superfamily cell-adhesion molecule possessing strong anti-tumor ability, protein 4.1B/DAL-1 regulates cell motility and actin rearrangements in cancer cells (11,26,43).

Breast cancer. Human chromosomal region 18p11.3 frequently undergoes a LOH in breast cancer (18). However, despite significant LOH, the mutational mechanisms acting on the EPB41L3 locus are undefined. A significant allelic imbalance and preferential retention of the C-containing allele after LOH at this locus have been reported, indicating that loss of the T-containing alleles confers an advantage to the pathogenesis of breast cancer, providing clues to the mechanisms responsible for the loss of 4.1B in early breast cancer (17). Furthermore, promoter hypermethylation of EPB41L3 in intraoperative sentinel lymph node biopsy samples from patients with breast cancer is frequently observed and holds potential clinical utility (44).

Several studies have evaluated the role of 4.1B/DAL-1 in breast cancer and have reported that its ability to modulate the activity of protein arginine methyltransferases PRMT3 and PRMT5 is via direct binding to the catalytic core domain of PRMTs in breast cancer cell lines (29,30). Notably, protein 4.1B/DAL-1 can enhance and inhibit PRMT5 catalytic activity in a substrate-specific manner (29,30). Furthermore, protein 4.1B/DAL-1 and protein methylation mediated by protein arginine methyltransferases cooperate to induce apoptosis via a caspase-8-dependent pathway in MCF-7 breast cancer cells (45). These findings suggest that 4.1B/DAL-1 influences breast cancer cell growth through the modulation of PRMT-dependent post-translational methylation.

Prostate cancer. Bisulfite sequencing and methylation-specific PCR revealed elevated levels of EPB41L3 hypermethylation in prostate cancer tissues and cell lines compared with the low methylation observed in normal prostate tissues (46-49). In particular, EPB41L3 expression increased through co-treatment with the DNA methyltransferase inhibitor (5-aza-2'-deoxycytidine) and a histone deacetylase inhibitor (SAHA) in Du145 and 22Rv1 prostate cancer cell lines, respectively (46). These studies illustrate that the loss of protein 4.1B/DAL-1 in prostate cancer is primarily associated with aberrant DNA methylation. Decreased expression of 4.1B in highly metastatic prostate cancer cells

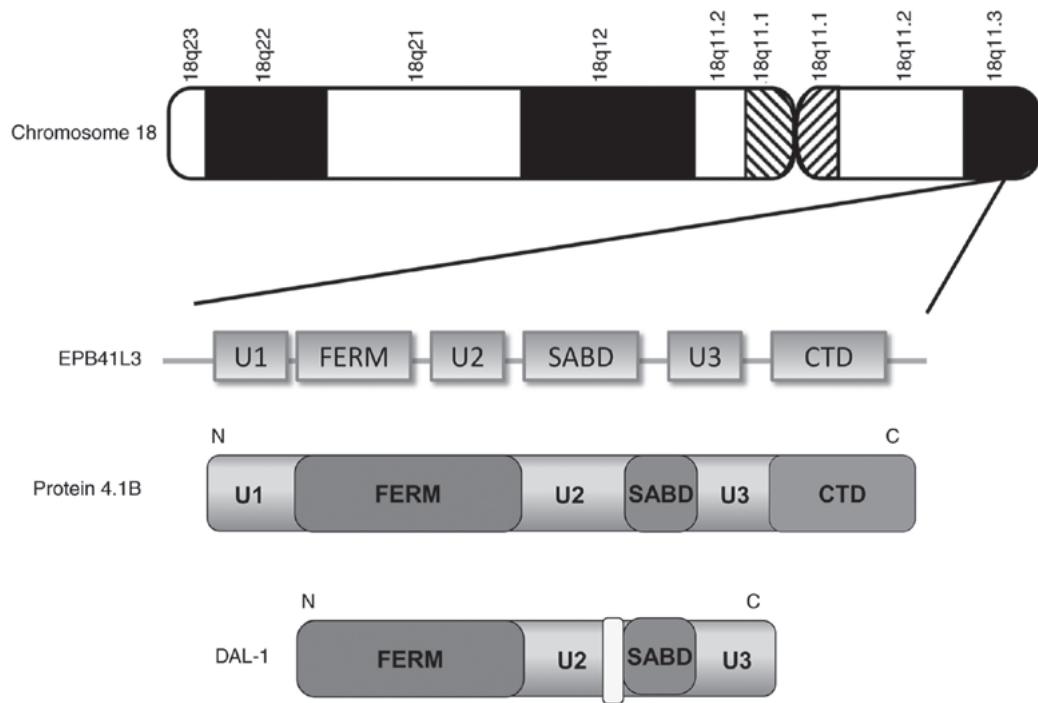


Figure 1. Gene localization of the EPB41L3 gene and domain structure of protein 4.1B/DAL-1. EPB41L3 is located on human chromosome 18p11.3. EPB41L3 possesses exons encoding FERM, SAB, CT, U1, U2 and U3 domains. The full-length protein encoded by EPB41L3 is termed protein 4.1B. DAL-1, a truncated form of protein 4.1B, which lacks N-terminal U1, parts of the U2 and SAB domains, and full-length C-terminal domain, is able to function as a tumor suppressor and inhibits cancer cell growth. EPB41L3, erythrocyte membrane protein band 4.1-like 3; FERM, four.one-ezrin-radixin-moesin; SAB, spectrin-actin-binding; CT, C-terminal.

Protein 4.1B	KPKSMQCKVILLDDGSEYTCDEKRSRGQVLFDKVCEHLNL	40
Protein 4.1R	KHRNMHCKVSLDDTVYECVVEKHAKGQDLLKRVCEHLNL	40
Protein 4.1N	KYKSAICRVTLDDASEYECEVEKHGRGQVLFDLVCEHLNL	40
Protein 4.1G	KTKTVQCKVTLDDGTEYSCDLEKHAKGQVLFDKVCEHLNL	40
Consensus	k c v l l d y c e k g q l v c e h l n l	
Protein 4.1B	LEKDYFGLTYRDAENQKNWLDPAKEIKKQVRSGAWHFSFN	80
Protein 4.1R	LEEDYFGLAIWNTTSKTWLDPAKEIKKQVRGVPWNFTFN	80
Protein 4.1N	LEKDYFGLTFCDADSQKNWLDPSKEIKKQIRSSPWNEFTN	80
Protein 4.1G	LEKDYFGLLFQESPEQKNWLDPAKEIKKQIRSNLPWLETFN	80
Consensus	l e d y f g l k w l d k e i k q r w f f	
Protein 4.1B	VKFYPPDPAQLSEDTIRYLLCLQLRQDIVSGLRPLCSFVTL	120
Protein 4.1R	VKFYPPDPAQLTEDITRYLLCLQLRQDIVAGRLPCSEFATL	120
Protein 4.1N	VKFYPPDPAQLTEDITRYLLCLQLRADIIITGRPLCSFVTH	120
Protein 4.1G	VKFYPPDPAQLTEDITRYLLCLQLRADIIASGLRPLCSFVTH	120
Consensus	v k f y p p d p q l e d i t r y l l c l q l r d i g r l p c s f t	
Protein 4.1B	ALLGSYTVQSELGDYDPECGSDYIIESEFRFAPNHTKELED	160
Protein 4.1R	ALLGSYTIQSELGDYDPELHGVYVSDFKLAPNQTKELEE	160
Protein 4.1N	ALLGSYAVQAEELGDYDAEEHVGNYVSELRFAPNQTRELEE	160
Protein 4.1G	ALLGSYTLQAEELGDYDPEEHGSIDLSEFQFAPNQTKELEE	160
Consensus	a l l g s y q e l g d y d s a p t e l e	
Protein 4.1B	KVIELHKSRRGMTPEAEEMHFLNNAKLSMYGVDLH	196
Protein 4.1R	KVMELHKSYSRMTPEAQDLEFLNNAKLSMYGVDLH	196
Protein 4.1N	RIMELHKTYSRMTPEAEIHFLENNAKLSMYGVDLH	196
Protein 4.1G	KVAELHKTYSRMTPEAQDSOFLENNAKLSMYGVDLH	196
Consensus	e l h k r p a f l e n a k l s m y g v d l h	

Figure 2. Sequence of the protein 4.1B N-terminal FERM domain aligned with other family members. Identical residues are highlighted in gray. FERM, four.one-ezrin-radixin-moesin.

indicates its role as a negative modulator of cancer progression and metastasis (21,47). Notably, 4.1B-deficient mice demonstrate increased susceptibility for aggressive and spontaneous prostate tumors in transgenic adenocarcinoma of the mouse prostate tumor models (21). Based on these findings, reduced 4.1B expression appears an important event in prostate cancer that causally contributes to an aggressive tumor phenotype.

Esophageal squamous cell carcinoma (ESCC). Hypermethylated CpG sites in the promoter region of EPB41L3 lead to loss of expression and are identified in ESCC, which is consistent with studies in other cancer types (50). By contrast, it has been reported that EPB41L3 is significantly upregulated in ESCC compared with normal tissue. The enhanced expression of EPB41L3 is associated with the response to neoadjuvant chemoradiation in ESCC (51). Hence, further studies investigating the expression of EPB41L3 in ESCC are necessary to elucidate the full spectrum of its functions in EPB41L3.

Protein 4.1B/DAL-1 plays a significant role in suppressing cell migration and invasion via inhibiting matrix metalloproteinase (MMP)2 and MMP9 in ESCC (52). EPB41L3 positively regulates apoptosis through activating caspase-3/8/9, and inducing G2/M arrest via the CDK1 pathway (53). The specific functions of protein 4.1B/DAL-1 in the pathogenesis of ESCC are relatively uncharacterized and require further exploration.

Cervical cancer. The methylation of EPB41L3 can distinguish precancerous lesions from invasive disease in cervical specimens. High methylation of EPB41L3 in women with cervical intraepithelial neoplasia grade 2/3 (CIN2/3) representing the pre-tumorigenic stage implies a higher cancer risk (54-57). Specifically, the methylation levels at CpG sites (438, 427 and 425) in EPB41L3 may distinguish CIN2/3 from negative intraepithelial lesions or malignancy/CIN1 and cancer from CIN2/3 (58). Recent studies targeting women living with HIV-1 (WLHIV) in Burkina Faso and South Africa revealed that the hypermethylation of EPB41L3 is frequent among WLHIV and occurs in conjunction with low CD4⁺ counts and a poor efficacy of antiretroviral therapy (59). Indeed, when

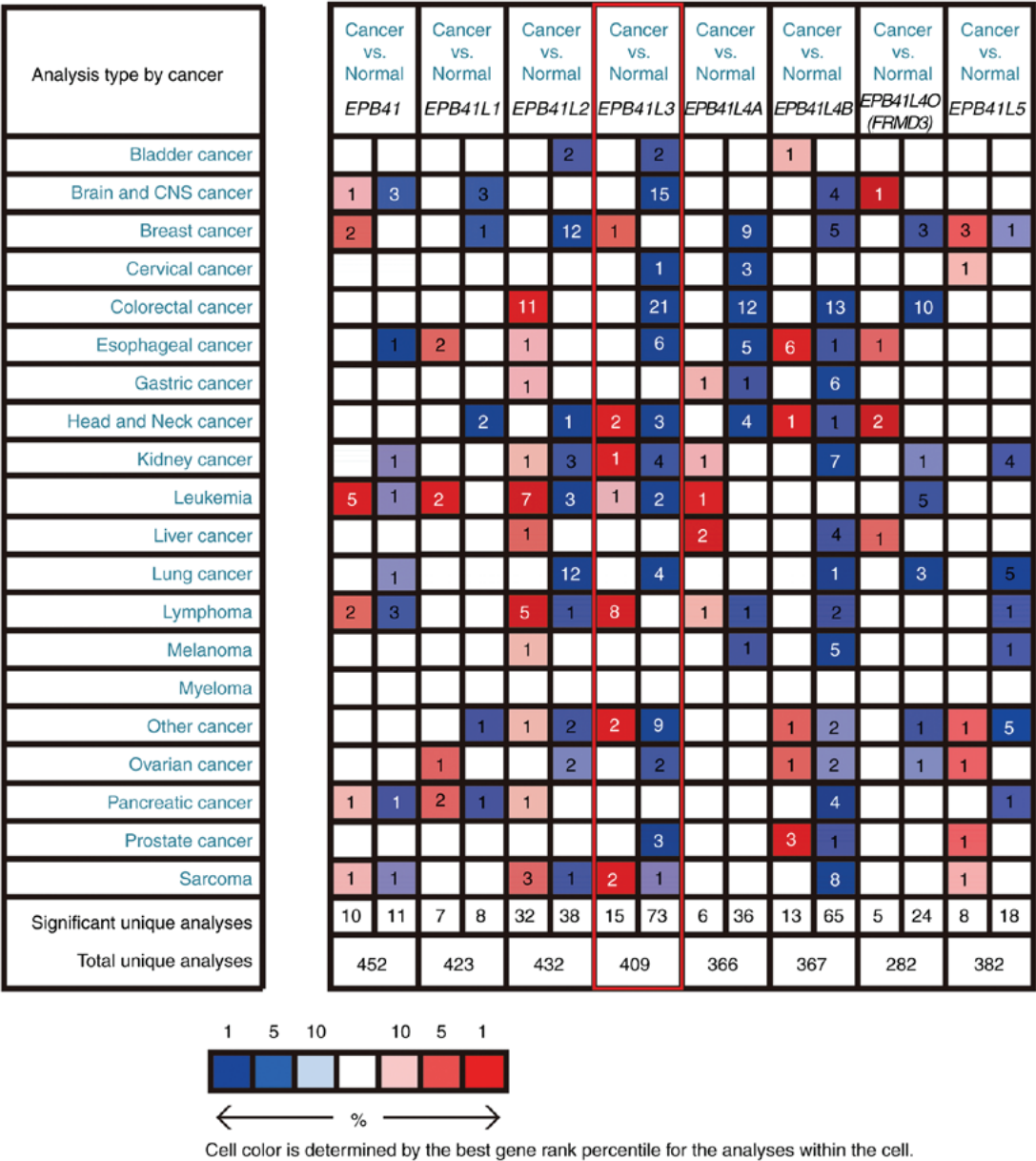


Figure 3. Transcription levels of EPB41L3 and other members of the 4.1 superfamily in different types of cancer. The data and figure are analyzed using the Oncomine database, indicating the numbers of datasets with statistically significant mRNA upregulation (red) or downregulation (blue). Different types of cancer vs. corresponding normal tissues. Threshold setting: P-value, 0.01; fold-change, 2; gene rank, top 10%. The number in the colored cell represents the number of datasets meeting the threshold. EPB41L3, erythrocyte membrane protein band 4.1-like 3.

screening for HPV infection, DNA methylation assessments of EPB41L3 offer promise as an objective molecular-based approach for early detection and diagnosis (60-69).

Meningiomas. A loss of protein 4.1B/DAL-1 occurs in $\geq 50\%$ of the cases of sporadic meningiomas. As a major cause of downregulating EPB41L3, a LOH on chromosome segment 18p11.3 is common in meningiomas. However, meningioma with 18p11.3 LOH does not correlate with the nucleotide inactivation of EPB41L3 (12-14). EPB41L3 hypermethylation is a prognostic factor for poor survival in diffuse gliomas, and treatment with the demethylating agent 5-aza-2'-deoxycytidine and the histone deacetylase inhibitor trichostatin A have been shown to restore EPB41L3 expression in glioma cells (70). A loss of protein 4.1B/DAL-1 is reported as an early event in meningioma

tumorigenesis, suggesting it plays a crucial role in growth regulation during meningioma pathogenesis (12). As a tumor suppressor, protein 4.1B/DAL-1 is associated with the activation of JNK in meningioma (16). Protein 4.1B/DAL-1 mediated growth suppression in meningioma requires the sequential activation of Src, Rac1 and JNK (16). Furthermore, the inhibition of Rac1 or JNK activation abrogates protein 4.1B-growth suppression. However, it is poorly understood how 4.1B/DAL-1 activates the JNK pathway.

Digestive system cancers. In addition to esophageal carcinoma, abnormal expression of EPB41L3 has been reported in other common digestive system cancer types, including gastric cancer, intestinal carcinoma, colorectal cancer, hepatocellular carcinoma and pancreatic carcinoma (71-75). Consistent with previous findings that *Helicobacter pylori* (HP)-induced

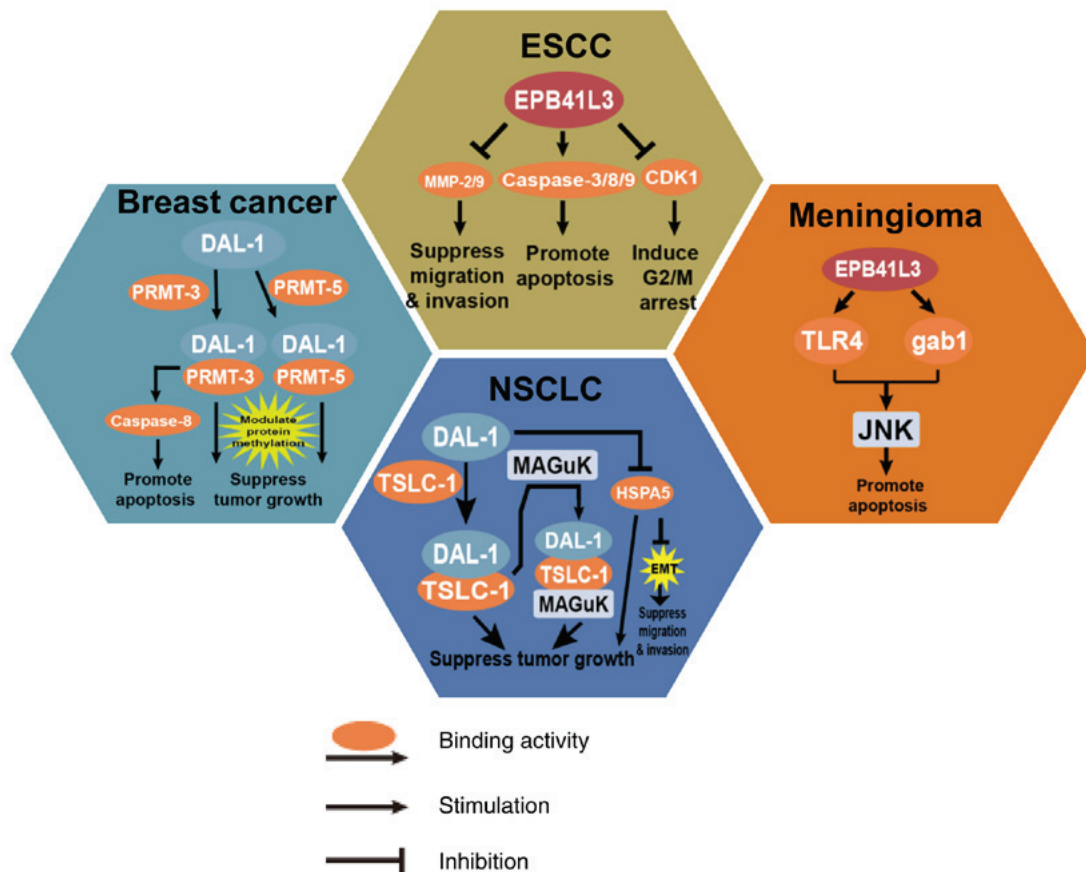


Figure 4. Multiple functions of EPB41L3 in several cancer including NSCLC, breast cancer, meningioma and ESCC. EPB41L3, erythrocyte membrane protein band 4.1-like 3; NSCLC, non-small cell lung cancer; ESCC, esophageal squamous cell carcinoma; TSLC-1, tumor suppressor in lung cancer 1; PRMT, protein arginine N-methyltransferase; HSPA5, heat shock 70 kDa protein 5; MAGuK, membrane-associated guanylate kinase; gab1, GRB2-associated binding protein 1; TLR4, toll-like receptor 4; EMT, epithelial-mesenchymal transition.

inflammatory response promotes EPB41L3 DNA methylation, the methylation of EPB41L3 is significantly lower in remnant gastric cancers with the decline of HP infection following distal gastrectomy (73,74). In colorectal cancer, protein 4.1B/DAL-1 has been shown to be downregulated through membrane proteomic analysis (72). In addition, a similar loss of 4.1B/DAL-1 occurs in intestinal epithelia malignant carcinomas of mouse and humans (71). Protein 4.1B/DAL-1 is also downregulated in hepatocellular carcinoma (HCC), and represses HCC cell migration and invasion (76). Using transgenic mouse models of pancreatic b-cell carcinogenesis (Rip1Tag2), a loss of 4.1B has been reported in the phenotypic transition from adenoma to carcinoma (75). In addition, EPB41L3 and HPV 16 methylation are markedly higher amongst oropharyngeal cancer (OPC) with high sensitivity and specificity for the detection of OPC from an oral gargle, suggesting that measurements of HPV 16 and EPB41L3 methylation have utility in identifying early OPC (77). High methylation of EPB41L3 is also associated with anal intraepithelial neoplasia (78).

Renal cell carcinoma. Whilst protein 4.1B is expressed in the proximal uriniferous tubules of the normal human kidney, its loss or downregulation through EPB41L3 promoter hypermethylation occurs in ~50% of renal clear cell carcinoma (RCCC) cases, implicating its association with EPB41L3 methylation, tumor grade and recurrence-free survival (79).

Furthermore, the protein 4.1B binding partner CADM4 serves an important role in the adhesion of the proximal uriniferous tubules that are the precursor cells of RCCC (27).

Other diseases. Similarly, promoter methylation of EPB41L3 is one of the common mechanisms of genetic downregulation in ovarian cancer (19). In addition, EPB41L3 is up-regulated with age and contributes to a decreased proliferation rate of human bone marrow-derived mesenchymal stem cells (80).

5. Future directions

Protein 4.1B/DAL-1 encoded by EPB41L3, functions as a tumor suppressor in human cancer (5,22). The pathological functions of protein 4.1B/DAL-1 are versatile through its regulation of various cellular processes during carcinogenesis (Fig. 4). Nevertheless, the functions and mechanisms of EPB41L3 in cancer remain unknown.

Promoter methylation is a major cause of EPB41L3 inactivation in cancer. Abnormal DNA methylation of EPB41L3 is identified in numerous cancer types, including NSCLC and breast cancer (9,44). Furthermore, aberrant EPB41L3 DNA methylation possesses high diagnostic and prognostic significance in cancer, indicating its potential use as a biomarker for cancer diagnosis (Table I).

Table I. Potential use of EPB41L3 in different cancer types.

Author, year	Cancer type	Status of EPB41L3	Potential use in cancer therapy	(Refs.)
Lorincz <i>et al</i> , 2017	Anal intraepithelial neoplasia	DNA hypermethylation	Diagnostic significance	(78)
Martín-Sánchez <i>et al</i> , 2016	Breast cancer	DNA hypermethylation	Prognostic significance	(44)
Vasiljević <i>et al</i> , 2014;	Cervical intraepithelial neoplasia and cancer	DNA hypermethylation	Diagnostic and significance prognostic	(55)
Louvanto <i>et al</i> , 2015;				(58)
Nedjai <i>et al</i> , 2018;				(63)
Lorincz <i>et al</i> , 2016;				(66)
Eijssink <i>et al</i> , 2012;				(68)
Lendvai <i>et al</i> , 2012				(69)
Kume <i>et al</i> , 2014	Colorectal cancer	Low expression	Diagnostic significance	(72)
Perez-Janices <i>et al</i> , 2015	Diffuse gliomas	DNA hypermethylation	Prognostic significance	(70)
Li <i>et al</i> , 2014;	Esophageal squamous cell carcinoma	DNA hypermethylation	Diagnostic and prognostic significance	(50,53)
Zeng <i>et al</i> , 2018				
Kikuchi <i>et al</i> , 2005	Non-small cell lung cancer	DNA hypermethylation	Prognostic significance	(9)
Giuliano <i>et al</i> , 2019	Oropharyngeal cancer	DNA hypermethylation	Diagnostic significance	(77)
Vasiljević <i>et al</i> , 2014;	Prostate cancer	DNA hypermethylation	Diagnostic significance	(48,49)
Schulz and Hoffmann, 2009				
Yamada <i>et al</i> , 2006	Renal clear cell carcinoma	DNA hypermethylation	Prognostic significance	(79)

EPB41L3, erythrocyte membrane protein band 4.1-like 3.

Re-expression of EPB41L3 results in significant suppression of cell growth in ovarian cancer, lung cancer and breast cancer (8,19,39). Particularly, reintroduction of EPB41L3 into 4.1B/DAL-1-null lung, breast and cervical cancer cell lines markedly suppresses cancer cell growth and promotes apoptosis (8,39,64). Hence, EPB41L3 represents an attractive therapeutic strategy. Treatment with DNA methyltransferase inhibitors can restore the expression of EPB41L3 (81). Engineered artificial transcription factors can reactivate EPB41L3 with tumor suppressive roles in breast, ovarian and cervical cancer cell lines. Treatment of human amniotic fluid stem with CXCR4 promoters and adenovirus vector expressing DAL-1 permits the targeting of lung cancer xenografts that overexpress DAL-1, representing a strategy to restore EPB41L3 function (82). Such epigenetic reprogramming tools to reintroduce silenced tumor suppressor genes represent an emerging potential therapeutic strategy (64).

6. Conclusions

Protein 4.1B/DAL-1 is essential for cytoskeleton-associated processes by interacting with a series of protein molecules through its conserved FERM, SAB and CT domains. A loss of protein 4.1B/DAL-1 expression, caused by aberrant DNA methylation and/or LOH, is frequently observed in cancer. Thus, protein 4.1B/DAL-1 can serve as a potential biomarker with high sensitivity and specificity for cancer diagnosis. In addition, reintroduction of protein 4.1B/DAL-1 by epigenetic

inhibitors or genome-editing tools significantly inhibits tumor progression, indicating its potential as a novel promising target for cancer therapy. Taken together, growing evidence highlights the importance of protein 4.1B/DAL-1 in cancer progression, and further studies will be required to elucidate the regulatory mechanisms of protein 4.1B/DAL-1 in human cancer.

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Availability of data and materials

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Authors' contributions

XY performed the original draft preparation. LP performed most of the revision work. XY and LP participated in the whole work. LW, XH and MZ contributed to figure preparation and revision, as well as editing of the manuscript. ZL provided financial support, edited the figures and contributed to editing

of the manuscript. All authors read and approved the final manuscript.

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.

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