biomolecules

Review

Cytoskeletal Protein 4.1R in Health and Diseases

Jiaojiao Liu [1], Cong Ding [2], Xin Liu [1,*] and Qiaozhen Kang [1,*]

[1] School of Life Science, Zhengzhou University, Zhengzhou 450001, China; ljj@gs.zzu.edu.cn
[2] Children's Hospital Affiliated of Zhengzhou University, Zhengzhou 450018, China; dingcong8866@163.com
* Correspondence: liux@zzu.edu.cn (X.L.); qzkang@zzu.edu.cn (Q.K.)

Abstract: The protein 4.1R is an essential component of the erythrocyte membrane skeleton, serving as a key structural element and contributing to the regulation of the membrane's physical properties, including mechanical stability and deformability, through its interaction with spectrin–actin. Recent research has uncovered additional roles of 4.1R beyond its function as a linker between the plasma membrane and the membrane skeleton. It has been found to play a crucial role in various biological processes, such as cell fate determination, cell cycle regulation, cell proliferation, and cell motility. Additionally, 4.1R has been implicated in cancer, with numerous studies demonstrating its potential as a diagnostic and prognostic biomarker for tumors. In this review, we provide an updated overview of the gene and protein structure of 4.1R, as well as its cellular functions in both physiological and pathological contexts.

Keywords: 4.1R; *EPB41*; cytoskeletal protein; diseases; cancer

check for updates

Citation: Liu, J.; Ding, C.; Liu, X.; Kang, Q. Cytoskeletal Protein 4.1R in Health and Diseases. *Biomolecules* **2024**, *14*, 214. https://doi.org/10.3390/biom14020214

Academic Editors: Viswanathan Natarajan and Masumi Eto

Received: 9 January 2024
Revised: 2 February 2024
Accepted: 7 February 2024
Published: 11 February 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The red blood cell cytoskeleton is essential for maintaining the membrane's physical characteristics, including mechanical stability and deformability. It is formed by a complex meshwork of proteins that imparts a great degree of elasticity. The red blood cell protein 4.1 is a crucial cytoskeletal protein found in mammalian erythrocytes. It plays a vital role in the mechanochemical properties of red blood cell membranes by facilitating the binding between spectrin and actin, and by aiding in the attachment of the membrane skeleton to the membrane [1]. Protein 4.1 was first discovered in human red blood cells. Its name derives from its identification as a specific band on an SDS gel of erythrocyte membranes ("band 4.1 protein") [2]. An examination of erythrocytes from a patient with homozygous elliptocytosis revealed a lack of band 4.1 protein in the cell membranes. Band 4.1-deficient erythrocytes exhibited reduced mechanical strength and a marked tendency for fragmentation [3].

Band 4.1 protein, hereafter referred to as 4.1R (the prototypical protein found first in red blood cells), is one member of the protein 4.1 family—the others being 4.1N (neuronal type), 4.1G (general type), and 4.1B (brain type). The corresponding genes are erythrocyte membrane protein band 4.1 (*EPB41*), erythrocyte membrane protein band 4.1 like 1 (*EPB41L1*), erythrocyte membrane protein band 4.1 like 2 (*EPB41L2*), and erythrocyte membrane protein band 4.1 like 3 (*EPB41L3*) [4]. This review will be devoted to 4.1R and will update our knowledge of the gene structure and expression of 4.1R, as well as its protein structure and functions. Additionally, it will explore the roles of 4.1R in pathology and tumorigenesis.

2. Characteristics of 4.1R Gene, mRNA, and Protein

The human *EPB41* gene is located on chr1p35.3, while the mouse *EPB41* gene is located on chr4. A total of 21 exons code for the sequence of 4.1R. Four main functional domains have been identified in 4.1R (Figure 1). The N-terminal highly ordered 30 kDa globular membrane binding domain (MBD), known as a FERM (four-point-one ezrin

radixin moesin) domain (amino acids 210–507), interacts with various proteins in erythroid and non-erythroid cells. It consists of three globular lobes (a "cloverleaf-like" structure), with distinct ligand binding capabilities in each lobe [5]. The N-terminal lobe (lobe A), which consists of the first 78 amino acids and 4 double-stranded β-strands, has a fold analogous to ubiquitin and binds to band 3 and the rhesus complex proteins Rh. The central lobe (lobe B), which consists of the next 90 amino acids, has an α-helical fold like acyl-CoA binding protein and binds to the transporter XK, the chemokine receptor Duffy, and glycophorin C (GPC). The C-terminal lobe (lobe C), which contains seven β-strands and ends with an α-helix, has a fold similar to a pleckstrin homology domain and binds to CD44, p55, and the phospholipid phosphatidylserine [6–8]. Phosphatidylinositol-4,5-biphosphate (PIP2) binds in a cleft between lobes A and C [9]. The overall folding structure of FERM domains is conserved, despite the level of sequence conservation being extremely low [4].

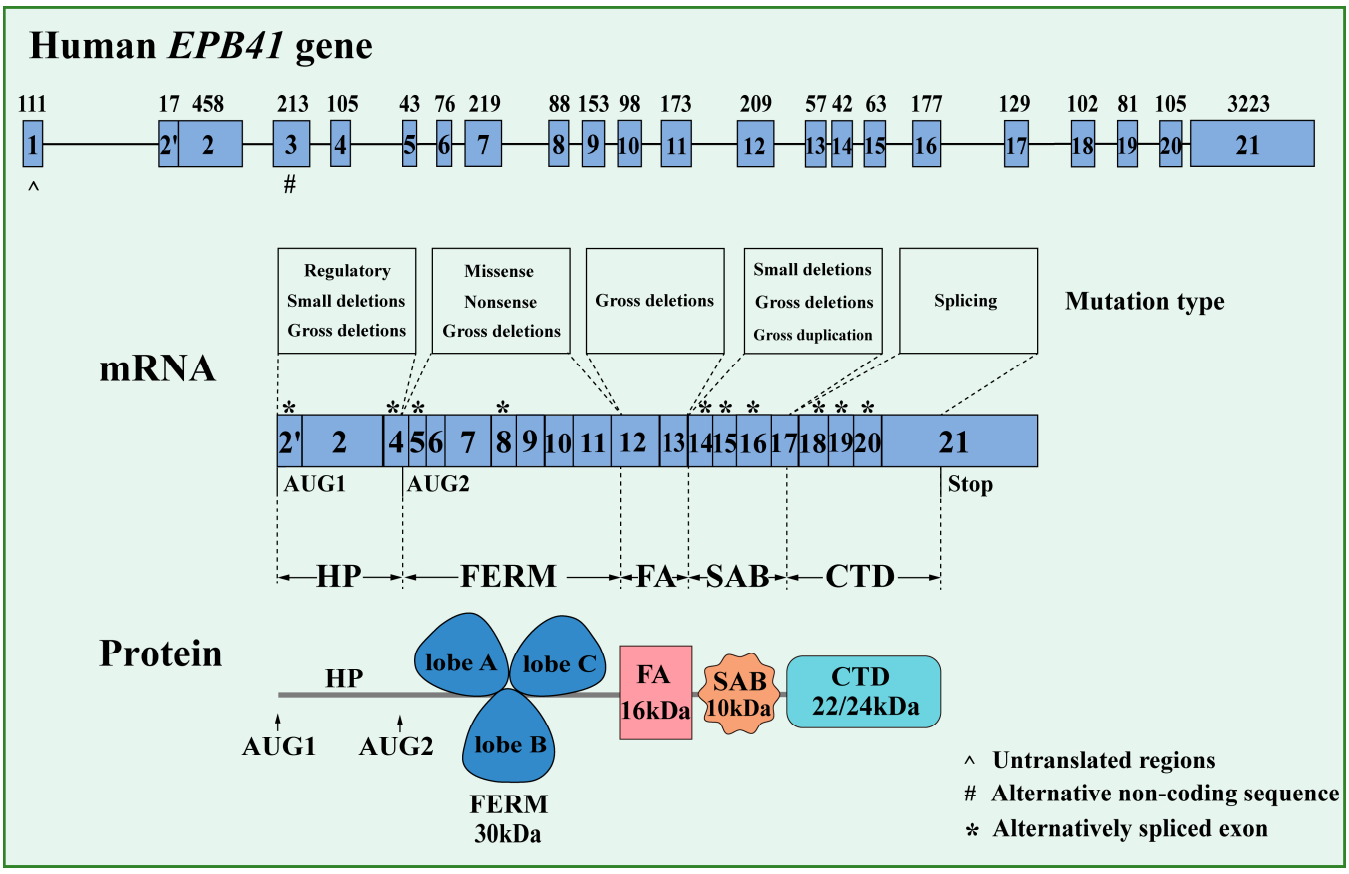


Figure 1. Gene, mRNA, and protein diagram of 4.1R. Mutation type: *EPB41* gene mutation associated with human disease. Protein domains: FERM—4.1 ezrin radixin moesin domain, FA—FERM-adjacent domain, SAB—spectrin–actin binding domain, and CTD—carboxyl terminal domain.

The second domain is the 16 kDa FERM-adjacent (FA) domain. This domain is phosphorylated by several protein kinases, including protein kinase C. Phosphorylation in this domain regulates interactions of both the FERM and spectrin–actin binding (SAB) domains [10].

The third domain, the 10 kDa SAB domain, strengthens the interaction between spectrin and actin and also binds to tropomyosin and myosin. The 21 amino acid peptides encoded by exon 16 in the SAB domain play an essential role in maintaining membrane stability by promoting spectrin/actin interactions [11].

The 22/24 kDa carboxyl-terminal domain (CTD) has been documented to exhibit binding affinity toward the immunophilin FKBP13 and the nuclear mitotic apparatus (NuMA) [12].

The *EPB41* pre-mRNA undergoes extensive alternative splicing, resulting in the production of multiple isoforms ranging from 30 to 210 kDa [13]. In mammals, the N-terminal of 4.1R has a variably spliced headpiece (also known as the U1 region, HP, amino acids 1–209). The HP region is an unstructured domain. Splicing of the HP region leads to the formation of the two most abundant isoforms of 4.1R, with apparent molecular masses of 135 kDa and 80 kDa on SDS gels [14]. The $4.1R^{135}$ isoform is exclusively expressed in early erythroblasts and other nucleated cells, while the $4.1R^{80}$ isoform is expressed during the late stages of erythroid differentiation and is the primary component of mature erythrocytes. Translation of $4.1R^{80}$ is initiated at AUG-2, located in exon 4, while translation of $4.1R^{135}$ is initiated at AUG-1, located in exon 2 [15]. The $4.1R^{135}$ isoform contains an additional HP region at the N-terminus compared to $4.1R^{80}$, and lacks a stretch of 21 amino acids for the interaction of 4.1R with spectrin [16]. Protein $4.1R^{135}$ has a theoretical molecular weight of ~100 kDa, and this difference is due to the unstructured nature of the HP region [17].

HP plays an important role in modulating the interaction of FERM with its two membrane binding partners, band 3 and GPC [16]. In comparison to the strong binding of $4.1R^{135}$ to band 3, its binding to GPC is notably weaker than that of $4.1R^{80}$. Another significant contrast between $4.1R^{135}$ and $4.1R^{80}$ is the calcium ion (Ca^{2+}) dependence of their binding to calmodulin (CaM). The binding of CaM to $4.1R^{80}$ is Ca^{2+}-independent, while the interaction of CaM with $4.1R^{135}$ is highly dependent on Ca^{2+}. This distinction is directly linked to the HP region of $4.1R^{135}$ [16]. Furthermore, CaM significantly reduces the binding of $4.1R^{135}$ to band 3 in a Ca^{2+}-dependent manner and eliminates its binding to GPC and p55. Unlike band 3 and GPC, which do not bind directly to the HP region, CaM binds to the HP region (a short polypeptide consisting of amino acids 70–80) in a Ca^{2+}-dependent manner. Therefore, it is inferred that the CaM binding site located in the HP region serves as the primary binding site in $4.1R^{135}$, and this site prevents the interaction of CaM with the Ca^{2+}-independent binding site in $4.1R^{80}$.

U2, which is located between the FERM domain and the SAB domain, is subject to variable splicing, as indicated by previous research [18]. Additionally, there is another variably spliced region, known as U3, situated between the SAB and CTD domains [19].

3. Function of 4.1R

Recent studies have found that 4.1R serves not only as a connector between the plasma membrane and the membrane skeleton, but also interacts with numerous proteins and plays a crucial role in a variety of biological processes, including cell fate determination, cell cycle regulation, cell proliferation, and cell movement (Table 1; Figure 2).

Table 1. Binding proteins of 4.1R.

Binding Proteins	Mechanism/Pathway	Function	Binding Domain	Detection Method	Reference
C-Kit	Ras-Raf-MAPKs and PI3K-AKT	Negatively regulates mast cell proliferation.	FERM	Co-immunoprecipitation and pull-down	[20]
GAT-1/GAT-2	—	Affects photodynamic therapy for B16 melanoma and affects the transport of 5-aminolevulinic acid into mouse embryonic fibroblast cells.	—	Immunofluorescence and co-immunoprecipitation	[21]
	—		—	Co-immunoprecipitation	[22]
von Hippel–Lindau (VHL)	Reverses myogenin ubiquitination and stability	Influences myogenesis.	—	Immunofluorescence and co-immunoprecipitation	[23]

Table 1. *Cont.*

Binding Proteins	Mechanism/Pathway	Function	Binding Domain	Detection Method	Reference
Toll-like receptor 4 (TLR4)	Canonical nuclear factor kappa-B (NF-κB) pathway	Modulates B-cell fate.	—	Immunofluorescence and co-immunoprecipitation	[24]
NuMA	Notch signaling	Regulates the asymmetric segregation of the Numb to mediate erythropoiesis.	CTD	co-immunoprecipitation	[25]
	4.1R alters the normal distribution of NuMA in the interphase nucleus	Organizes the nuclear architecture, mitotic spindle, and spindle poles.	CTD	Yeast two-hybrid assay, immunofluorescence, co-immunoprecipitation, and pull-down	[26]
Linker for activation of T cells (LAT)1/LAT2	FcεRI Signaling	Acts as a positive regulator in the early activation events in mast cells.	—	Co-immunoprecipitation	[27]
LAT	4.1R negatively regulates signaling from the T-cell antigen receptor (TCR) through LAT to the MAP kinase pathway	Negatively regulates T-cell activation.	FERM	Co-immunoprecipitation and pull-down	[28]
	TCR-mediated signal transduction	Negatively regulates CD8 T-cell activation.	—	Co-immunoprecipitation	[29]
Transient receptor potential canonical 4 (TRPC4)	4.1R interacts with TRPC4 and the membrane skeleton	Activates the endothelial I_{SOC} channel.	—	Co-immunoprecipitation	[30]
IQ motif-containing GTPase-activating protein 1 (IQGAP1)	4.1R is necessary for the localization of IQGAP1 to the leading edge of cells	Cell migration and the recruitment of the scaffold protein IQGAP1 to the cell front.	FERM	Immunofluorescence, co-immunoprecipitation, and pull-down	[31]
Epidermal growth factor receptor (EGFR)	Akt/ERK signaling	Regulation of EGFR activation and EGFR signaling in keratinocytes.	—	Immunofluorescence and co-immunoprecipitation	[32]
β1 integrin	Modulating surface expression of beta1 integrin	Regulates cell adhesion, spreading, migration, and motility of mouse keratinocytes.	FERM	Co-immunoprecipitation and pull-down	[33]
β-catenin	Linking the cadherin/catenin complex to the cytoskeleton through its direct interaction with β-catenin	Regulates the integrity of adherens junction in the gastric epithelial cells.	FERM	Immunofluorescence, co-immunoprecipitation, and pull-down	[34]
Cell adhesion molecule 1 (CADM1)	Membranous co-localization of CADM1 and 4.1R	Promotes malignant features of small cell lung cancer (SCLC).	—	Immunofluorescence, co-immunoprecipitation, and immunohistochemistry	[35]
Eukaryotic translation initiation factor 3 (elF3-p44)	4.1R direct association with elF3-p44	An anchor protein that links the cytoskeleton network to the translation apparatus.	CTD	Yeast two-hybrid assay, co-immunoprecipitation, and pull-down	[36]
Cytoplasmic linker-associated protein-2 (CLASP2)	4.1R controls CLASP2 behavior, CLASP2 cortical platform turnover, and GSK3 activity	Correct MT organization and dynamics essential for cell polarity.	FERM	Immunofluorescence, co-immunoprecipitation, and pull-down	[37]
ICln	Affecting 4.1R interaction with β-actin	Cell volume regulation and cell morphology.	FERM	Yeast two-hybrid assay, co-immunoprecipitation, pull-down, and fluorescence resonance energy transfer (FRET)	[38,39]
ZO-2	The link between the tight junction and the actin cytoskeleton	Organizes the tight junction.	CTD	Yeast two-hybrid assay, immunofluorescence, co-immunoprecipitation, and pull-down	[40]

Table 1. *Cont.*

Binding Proteins	Mechanism/Pathway	Function	Binding Domain	Detection Method	Reference
Voltage-gated Sodium Channel 1.5 (NaV1.5)	4.1R interaction with ion channel proteins	Involved in the occurrence and development of heart failure (HF).	—	Immunofluorescence and co-immunoprecipitation	[41]
Metabotropic glutamate receptor type 8 (mGluR8)	mGluR8-mediated signal transduction	Correct regulation of neurotransmitter receptors.	CTD	Yeast two-hybrid assay	[42]
Plasma membrane calcium ATPase 1b (PMCA1b)	Regulation of membrane expression of PMCA1b	Regulates intestinal Ca^{2+} absorption.	FERM	Immunofluorescence, co-immunoprecipitation, and pull-down	[43]
Human discs large isoform (hDlg-I_3)	—	Recruits hDlg to the lateral membrane in polarized epithelial cells.	FERM	Pull-down	[44]
CD44	—	Acts as an important tumor suppressor in the molecular pathogenesis of meningioma.	FERM	Co-immunoprecipitation	[45]
Aldolase C (ALDOC)	Wnt signaling	Inhibits non-small cell lung cancer (NSCLC) proliferation, invasion, and metastasis in vitro and in vivo.	—	Immunofluorescence and co-immunoprecipitation	[46]
Centrosomal P4.1-associated protein (CPAP)	—	Cell division and centrosome function.	HP	Yeast two-hybrid assay, co-immunoprecipitation, and pull-down	[47]

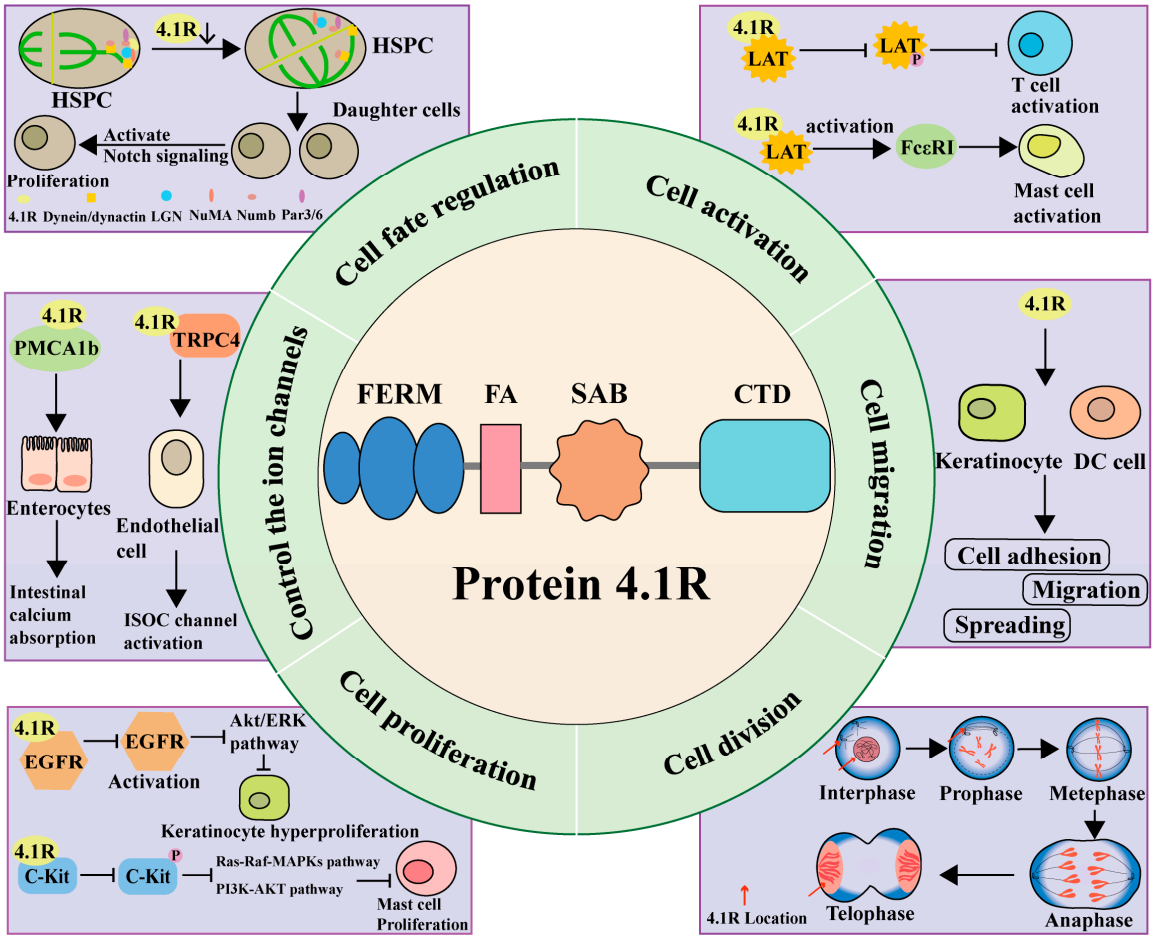


Figure 2. Structure and function of cytoskeleton protein 4.1R.

3.1. Cell Fate Regulation

In B cells, 4.1R acts as a regulator of B-cell fate by inhibiting the canonical NF-κB signaling pathway, rather than non-canonical NF-κB regulating B-cell class switch recombination and plasma cell differentiation [24]. In addition, Huang et al. found a crucial function of 4.1R in the regulation of the unequal distribution of Numb and mediated the balance of fate in hematopoietic stem cells or erythropoiesis progenitor cells through its interaction with NuMA. Depletion of 4.1R resulted in the disconnection of LGN and NuMA from the cell cortex, leading to misorientation of the spindle. Despite this, dynein/dynactin can still be loaded onto the cell cortex through its direct interaction with Par3, allowing Numb to be transported to the cell cortex. However, Par3 alone is insufficient to target Numb in the cell cortex in the absence of 4.1R-LGN-NuMA, potentially leading to certain Numb components being unable to load into the cell cortex. Consequently, these depletions notably increase the proportion of symmetric distribution of Numb. Knockdown of 4.1R reduces the size of Numb in the daughter cells and enhances Notch signaling, directly promoting cell proliferation and delaying cell maturation [25].

3.2. Cell Activation

In T cells, 4.1R has been found to negatively regulate T-cell activation by directly interacting with LAT, leading to the inhibition of LAT phosphorylation and its downstream signaling molecule extracellular signal-regulated kinase (ERK) [28]. Additional investigations have demonstrated that 4.1R suppresses the activation of $CD4^+$ T cells, thereby mitigating pathogenic autoimmunity in the progression of multiple sclerosis and experimental autoimmune encephalomyelitis progression [48]. Recent research has also indicated that 4.1R is a negative regulator of TCR signaling in CD8 T cells through its direct association with LAT [29]. In mast cells, Draberova et al. indicated that 4.1R acts as a positive regulator in the initial activation events following FcεRI triggering through its direct interaction with both LAT1 and LAT2 [27].

3.3. Cell Proliferation

A study by Ding et al. found that 4.1R inhibits mast cell proliferation by directly binding to the tyrosine kinase receptor C-Kit, thereby inhibiting C-Kit phosphorylation. It also negatively regulates the activation of the Ras-Raf-MAPKs and PI3K-AKT signal pathways [20]. In keratinocytes, a deficiency in 4.1R plays a role in sustaining abnormal EGFR-mediated cellular signaling and increasing the excessive proliferation potential of keratinocytes [32].

3.4. Cell Migration

Chen and colleagues observed a notable decrease in cell adhesion, spreading, migration, and motility in 4.1R-deficient keratinocytes, along with a reduction in the surface expression of β1 integrin. These findings indicate that 4.1R plays a functional role in keratinocytes by influencing the surface expression of β1 integrin through a direct interaction between 4.1R and β1 integrin [33]. In addition, Ruiz-Sáenz et al. indicated that 4.1R plays a crucial role in cell migration and the localization of the scaffold protein IQGAP1 to the leading edge of cells migrating into a wound [31]. The microtubule (MT) cytoskeleton is essential for cell polarity and migration. In a subsequent study, Ruiz-Saenz et al. showed that 4.1R associates with CLASP2 independently of MTs. They also found that 4.1R locally controls CLASP2 behavior, CLASP2 cortical platform turnover, and the organization, dynamics, and attachment of MTs to the cell cortex [37].

In addition, previous studies have found that there is an increased presence of 4.1R in the thymus of individuals with myasthenia gravis (MG). 4.1R may have a significant impact on the pathogenesis of MG in dendritic cells (DC). Silencing the expression of 4.1R led to a decrease in their ability to migrate, arrest in the cell cycle, and an increase in surface antigens in DC cells. This suggests that 4.1R plays a role in the autoimmune response in MG [49].

3.5. Control the Ion Channels

In endothelial cells, the interaction between 4.1R and TRPC4 is necessary for the activation of the store-operated calcium channel [30]. Liu showed that 4.1R$^{-/-}$ mice displayed notable deficiencies in the absorption of calcium in the small intestine, as well as reduced levels of PMCA1b expression in enterocytes. These results indicate that 4.1R is directly related to PMCA1b, and the functional role of 4.1R in small intestine calcium absorption could be defined by regulating the membrane expression of PMCA1b [43].

3.6. Cell Division

As an adapter protein within nucleated cells, 4.1R is capable of integrating structural components of centrosome and is crucial for ensuring the fidelity of centrosome function [50]. 4.1R is rearranged during cell division [51]. It is located within the nucleus and centrosomes of cells during the interphase stage and rapidly redistributes to the developing spindle poles when the nuclear envelope disassembles in prometaphase. Additionally, it is detected in the perichromatin during telophase and in the midbody during cytokinesis [52,53]. These results indicate that 4.1R may have a substantial impact on nuclear structure and ultimately influence nuclear function. Initially, Mattagajasingh et al. discovered that 4.1R was linked to the spindle pole protein NuMA within the interphase nucleus. During the process of cell division, it also interacts with spindle pole organizing proteins, NuMA, dynein, and dynactin to form a complex. 4.1R may have a significant impact on the organization of nuclear architecture, mitotic spindle, and spindle poles [26]. Subsequently, Huang et al. proposed the significance of a 135-kDa non-erythroid 4.1R protein in cellular division. This protein is involved in the assembly of mitotic spindles and spindle poles by interacting with mitotic microtubules [54]. Krauss and colleagues demonstrated that immunodepletion of 4.1R disrupted microtubule arrays and mislocalized NuMA. They identified two 4.1R domains critical for its function: the SAB domain and the NuMA binding C-terminal domain [55]. Downregulation of 4.1R affected cell cycle progression and caused abnormalities in mitotic spindles and anaphase. Their findings provided functional evidence supporting the significant role of 4.1R in maintaining the structural integrity of centrosomes and mitotic spindles [56]. Mattagajasingh et al. identified the amino acids of 4.1R and NuMA that sustained their interaction. They demonstrated that the inhibition of the interaction between the protein 4.1R and NuMA through mutagenization of their binding sites resulted in the abrogation of nuclear localization of 4.1R [57]. Meyer and colleagues presented proof that 4.1R has significant functional interactions with the nuclear envelope protein emerin and the intermediate filament protein lamin A. These connections affect the nuclear structure, the association between the centrosome and nuclear envelope, and the regulation of β-catenin transcriptional co-activator activity, which relies on β-catenin nuclear export [58]. Recently, Huang et al. suggested that 4.1R regulates the asymmetric segregation of the Notch signaling regulatory protein Numb during terminal erythroid maturation. They also identified a critical role for 4.1R in mediating erythropoiesis [25].

4. 4.1R and Disease

EPB41 gene mutations, as well as a quantitative deficiency of protein 4.1R or defective assembly of structurally altered protein 4.1R, can lead to human disease (Table 2; Figure 3).

Table 2. All published *EPB41* gene lesions responsible for human inherited disease. Data from the Human Gene Mutation Database (HGMD) [59].

Mutation Type	Mutation Data by Type			Mutation Position	Phenotype	Reference
	Codon change	**Amino acid change**	**Codon number**			
Missense/nonsense	AUG-ACG	Met-Thr	1	AUG2	Elliptocytosis	[60]
	AUG-AGG	Met-Arg	1	AUG2	Elliptocytosis	[61]
	TAT-TAA	Tyr-Ter	233	FERM	Hemolytic anemia	[62]
	CGA-TGA	Arg-Ter	262	FERM	Elliptocytosis	[63]
	ACA-ATA	Thr-Ile	283	FERM	Spherocytosis	[64]

Table 2. *Cont.*

Mutation Type	Mutation Data by Type	Mutation Position	Phenotype	Reference
Splicing	**Splicing mutation** a base substitution at position 2720 (G→A)	CTD	Elliptocytosis	[12]
Regulatory	**Sequence** −278 relative to transcription initiation site	HP	Hepatocellular carcinoma (HCC), increased risk	[65]
Small deletions	**Deletion** GAATCAG A AAA	HP SAB SAB	Elliptocytosis Elliptocytosis Elliptocytosis	[66] [67] [68]
Gross deletions	**Description** exons 2 to 12 240 bp (Lys407-Gly486) 318 bp 50 kb of genomic DNA, exons 2 and 4	HP, FERM, and FA SAB HP and FERM HP	Elliptocytosis Elliptocytosis Elliptocytosis Elliptocytosis	[69] [70] [71] [72]
Gross duplication	**Description** 369 bp (Lys407-Gln529)	SAB	Elliptocytosis	[70]

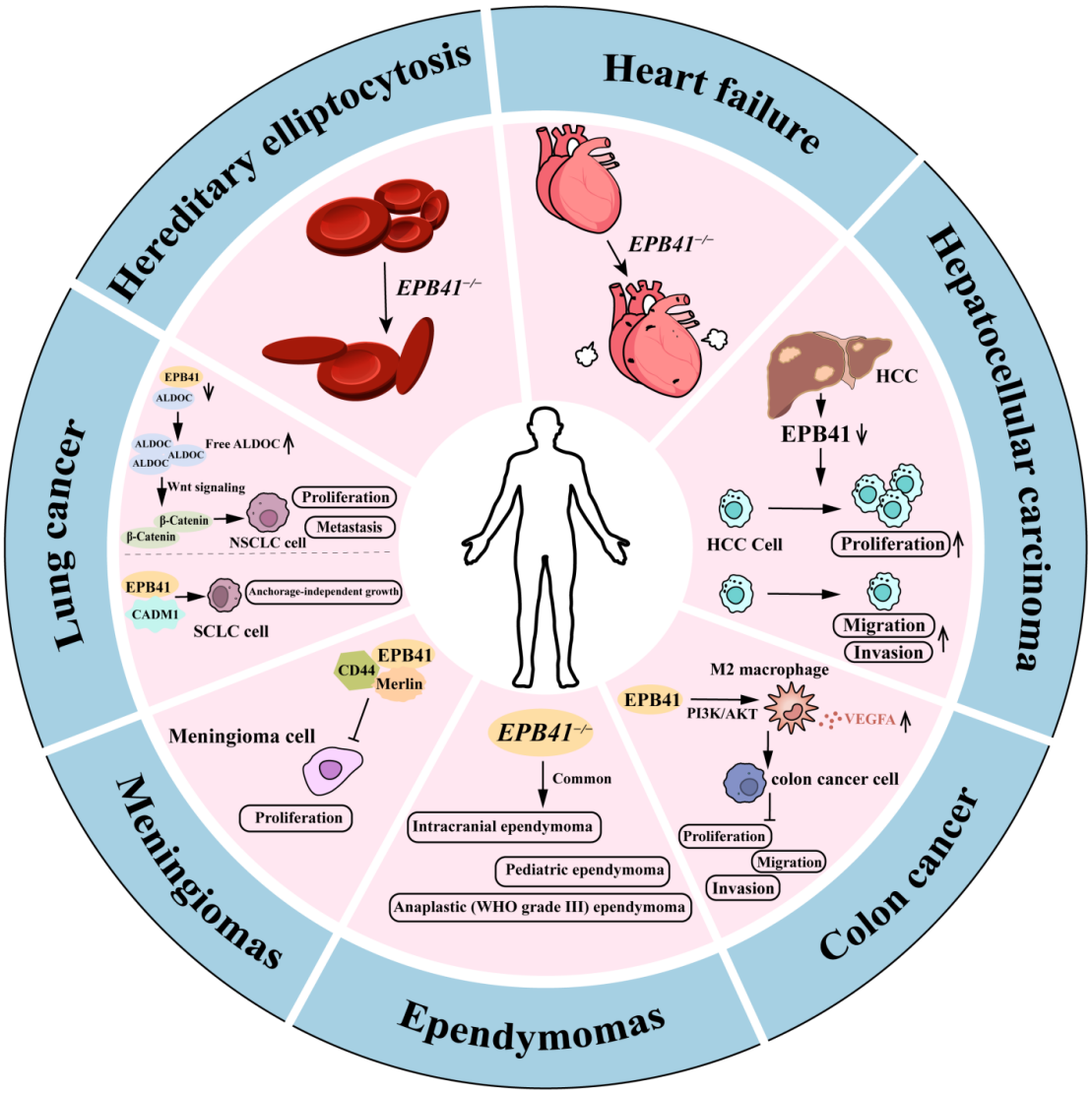


Figure 3. Overview of 4.1R and human disease. The figure shows the dysfunction of 4.1R in HE, HF, and tumors. The direction of the arrows representing rising or falling.

4.1. Hereditary Elliptocytosis (HE)

HE, also referred to as hereditary ovalocytosis, is a genetically heterogeneous blood disorder characterized by oval-shaped erythrocytes with variable levels of hemolytic anemia [73]. HE is distributed globally, with a higher incidence in regions endemic to malaria, particularly among individuals of African and Mediterranean descent [74]. The actual prevalence of HE remains uncertain due to the variability in its clinical severity and the presence of many asymptomatic patients. Nevertheless, symptomatic patients should receive treatment involving blood transfusion and splenectomy [75]. Elliptocytosis is typically inherited as an autosomal dominant trait and is caused by mutations in various genes encoding proteins of the red blood cell cytoskeleton. The instability of membrane skeletons, cell membranes, and erythrocytes can lead to the fragmentation and hemolysis of red blood cells [76]. The primary cause of HE-associated lesions is attributed to qualitative and quantitative defects in the red blood cell membrane skeleton proteins, such as alpha spectrin, beta spectrin, protein 4.1R, band 3, and rarely, GPC [77–79].

Heterozygous or homozygous mutation of the *EPB41* gene causes HE-1 [80]. A partial deficiency in protein 4.1R has been linked to mild, dominant HE, whereas a complete deficiency is associated with severe hemolytic disease [61,81]. In a family from Algeria, people with HE exhibited severe hemolytic anemia due to protein 4.1R deficiency. The mutant gene had a DNA rearrangement from the upstream translation initiation codon. Additionally, the mRNA from the mutant gene was spliced in an abnormal manner [82]. Variants of protein 4.1R with abnormal molecular weights have also been observed in individuals with HE, predominantly by deletions or duplications of the exons located around the SAB domain [70,83]. A higher molecular weight form of 4.1R (95 kDa) is related to mild elliptocytosis without anemia, whereas a lower molecular weight form (65/68 kDa) is associated with moderate elliptocytosis and anemia. Erythrocyte membranes containing 4.1R (95 kDa) showed similar mechanical stability to normal membranes, consistent with the presence of an intact SAB domain in protein 4.1R. On the contrary, membranes containing 4.1R (65/68 kDa) displayed significantly reduced mechanical stability due to the absence of the SAB domain [84].

The constitutional deficit of protein 4.1R has been linked to HE. Acquired deficits of 4.1R have been reported in myelodysplastic syndromes with elliptocytosis [85]. Eight cases of acquired elliptocytosis have already been reported in myelodysplastic syndrome (MDS) or chronic myeloproliferative disorders (CMPD) [86–88]. Alanio-Bréchot et al. reported six patients with MDS or CMPDs and an acquired CMPD who had a deficiency in protein 4.1R. Alanio-Bréchot et al. confirmed that a deficiency in protein 4.1R is a recurring occurrence in myeloid malignancies when the deletion del (20q) is present. They discovered this chromosomal abnormality in four out of six patients. They found that the expression of the *EPB41* gene, rather than its structure, was disturbed due to the malignancy [89].

4.2. HF

HF is a prominent contributor to mortality in cardiovascular diseases worldwide, characterized by intricate clinical symptoms and a high rate of mortality [90]. Research has indicated that the myocardial cytoskeleton plays an important role by stabilizing the myocardium, detecting mechanical stretching, and coordinating cellular organization and intercellular signaling [91]. 4.1R is expressed in the heart, but its functional role in the myocardium is unknown. Stagg and colleagues published the initial findings of a cardiac phenotype linked to the absence of 4.1R [92]. In this study, Stagg et al. reported that $4.1R^{-/-}$ mice displayed a reduced heart rate along with a prolonged Q-T interval. The isolated $4.1R^{-/-}$ ventricular cardiomyocytes displayed prolonged action potentials, aberrant Ca^{2+} transients, increased sarcoplasmic reticulum Ca^{2+} stores, and increased spark frequency. The data indicated that 4.1R plays an unexpected role in regulating the functional properties of several cardiac ion transporters, thereby influencing cardiac electrophysiology. This suggests that 4.1R may have an important role in both normal heart function and disease. Then, Pinder et al. characterized the expression, distribution, and novel activities of 4.1R in

the left ventricle. They detected an 80 kDa isoform of 4.1R in subcellular fractions that were enriched in intercalated discs. The presence of 4.1R overlapped with the plasma membrane signaling proteins, including the Na/K-ATPase and the Na/Ca exchanger NCX1, at the intercalated disc [93].

Wei and colleagues assessed the expression of 4.1R in cardiomyocytes and determined its potential role in the development of HF. Their findings revealed a significantly higher proportion of 4.1R-positive cells in the HF group compared to the control group. Their study also observed that 4.1R was primarily localized to the plasma membrane of myocardial cells and was upregulated as HF progressed [94]. Recently, Ning et al. detected co-localization and interaction between 4.1R and Nav1.5. These results suggest that 4.1R may play a role in the occurrence and progression of HF by interacting with ion channel proteins [41]. These findings suggest that there may be an association between 4.1R and the progression of HF, making it a promising therapeutic target for HF.

4.3. Tumors

4.1R exhibits various expressions and functions across different types of tumors. It is a potential marker for tumor prognosis and a target for tumor treatment.

4.3.1. The Role of 4.1R in Tumors

A study by Yang et al. reported that there is a decreased expression of 4.1R in HCC tissues compared to adjacent normal tissues. Furthermore, the study found that 4.1R can significantly suppress the growth and progression of HCC [65].

Yuan et al. indicated that *EPB41* is a novel tumor suppressor in NSCLC. Their study demonstrated that the inhibition of *EPB41* expression in cancer cells increased the levels of ALDOC protein released from the EPB41-ALDOC complex. This, in turn, resulted in the upregulation of multiple oncogenes through the β-Catenin/TCF/LEF TF complex, leading to the pathogenesis of NSCLC [46].

SCLC is another malignancy with high expression of CADM1, leading to increased malignant characteristics. Funaki et al. found that 4.1R was necessary for the oncogenic effect of CADM1 in SCLC. CADM1 expression was observed to correlate with the membrane localization of 4.1R in both SCLC primary and cell lines. Additionally, the co-localization of CADM1 and 4.1R on the cell membrane was associated with a more advanced tumor stage. These findings indicate that the formation of the CADM1-4.1R complex contributes to the malignant features of SCLC [35]. Further investigation is needed to elucidate the mechanism of the CADM1-4.1R complex in the development and progression of SCLC.

Meningiomas are prevalent neoplasms of the central nervous system (CNS). One of the most common events associated with meningioma tumorigenesis is the deletion of chromosome 22q and the inactivation of the neurofibromatosis 2 gene [95,96]. Robb et al. observed a loss of 4.1R expression in 2 meningioma cell lines (IOMM-Lee and CH157-MN) as well as in 6 of 15 sporadic meningiomas. They demonstrated that 4.1R was a tumor suppressor in the molecular pathogenesis of meningioma [45]. However, Piaskowski et al. observed that the expression of 4.1R mRNA was unchanged in all analyzed meningiomas and suggested that the role of 4.1R in meningioma development should be reconsidered [97].

Ependymomas are prevalent malignant tumors affecting both pediatric and adult CNSs. Rajaram et al. found that losses of 4.1R expression and 4.1B (18p11.3) deletions were more frequently observed in pediatric, intracranial, and/or anaplastic (WHO grade III) ependymoma subtypes. Furthermore, the deletion of 4.1G (6q23) was linked to a more aggressive clinical disease. The researchers determined that alterations in protein 4.1 family members were widespread in ependymal tumors, and specific alterations were linked to distinct clinicopathologic subsets [98].

Li et al. revealed that protein 4.1R interacts with GAT-1 and GAT-2, leading to an impact on the transmembrane transport of 5-ALA. This interaction results in a decreased sensitivity of B16 cells to photodynamic therapy (PDT) and downregulates the anti-tumor immune response triggered by PDT [21].

M2 macrophages play a critical role in the tumor microenvironment and have been demonstrated to be closely associated with tumor progression. Lu et al. reported that 4.1R downregulated the secretion of vascular endothelial growth factor A (VEGFA) in M2 macrophages, thereby delaying colon cancer progression through the inhibition of the PI3K/AKT signaling pathway [99].

4.3.2. Prognostic Marker of Disease

Individuals who receive a diagnosis of cancer at earlier or intermediate stages generally have more favorable prognoses compared to those diagnosed with advanced disease. So, early prediction and intervention represent the most efficacious approaches for enhancing the clinical outcomes of patients [100]. In recent years, numerous reports have confirmed that the expression of 4.1R is altered in various malignancies, either decreased or increased. Abnormal expression of 4.1R has been found to contribute to tumorigenesis and tumor progression. Feng et al. found that high expressions of 4.1R mRNA were associated with better survival in breast cancer patients. They concluded that 4.1R can be considered a novel biomarker and a potential therapeutic target for breast cancer [101]. In addition, it was found that the expression of 4.1R was weak in NSCLC tissues compared to normal tissues. Low expression of 4.1R was associated with a poor prognosis for lung cancer patients [46,102,103]. Liu et al. identified a two-gene (*PML-EPB41*) signature as a prognostic predictor for patients with osteosarcoma. This signature was validated and analyzed by an external dataset and biological experiment [104]. Yin et al. identified *EPB41* as a prognostic risk biomarker (PRB) with high potential as a drug target for the treatment of colon adenocarcinoma [105].

These studies elucidated the potential of *EPB41* as a future therapeutic target for cancer.

5. Conclusions and Future Perspectives

In this review, we summarize the structural characteristics, physiological functions, and pathological functions of 4.1R. As a structural protein, the function of 4.1R in mature erythrocytes has been extensively studied. In recent years, many studies have been carried out on its function in nucleated cells. 4.1R is involved in many cellular processes by regulating the cytoskeleton and signaling pathways. Any interference with the expression of 4.1R leads to disruptions in normal cell function and pathological outcomes.

Genetic mutations associated with human disease have been reported to occur in different domains of the protein 4.1R, but many details remain unknown. In order to better understand the relationship between the structure and function of 4.1R and human diseases, the regulatory functions and mechanisms of different 4.1R domains in human diseases need to be further studied. Multiple isoforms of protein 4.1R are expressed in various tissues through complex pre-mRNA splicing events. During cell differentiation, the expression and localization of the *EPB41* gene are regulated. Therefore, the regulatory mechanisms of different subtypes or multiple subtypes of 4.1R in different diseases still need to be further studied. Elucidation of the regulatory functions and molecular mechanisms of 4.1R in diseases will provide a foundation for clinical diagnosis and personalized gene therapy for patients with *EPB41* mutations or expression deficiencies.

Author Contributions: All authors contributed to the present study's conception and design. J.L. wrote the first draft of the manuscript. C.D. illustrated the figures. X.L. and Q.K. reviewed and edited the draft. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the grants from the National Natural Science Foundation of China (No. 82370113) and the Open Project of the provincial scientific research platform of Henan Province Children's Hospital (SS202202).

Acknowledgments: Figures 1–3 presented in this manuscript were designed using Adobe Illustrator software (version 24.0.1).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Lux, S.E.t. Anatomy of the red cell membrane skeleton: Unanswered questions. *Blood* **2016**, *127*, 187–199. [CrossRef] [PubMed]
2. Yu, J.; Fischman, D.A.; Steck, T.L. Selective solubilization of proteins and phospholipids from red blood cell membranes by nonionic detergents. *J. Supramol. Struct.* **1973**, *1*, 233–248. [CrossRef]
3. Feo, C.J.; Fischer, S.; Piau, J.P.; Grange, M.J.; Tchernia, G. 1st instance of the absence of an erythrocyte membrane protein (band 4(1)) in a case of familial elliptocytic anemia. *Nouv. Rev. Fr. Hematol.* **1980**, *22*, 315–325. [PubMed]
4. Baines, A.J.; Lu, H.C.; Bennett, P.M. The Protein 4.1 family: Hub proteins in animals for organizing membrane proteins. *Biochim. Biophys. Acta* **2014**, *1838*, 605–619. [CrossRef]
5. Baines, A.J.; Bennett, P.M.; Carter, E.W.; Terracciano, C. Protein 4.1 and the control of ion channels. *Blood Cells Mol. Dis.* **2009**, *42*, 211–215. [CrossRef]
6. Salomao, M.; Zhang, X.; Yang, Y.; Lee, S.; Hartwig, J.H.; Chasis, J.A.; Mohandas, N.; An, X. Protein 4.1R-dependent multiprotein complex: New insights into the structural organization of the red blood cell membrane. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 8026–8031. [CrossRef]
7. Han, B.G.; Nunomura, W.; Takakuwa, Y.; Mohandas, N.; Jap, B.K. Protein 4.1R core domain structure and insights into regulation of cytoskeletal organization. *Nat. Struct. Biol.* **2000**, *7*, 871–875. [CrossRef]
8. An, X.L.; Takakuwa, Y.; Manno, S.; Han, B.G.; Gascard, P.; Mohandas, N. Structural and functional characterization of protein 4.1R-phosphatidylserine interaction: Potential role in 4.1R sorting within cells. *J. Biol. Chem.* **2001**, *276*, 35778–35785. [CrossRef]
9. An, X.; Zhang, X.; Debnath, G.; Baines, A.J.; Mohandas, N. Phosphatidylinositol-4,5-biphosphate (PIP2) differentially regulates the interaction of human erythrocyte protein 4.1 (4.1R) with membrane proteins. *Biochemistry* **2006**, *45*, 5725–5732. [CrossRef] [PubMed]
10. Baines, A.J. A FERM-adjacent (FA) region defines a subset of the 4.1 superfamily and is a potential regulator of FERM domain function. *BMC Genom.* **2006**, *7*, 85. [CrossRef] [PubMed]
11. Bennett, V.; Baines, A.J. Spectrin and ankyrin-based pathways: Metazoan inventions for integrating cells into tissues. *Physiol. Rev.* **2001**, *81*, 1353–1392. [CrossRef] [PubMed]
12. Morinière, M.; Ribeiro, L.; Dalla Venezia, N.; Deguillien, M.; Maillet, P.; Cynober, T.; Delhommeau, F.; Almeida, H.; Tamagnini, G.; Delaunay, J.; et al. Elliptocytosis in patients with C-terminal domain mutations of protein 4.1 correlates with encoded messenger RNA levels rather than with alterations in primary protein structure. *Blood* **2000**, *95*, 1834–1841. [CrossRef]
13. Conboy, J.G. Structure, function, and molecular genetics of erythroid membrane skeletal protein 4.1 in normal and abnormal red blood cells. *Semin. Hematol.* **1993**, *30*, 58–73.
14. Conboy, J.G.; Chan, J.; Mohandas, N.; Kan, Y.W. Multiple protein 4.1 isoforms produced by alternative splicing in human erythroid cells. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 9062–9065. [CrossRef] [PubMed]
15. Nunomura, W.; Gascard, P.; Takakuwa, Y. Insights into the Function of the Unstructured N-Terminal Domain of Proteins 4.1R and 4.1G in Erythropoiesis. *Int. J. Cell Biol.* **2011**, *2011*, 943272. [CrossRef]
16. Nunomura, W.; Parra, M.; Hebiguchi, M.; Sawada, K.; Mohandas, N.; Takakuwa, Y. Marked difference in membrane-protein-binding properties of the two isoforms of protein 4.1R expressed at early and late stages of erythroid differentiation. *Biochem. J.* **2009**, *417*, 141–148. [CrossRef] [PubMed]
17. Diakowski, W.; Grzybek, M.; Sikorski, A.F. Protein 4.1, a component of the erythrocyte membrane skeleton and its related homologue proteins forming the protein 4.1/FERM superfamily. *Folia Histochem. Cytobiol.* **2006**, *44*, 231–248.
18. Conboy, J. The role of alternative pre-mRNA splicing in regulating the structure and function of skeletal protein 4.1. *Proc. Soc. Exp. Biol. Med.* **1999**, *220*, 73–78. [CrossRef]
19. Parra, M.; Gee, S.; Chan, N.; Ryaboy, D.; Dubchak, I.; Mohandas, N.; Gascard, P.D.; Conboy, J.G. Differential domain evolution and complex RNA processing in a family of paralogous *EPB41* (protein 4.1) genes facilitate expression of diverse tissue-specific isoforms. *Genomics* **2004**, *84*, 637–646. [CrossRef]
20. Ding, C.; Guo, Y.; Liang, T.; Liu, J.; Yang, L.; Wang, T.; Liu, X.; Kang, Q. Protein 4.1R negatively regulates P815 cells proliferation by inhibiting C-Kit-mediated signal transduction. *Exp. Cell Res.* **2021**, *398*, 112403. [CrossRef]
21. Li, B.; Zhang, X.; Lu, Y.; Zhao, L.; Guo, Y.; Guo, S.; Kang, Q.; Liu, J.; Dai, L.; Zhang, L.; et al. Protein 4.1R affects photodynamic therapy for B16 melanoma by regulating the transport of 5-aminolevulinic acid. *Exp. Cell Res.* **2021**, *399*, 112465. [CrossRef]
22. Ning, S.; Kang, Q.; Fan, D.; Liu, J.; Xue, C.; Zhang, X.; Ding, C.; Zhang, J.; Peng, Q.; Ji, Z. Protein 4.1R is Involved in the Transport of 5-Aminolevulinic Acid by Interaction with GATs in MEF Cells. *Photochem. Photobiol.* **2018**, *94*, 173–178. [CrossRef]
23. Huang, S.C.; Zhou, A.; Nguyen, D.T.; Zhang, H.S.; Benz, E.J., Jr. Protein 4.1R Influences Myogenin Protein Stability and Skeletal Muscle Differentiation. *J. Biol. Chem.* **2016**, *291*, 25591–25607. [CrossRef]
24. Liang, T.; Guo, Y.; Li, M.; Ding, C.; Sang, S.; Zhou, T.; Shao, Q.; Liu, X.; Lu, J.; Ji, Z.; et al. Cytoskeleton protein 4.1R regulates B-cell fate by modulating the canonical NF-κB pathway. *Immunology* **2020**, *161*, 314–324. [CrossRef]
25. Huang, S.C.; Vu, L.V.; Yu, F.H.; Nguyen, D.T.; Benz, E.J., Jr. Multifunctional protein 4.1R regulates the asymmetric segregation of Numb during terminal erythroid maturation. *J. Biol. Chem.* **2021**, *297*, 101051. [CrossRef]
26. Mattagajasingh, S.N.; Huang, S.C.; Hartenstein, J.S.; Snyder, M.; Marchesi, V.T.; Benz, E.J. A nonerythroid isoform of protein 4.1R interacts with the nuclear mitotic apparatus (NuMA) protein. *J. Cell Biol.* **1999**, *145*, 29–43. [CrossRef]
27. Draberova, L.; Draberova, H.; Potuckova, L.; Halova, I.; Bambouskova, M.; Mohandas, N.; Draber, P. Cytoskeletal Protein 4.1R Is a Positive Regulator of the FcεRI Signaling and Chemotaxis in Mast Cells. *Front. Immunol.* **2019**, *10*, 3068. [CrossRef]

28. Kang, Q.; Yu, Y.; Pei, X.; Hughes, R.; Heck, S.; Zhang, X.; Guo, X.; Halverson, G.; Mohandas, N.; An, X. Cytoskeletal protein 4.1R negatively regulates T-cell activation by inhibiting the phosphorylation of LAT. *Blood* **2009**, *113*, 6128–6137. [CrossRef] [PubMed]
29. Fan, D.; Li, J.; Li, Y.; Guo, Y.; Zhang, X.; Wang, W.; Liu, X.; Liu, J.; Dai, L.; Zhang, L.; et al. Protein 4.1R negatively regulates $CD8^+$ T-cell activation by modulating phosphorylation of linker for activation of T cells. *Immunology* **2019**, *157*, 312–321. [CrossRef] [PubMed]
30. Cioffi, D.L.; Wu, S.; Alexeyev, M.; Goodman, S.R.; Zhu, M.X.; Stevens, T. Activation of the endothelial store-operated ISOC Ca^{2+} channel requires interaction of protein 4.1 with TRPC4. *Circ. Res.* **2005**, *97*, 1164–1172. [CrossRef] [PubMed]
31. Ruiz-Sáenz, A.; Kremer, L.; Alonso, M.A.; Millán, J.; Correas, I. Protein 4.1R regulates cell migration and IQGAP1 recruitment to the leading edge. *J. Cell Sci.* **2011**, *124*, 2529–2538. [CrossRef]
32. Chen, L.; Wang, T.; Ji, X.; Ding, C.; Liang, T.; Liu, X.; Lu, J.; Guo, X.; Kang, Q.; Ji, Z. Cytoskeleton protein 4.1R suppresses murine keratinocyte cell hyperproliferation via activating the Akt/ERK pathway in an EGFR-dependent manner. *Exp. Cell Res.* **2019**, *384*, 111648. [CrossRef] [PubMed]
33. Chen, L.; Hughes, R.A.; Baines, A.J.; Conboy, J.; Mohandas, N.; An, X. Protein 4.1R regulates cell adhesion, spreading, migration and motility of mouse keratinocytes by modulating surface expression of beta1 integrin. *J. Cell Sci.* **2011**, *124*, 2478–2487. [CrossRef] [PubMed]
34. Yang, S.; Guo, X.; Debnath, G.; Mohandas, N.; An, X. Protein 4.1R links E-cadherin/beta-catenin complex to the cytoskeleton through its direct interaction with beta-catenin and modulates adherens junction integrity. *Biochim. Biophys. Acta* **2009**, *1788*, 1458–1465. [CrossRef] [PubMed]
35. Funaki, T.; Ito, T.; Tanei, Z.I.; Goto, A.; Niki, T.; Matsubara, D.; Murakami, Y. CADM1 promotes malignant features of small-cell lung cancer by recruiting 4.1R to the plasma membrane. *Biochem. Biophys. Res. Commun.* **2021**, *534*, 172–178. [CrossRef] [PubMed]
36. Hou, C.L.; Tang, C.; Roffler, S.R.; Tang, T.K. Protein 4.1R binding to eIF3-p44 suggests an interaction between the cytoskeletal network and the translation apparatus. *Blood* **2000**, *96*, 747–753. [CrossRef] [PubMed]
37. Ruiz-Saenz, A.; van Haren, J.; Sayas, C.L.; Rangel, L.; Demmers, J.; Millán, J.; Alonso, M.A.; Galjart, N.; Correas, I. Protein 4.1R binds to CLASP2 and regulates dynamics, organization and attachment of microtubules to the cell cortex. *J. Cell Sci.* **2013**, *126*, 4589–4601. [CrossRef] [PubMed]
38. Bazzini, C.; Benedetti, L.; Civello, D.; Zanoni, C.; Rossetti, V.; Marchesi, D.; Garavaglia, M.L.; Paulmichl, M.; Francolini, M.; Meyer, G.; et al. ICln: A new regulator of non-erythroid 4.1R localisation and function. *PLoS ONE* **2014**, *9*, e108826. [CrossRef] [PubMed]
39. Tang, C.J.; Tang, T.K. The 30-kD domain of protein 4.1 mediates its binding to the carboxyl terminus of pICln, a protein involved in cellular volume regulation. *Blood* **1998**, *92*, 1442–1447. [CrossRef]
40. Mattagajasingh, S.N.; Huang, S.C.; Hartenstein, J.S.; Benz, E.J., Jr. Characterization of the interaction between protein 4.1R and ZO-2. A possible link between the tight junction and the actin cytoskeleton. *J. Biol. Chem.* **2000**, *275*, 30573–30585. [CrossRef]
41. Ning, S.; Hua, L.; Ji, Z.; Fan, D.; Meng, X.; Li, Z.; Wang, Q.; Guo, Z. Protein 4.1 family and ion channel proteins interact to regulate the process of heart failure in rats. *Acta Histochem.* **2021**, *123*, 151748. [CrossRef]
42. Rose, M.; Dütting, E.; Enz, R. Band 4.1 proteins are expressed in the retina and interact with both isoforms of the metabotropic glutamate receptor type 8. *J. Neurochem.* **2008**, *105*, 2375–2387. [CrossRef]
43. Liu, C.; Weng, H.; Chen, L.; Yang, S.; Wang, H.; Debnath, G.; Guo, X.; Wu, L.; Mohandas, N.; An, X. Impaired intestinal calcium absorption in protein 4.1R-deficient mice due to altered expression of plasma membrane calcium ATPase 1b (PMCA1b). *J. Biol. Chem.* **2013**, *288*, 11407–11415. [CrossRef]
44. Hanada, T.; Takeuchi, A.; Sondarva, G.; Chishti, A.H. Protein 4.1-mediated membrane targeting of human discs large in epithelial cells. *J. Biol. Chem.* **2003**, *278*, 34445–34450. [CrossRef]
45. Robb, V.A.; Li, W.; Gascard, P.; Perry, A.; Mohandas, N.; Gutmann, D.H. Identification of a third Protein 4.1 tumor suppressor, Protein 4.1R, in meningioma pathogenesis. *Neurobiol. Dis.* **2003**, *13*, 191–202. [CrossRef] [PubMed]
46. Yuan, J.; Xing, H.; Li, Y.; Song, Y.; Zhang, N.; Xie, M.; Liu, J.; Xu, Y.; Shen, Y.; Wang, B.; et al. *EPB41* suppresses the Wnt/β-catenin signaling in non-small cell lung cancer by sponging ALDOC. *Cancer Lett.* **2021**, *499*, 255–264. [CrossRef] [PubMed]
47. Hung, L.Y.; Tang, C.J.; Tang, T.K. Protein 4.1 R-135 interacts with a novel centrosomal protein (CPAP) which is associated with the γ-tubulin complex. *Mol. Cell Biol.* **2000**, *20*, 7813–7825. [CrossRef] [PubMed]
48. Liu, X.; Zhou, Q.; Ji, Z.; Fu, G.; Li, Y.; Zhang, X.; Shi, X.; Wang, T.; Kang, Q. Protein 4.1R attenuates autoreactivity in experimental autoimmune encephalomyelitis by suppressing $CD4^+$ T cell activation. *Cell Immunol.* **2014**, *292*, 19–24. [CrossRef] [PubMed]
49. Zhao, Q.; Li, Y.; Li, Y.; Ji, X.; Li, H.; Wu, D.; Wei, W.; Xinchun, W. Silencing *EPB41* Gene Expression Leads to Cell Cycle Arrest, Migration Inhibition, and Upregulation of Cell Surface Antigen in DC2.4 Cells. *Med. Sci. Monit.* **2020**, *26*, e920594. [CrossRef] [PubMed]
50. Hinchcliffe, E.H.; Sluder, G. Centrosome duplication: Three kinases come up a winner! *Curr. Biol.* **2001**, *11*, R698–R701. [CrossRef] [PubMed]
51. Krauss, S.W.; Chasis, J.A.; Rogers, C.; Mohandas, N.; Krockmalnic, G.; Penman, S. Structural protein 4.1 is located in mammalian centrosomes. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 7297–7302. [CrossRef]
52. de Cárcer, G.; Lallena, M.J.; Correas, I. Protein 4.1 is a component of the nuclear matrix of mammalian cells. *Biochem. J.* **1995**, *312 Pt 3*, 871–877. [CrossRef] [PubMed]
53. Krauss, S.W.; Larabell, C.A.; Lockett, S.; Gascard, P.; Penman, S.; Mohandas, N.; Chasis, J.A. Structural protein 4.1 in the nucleus of human cells: Dynamic rearrangements during cell division. *J. Cell Biol.* **1997**, *137*, 275–289. [CrossRef] [PubMed]

54. Huang, S.C.; Jagadeeswaran, R.; Liu, E.S.; Benz, E.J., Jr. Protein 4.1R, a microtubule-associated protein involved in microtubule aster assembly in mammalian mitotic extract. *J. Biol. Chem.* **2004**, *279*, 34595–34602. [CrossRef] [PubMed]
55. Krauss, S.W.; Lee, G.; Chasis, J.A.; Mohandas, N.; Heald, R. Two protein 4.1 domains essential for mitotic spindle and aster microtubule dynamics and organization in vitro. *J. Biol. Chem.* **2004**, *279*, 27591–27598. [CrossRef] [PubMed]
56. Krauss, S.W.; Spence, J.R.; Bahmanyar, S.; Barth, A.I.; Go, M.M.; Czerwinski, D.; Meyer, A.J. Downregulation of protein 4.1R, a mature centriole protein, disrupts centrosomes, alters cell cycle progression, and perturbs mitotic spindles and anaphase. *Mol. Cell Biol.* **2008**, *28*, 2283–2294. [CrossRef] [PubMed]
57. Mattagajasingh, S.N.; Huang, S.C.; Benz, E.J., Jr. Inhibition of protein 4.1 R and NuMA interaction by mutagenization of their binding-sites abrogates nuclear localization of 4.1 R. *Clin. Transl. Sci.* **2009**, *2*, 102–111. [CrossRef] [PubMed]
58. Meyer, A.J.; Almendrala, D.K.; Go, M.M.; Krauss, S.W. Structural protein 4.1R is integrally involved in nuclear envelope protein localization, centrosome-nucleus association and transcriptional signaling. *J. Cell Sci.* **2011**, *124*, 1433–1444. [CrossRef]
59. Stenson, P.D.; Ball, E.V.; Mort, M.; Phillips, A.D.; Shiel, J.A.; Thomas, N.S.; Abeysinghe, S.; Krawczak, M.; Cooper, D.N. Human Gene Mutation Database (HGMD): 2003 update. *Hum. Mutat.* **2003**, *21*, 577–581. [CrossRef]
60. Garbarz, M.; Devaux, I.; Bournier, O.; Grandchamp, B.; Dhermy, D. Protein 4.1 Lille, a novel mutation in the downstream initiation codon of protein 4.1 gene associated with heterozygous 4,1(-) hereditary elliptocytosis. *Hum. Mutat.* **1995**, *5*, 339–340. [CrossRef]
61. Dalla Venezia, N.; Gilsanz, F.; Alloisio, N.; Ducluzeau, M.T.; Benz, E.J., Jr.; Delaunay, J. Homozygous 4.1(-) hereditary elliptocytosis associated with a point mutation in the downstream initiation codon of protein 4.1 gene. *J. Clin. Investig.* **1992**, *90*, 1713–1717. [CrossRef]
62. Del Orbe Barreto, R.; Arrizabalaga, B.; De la Hoz, A.B.; García-Orad, Á.; Tejada, M.I.; Garcia-Ruiz, J.C.; Fidalgo, T.; Bento, C.; Manco, L.; Ribeiro, M.L. Detection of new pathogenic mutations in patients with congenital haemolytic anaemia using next-generation sequencing. *Int. J. Lab. Hematol.* **2016**, *38*, 629–638. [CrossRef]
63. Niss, O.; Chonat, S.; Dagaonkar, N.; Almansoori, M.O.; Kerr, K.; Rogers, Z.R.; McGann, P.T.; Quarmyne, M.O.; Risinger, M.; Zhang, K.; et al. Genotype-phenotype correlations in hereditary elliptocytosis and hereditary pyropoikilocytosis. *Blood Cells Mol. Dis.* **2016**, *61*, 4–9. [CrossRef]
64. Choi, H.S.; Choi, Q.; Kim, J.A.; Im, K.O.; Park, S.N.; Park, Y.; Shin, H.Y.; Kang, H.J.; Kook, H.; Kim, S.Y.; et al. Molecular diagnosis of hereditary spherocytosis by multi-gene target sequencing in Korea: Matching with osmotic fragility test and presence of spherocyte. *Orphanet J. Rare Dis.* **2019**, *14*, 114. [CrossRef]
65. Yang, X.; Yu, D.; Ren, Y.; Wei, J.; Pan, W.; Zhou, C.; Zhou, L.; Liu, Y.; Yang, M. Integrative Functional Genomics Implicates *EPB41* Dysregulation in Hepatocellular Carcinoma Risk. *Am. J. Hum. Genet.* **2016**, *99*, 275–286. [CrossRef]
66. Lacy, J.N.; Ulirsch, J.C.; Grace, R.F.; Towne, M.C.; Hale, J.; Mohandas, N.; Lux, S.E.; Agrawal, P.B.; Sankaran, V.G. Exome sequencing results in successful diagnosis and treatment of a severe congenital anemia. *Cold Spring Harb. Mol. Case Stud.* **2016**, *2*, a000885. [CrossRef] [PubMed]
67. Maillet, P.; Dalla Venezia, N.; Lorenzo, F.; Morinière, M.; Bozon, M.; Noël, B.; Delaunay, J.; Baklouti, F. A premature termination codon within an alternative exon affecting only the metabolism of transcripts that retain this exon. *Hum. Mutat.* **1999**, *14*, 145–155. [CrossRef]
68. Lorenzo, F.; Dalla Venezia, N.; Morlé, L.; Baklouti, F.; Alloisio, N.; Ducluzeau, M.T.; Roda, L.; Lefrançois, P.; Delaunay, J. Protein 4.1 deficiency associated with an altered binding to the spectrin-actin complex of the red cell membrane skeleton. *J. Clin. Investig.* **1994**, *94*, 1651–1656. [CrossRef] [PubMed]
69. Venezia, N.D.; Maillet, P.; Morlé, L.; Roda, L.; Delaunay, J.; Baklouti, F. A large deletion within the protein 4.1 gene associated with a stable truncated mRNA and an unaltered tissue-specific alternative splicing. *Blood* **1998**, *91*, 4361–4367. [CrossRef] [PubMed]
70. Conboy, J.; Marchesi, S.; Kim, R.; Agre, P.; Kan, Y.W.; Mohandas, N. Molecular analysis of insertion/deletion mutations in protein 4.1 in elliptocytosis. II. Determination of molecular genetic origins of rearrangements. *J. Clin. Investig.* **1990**, *86*, 524–530. [CrossRef] [PubMed]
71. Conboy, J.G.; Chasis, J.A.; Winardi, R.; Tchernia, G.; Kan, Y.W.; Mohandas, N. An isoform-specific mutation in the protein 4.1 gene results in hereditary elliptocytosis and complete deficiency of protein 4.1 in erythrocytes but not in nonerythroid cells. *J. Clin. Investig.* **1993**, *91*, 77–82. [CrossRef] [PubMed]
72. Baklouti, F.; Morinière, M.; Haj-Khélil, A.; Fénéant-Thibault, M.; Gruffat, H.; Couté, Y.; Ninot, A.; Guitton, C.; Delaunay, J. Homozygous deletion of *EPB41* genuine AUG-containing exons results in mRNA splicing defects, NMD activation and protein 4.1R complete deficiency in hereditary elliptocytosis. *Blood Cells Mol. Dis.* **2011**, *47*, 158–165. [CrossRef] [PubMed]
73. Iolascon, A.; Andolfo, I.; Russo, R. Advances in understanding the pathogenesis of red cell membrane disorders. *Br. J. Haematol.* **2019**, *187*, 13–24. [CrossRef] [PubMed]
74. Glele-Kakai, C.; Garbarz, M.; Lecomte, M.C.; Leborgne, S.; Galand, C.; Bournier, O.; Devaux, I.; Gautero, H.; Zohoun, I.; Gallagher, P.G.; et al. Epidemiological studies of spectrin mutations related to hereditary elliptocytosis and spectrin polymorphisms in Benin. *Br. J. Haematol.* **1996**, *95*, 57–66. [CrossRef] [PubMed]
75. Mohandas, N. Inherited hemolytic anemia: A possessive beginner's guide. *Hematol. Am. Soc. Hematol. Educ. Program.* **2018**, *2018*, 377–381. [CrossRef] [PubMed]
76. Shin, S.; Hwang, K.A.; Paik, K.; Park, J. A novel *EPB41* p.Trp704* mutation in a Korean patient with hereditary elliptocytosis: A case report. *Hematology* **2020**, *25*, 321–326. [CrossRef] [PubMed]

77. del Giudice, E.M.; Ducluzeau, M.T.; Alloisio, N.; Wilmotte, R.; Delaunay, J.; Perrotta, S.; Cutillo, S.; Iolascon, A. Alpha I/65 hereditary elliptocytosis in southern Italy: Evidence for an African origin. *Hum. Genet.* **1992**, *89*, 553–556. [CrossRef]
78. Gallagher, P.G.; Tse, W.T.; Coetzer, T.; Lecomte, M.C.; Garbarz, M.; Zarkowsky, H.S.; Baruchel, A.; Ballas, S.K.; Dhermy, D.; Palek, J.; et al. A common type of the spectrin alpha I 46-50a-kD peptide abnormality in hereditary elliptocytosis and pyropoikilocytosis is associated with a mutation distant from the proteolytic cleavage site. Evidence for the functional importance of the triple helical model of spectrin. *J. Clin. Investig.* **1992**, *89*, 892–898. [CrossRef]
79. Zaidi, A.U.; Buck, S.; Gadgeel, M.; Herrera-Martinez, M.; Mohan, A.; Johnson, K.; Bagla, S.; Johnson, R.M.; Ravindranath, Y. Clinical Diagnosis of Red Cell Membrane Disorders: Comparison of Osmotic Gradient Ektacytometry and Eosin Maleimide (EMA) Fluorescence Test for Red Cell Band 3 (AE1, SLC4A1) Content for Clinical Diagnosis. *Front. Physiol.* **2020**, *11*, 636. [CrossRef]
80. Gallagher, P.G. Hereditary elliptocytosis: Spectrin and protein 4.1R. *Semin. Hematol.* **2004**, *41*, 142–164. [CrossRef]
81. Tchernia, G.; Mohandas, N.; Shohet, S.B. Deficiency of skeletal membrane protein band 4.1 in homozygous hereditary elliptocytosis. Implications for erythrocyte membrane stability. *J. Clin. Investig.* **1981**, *68*, 454–460. [CrossRef]
82. Conboy, J.; Mohandas, N.; Tchernia, G.; Kan, Y.W. Molecular basis of hereditary elliptocytosis due to protein 4.1 deficiency. *N. Engl. J. Med.* **1986**, *315*, 680–685. [CrossRef]
83. McGuire, M.; Smith, B.L.; Agre, P. Distinct variants of erythrocyte protein 4.1 inherited in linkage with elliptocytosis and Rh type in three white families. *Blood* **1988**, *72*, 287–293. [CrossRef]
84. Marchesi, S.L.; Conboy, J.; Agre, P.; Letsinger, J.T.; Marchesi, V.T.; Speicher, D.W.; Mohandas, N. Molecular analysis of insertion/deletion mutations in protein 4.1 in elliptocytosis. I. Biochemical identification of rearrangements in the spectrin/actin binding domain and functional characterizations. *J. Clin. Investig.* **1990**, *86*, 516–523. [CrossRef] [PubMed]
85. Ideguchi, H.; Yamada, Y.; Kondo, S.; Tamura, K.; Makino, S.; Hamasaki, N. Abnormal erythrocyte band 4.1 protein in myelodysplastic syndrome with elliptocytosis. *Br. J. Haematol.* **1993**, *85*, 387–392. [CrossRef] [PubMed]
86. Rummens, J.L.; Verfaillie, C.; Criel, A.; Hidajat, M.; Vanhoof, A.; Van den Berghe, H.; Louwagie, A. Elliptocytosis and schistocytosis in myelodysplasia: Report of two cases. *Acta Haematol.* **1986**, *75*, 174–177. [CrossRef]
87. Ishida, F.; Shimodaira, S.; Kobayashi, H.; Saito, H.; Kaku, M.; Kanzaki, A.; Yawata, Y.; Kitano, K.; Kiyosawa, K. Elliptocytosis in myelodysplastic syndrome associated with translocation (1;5)(p10;q10) and deletion of 20q. *Cancer Genet. Cytogenet.* **1999**, *108*, 162–165. [CrossRef]
88. Hur, M.; Lee, K.M.; Cho, H.C.; Park, Y.I.; Kim, S.H.; Chang, Y.W.; Kim, Y.R.; Cho, H.I. Protein 4.1 deficiency and deletion of chromosome 20q are associated with acquired elliptocytosis in myelodysplastic syndrome. *Clin. Lab. Haematol.* **2004**, *26*, 69–72. [CrossRef] [PubMed]
89. Alanio-Bréchot, C.; Schischmanoff, P.O.; Fénéant-Thibault, M.; Cynober, T.; Tchernia, G.; Delaunay, J.; Garçon, L. Association between myeloid malignancies and acquired deficit in protein 4.1R: A retrospective analysis of six patients. *Am. J. Hematol.* **2008**, *83*, 275–278. [CrossRef]
90. McMurray, J.J.; Pfeffer, M.A. Heart failure. *Lancet* **2005**, *365*, 1877–1889. [CrossRef]
91. LeBar, K.; Liu, W.; Pang, J.; Chicco, A.; Wang, Z. Role of the Microtubule Network in the Passive Anisotropic Viscoelasticity of Right Ventricle with Pulmonary Hypertension Progression. *Acta Biomater.* 2024; *in press*. [CrossRef]
92. Stagg, M.A.; Carter, E.; Sohrabi, N.; Siedlecka, U.; Soppa, G.K.; Mead, F.; Mohandas, N.; Taylor-Harris, P.; Baines, A.; Bennett, P.; et al. Cytoskeletal protein 4.1R affects repolarization and regulates calcium handling in the heart. *Circ. Res.* **2008**, *103*, 855–863. [CrossRef] [PubMed]
93. Pinder, J.C.; Taylor-Harris, P.M.; Bennett, P.M.; Carter, E.; Hayes, N.V.; King, M.D.; Holt, M.R.; Maggs, A.M.; Gascard, P.; Baines, A.J. Isoforms of protein 4.1 are differentially distributed in heart muscle cells: Relation of 4.1R and 4.1G to components of the Ca^{2+} homeostasis system. *Exp. Cell Res.* **2012**, *318*, 1467–1479. [CrossRef]
94. Wei, Z.; Yang, G.; Xu, R.; Zhu, C.; He, F.; Dou, Q.; Tang, J. Correlation between protein 4.1R and the progression of heart failure in vivo. *Genet. Mol. Res.* **2016**, *15*, gmr.15028648. [CrossRef] [PubMed]
95. Rutland, J.W.; Gill, C.M.; Loewenstern, J.; Arib, H.; Pain, M.; Umphlett, M.; Kinoshita, Y.; McBride, R.B.; Bederson, J.; Donovan, M.; et al. NF2 mutation status and tumor mutational burden correlate with immune cell infiltration in meningiomas. *Cancer Immunol. Immunother.* **2021**, *70*, 169–176. [CrossRef] [PubMed]
96. Ueki, K.; Wen-Bin, C.; Narita, Y.; Asai, A.; Kirino, T. Tight association of loss of merlin expression with loss of heterozygosity at chromosome 22q in sporadic meningiomas. *Cancer Res.* **1999**, *59*, 5995–5998.
97. Piaskowski, S.; Rieske, P.; Szybka, M.; Wozniak, K.; Bednarek, A.; Płuciennik, E.; Jaskolski, D.; Sikorska, B.; Liberski, P.P. *GADD45A* and *EPB41* as tumor suppressor genes in meningioma pathogenesis. *Cancer Genet. Cytogenet.* **2005**, *162*, 63–67. [CrossRef] [PubMed]
98. Rajaram, V.; Gutmann, D.H.; Prasad, S.K.; Mansur, D.B.; Perry, A. Alterations of protein 4.1 family members in ependymomas: A study of 84 cases. *Mod. Pathol.* **2005**, *18*, 991–997. [CrossRef]
99. Lu, Y.; Fan, D.; Wang, W.; Gao, X.; Li, H.; Guo, S.; Zhao, L.; Guo, Y.; Li, B.; Zhong, Y.; et al. The protein 4.1R downregulates VEGFA in M2 macrophages to inhibit colon cancer metastasis. *Exp. Cell Res.* **2021**, *409*, 112896. [CrossRef]
100. Luo, H.; Zhao, Q.; Wei, W.; Zheng, L.; Yi, S.; Li, G.; Wang, W.; Sheng, H.; Pu, H.; Mo, H.; et al. Circulating tumor DNA methylation profiles enable early diagnosis, prognosis prediction, and screening for colorectal cancer. *Sci. Transl. Med.* **2020**, *12*, eaax7533. [CrossRef]

101. Feng, G.; Guo, K.; Yan, Q.; Ye, Y.; Shen, M.; Ruan, S.; Qiu, S. Expression of Protein 4.1 Family in Breast Cancer: Database Mining for 4.1 Family Members in Malignancies. *Med. Sci. Monit.* **2019**, *25*, 3374–3389. [CrossRef] [PubMed]
102. Zheng, X.Y.; Qi, Y.M.; Gao, Y.F.; Wang, X.Y.; Qi, M.X.; Shi, X.F.; An, X.L. Expression and significance of membrane skeleton protein 4.1 family in non-small cell lung cancer. *Ai Zheng* **2009**, *28*, 679–684. [CrossRef]
103. Xiang, Y.; Shan, F.; Feng, G.; Guo, K.; Ruan, S.; Huang, D. The prognostic value of 4.1 mRNA expression in non-small cell lung cancer. *Transl. Cancer Res.* **2021**, *10*, 1216–1228. [CrossRef]
104. Liu, S.; Liu, J.; Yu, X.; Shen, T.; Fu, Q. Identification of a Two-Gene (*PML-EPB41*) Signature With Independent Prognostic Value in Osteosarcoma. *Front. Oncol.* **2019**, *9*, 1578. [CrossRef] [PubMed]
105. Yin, Z.; Yan, X.; Wang, Q.; Deng, Z.; Tang, K.; Cao, Z.; Qiu, T. Detecting Prognosis Risk Biomarkers for Colon Cancer Through Multi-Omics-Based Prognostic Analysis and Target Regulation Simulation Modeling. *Front. Genet.* **2020**, *11*, 524. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.