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The myristoylated alanine-rich C-kinase substrates (MARCKS): A membrane-anchored mediator of the cell function

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Abstract

The myristoylated alanine-rich C-kinase substrate (MARCKS) and the MARCKS-related protein (MARCKSL1) are ubiquitous, highly conserved membrane-associated proteins involved in the structural modulation of the actin cytoskeleton, chemotaxis, motility, cell adhesion, phagocytosis, and exocytosis. MARCKS includes an N-terminal myristoylated domain for membrane binding, a highly conserved MARCKS Homology 2 (MH2) domain, and an effector domain (which is the phosphorylation site). MARCKS can sequester phosphatidylinositol-4, 5-diphosphate (PIP2) at lipid rafts in the plasma membrane of quiescent cells, an action reversed by protein kinase C (PKC), ultimately modulating the immune function. Being expressed mostly in innate immune cells, MARCKS promotes the inflammation-driven migration and adhesion of cells and the secretion of cytokines such as tumor necrosis factor (TNF). From a clinical point of view, MARCKS is overexpressed in patients with schizophrenia and bipolar disorders, while the brain level of MARCKS phosphorylation is associated with Alzheimer's disease. Furthermore, MARCKS is associated with the development and progression of numerous types of cancers. Data in autoimmune diseases are limited to rheumatoid arthritis models in which a connection between MARCKS and the JAK-STAT pathway is mediated by miRNAs.

We provide a comprehensive overview of the structure of MARCKS, its molecular characteristics and functions from a biological and pathogenetic standpoint, and will discuss the clinical implications of this pathway.

Keywords

Protein kinase C; Cell function; Inflammation; Immune modulation; Autoimmunity; Schizophrenia; Psychosis; Dementia; Cancer; Personalized medicine

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Conflicts of interest

The authors declare that they have no conflict of interests.

1. The general terms of MARCKS

The myristoylated alanine-rich C-kinase substrate (MARCKS) was originally identified as *Mr* 87 kDa protein in the nerve terminals of rat cerebral cortex and is a member of a family (also named "MARCKS) of ubiquitous, highly conserved membrane-associated proteins. These were originally recognized as the major target of protein kinase C (PKC), and are integral to embryo development and postnatal development of the cytoskeleton, along with numerous other cell functions, including chemotaxis and motility, adhesion, migration, secretion, phagocytosis, and exocytosis. The two main members of the MARCKS family are (i) MARCKS, an acidic protein ubiquitously distributed in different tissues; (ii) MARCKSL1 (also denoted MRP, MLP, F52 MACMARCKS and MARCKS-related protein), a 20-kDa acidic protein mainly expressed in the brain, reproductive tissues, and macrophages. MARCKS and MARCKSL1 structures include a basic effector domain that binds Ca^{2+}- calmodulin, F-actin, and acidic phospholipids and thus participates to the inhibition of fibrin formation [1] and platelet function [2]. The effector domain is also involved in the phosphorylation of PKC, which in turn phosphorylates the key serine residues within the effector domain [3]. The $Ca2^{+}$ influx into the cytosol leads to phosphorylation of MARCKS [4] which in turn regulates neurological development and immunological functions. The clinical role of MARCKS has been established in neurological conditions, cancer, and inflammation, with potential therapeutic implications as shown by the effect of MARCKS inhibition in chronic obstructive pulmonary disease [5]. MARCKS is expressed at the highest levels in the brain during embryogenesis but is expressed widely throughout adulthood [6,7]. MARCKS mRNA levels are significantly upregulated in schizophrenia and bipolar disorders [8] while the phosphorylation of MARCKS, especially at Ser46, predates the aggregation of Aβ, and is sustained throughout the course of Alzheimer's disease in human and mouse brains [9]. MARCKS might participate in microcephaly with pontine and cerebellar hypoplasia [10]. MARCKS is also involved in the development and progression of cancer and the hyperphosphorylated MARCKS affects cell proliferation, migration, drug sensitivity being associated with a poor prognosis in cancer patients. In the case of cholangiocarcinoma, phosphorylated MARCKS promotes cell migration, while unphosphorylated MARCKS promotes cell attachment thus modulating the disease prognosis [11]. In bladder carcinoma, MARCKS is hyper-phosphorylated and modulates metastasis formation [12] while in breast and lung cancer, increased phosphorylated MARCKS promotes the proliferation, invasion, and possibly resistance to chemotherapy [13–17]. Similarly, hyperphosphorylated MARCKS contributes to tumorigenesis and invasiveness of kidney [18], pancreas and skin [19–21], colon [22], and hepatocellular [23] cancers. MARCKS expression is increased during liver tumorigenesis [24] and mouse colitis associated with a worse outcome [25]. Finally, the stromal MARCKS hyperexpression contributes to activating cancer-associated fibroblasts in epithelial ovarian cancer [26].

During inflammation, MARCKS promotes the migration of inflammatory cells and the secretion of cytokines [27] mediated by PKC [28], particularly in macrophages [29]. MARCKS also modulates neutrophil migration and adhesion [30–32] by interfering with phosphorylation, actin-bundling, and cytoplasm translocation [33,34].

2. MARCKS and cell functions

The M_r "87 k" unique Ca^{++}/phosphoprotein was first discovered in nerve terminals from rat cerebral cortex in 1982, where it modulated neurotransmission, and was later renamed MARCKS [4] based on evidence obtained from the bovine brain as [35].

MARCKS is found as a monomer or dimer, regardless of its phosphorylation status and regulates the intracellular signaling cascades via translocation and reorganization, while influencing the localization of multiple signal message molecules or complexes, thereby regulating cell polarity and domain-relative function [36].

MARCKS is an acidic, rod-shaped ubiquitous protein [37]. MARCKS and MARCKSL1 are negatively charged at neutral pH with an isoelectric point at approximately pH 4.4 while the effector domain is rich in positively charged residues (lysine), phenylalanine, and serine susceptible to phosphorylation [37] (Table 1, Fig. 1). The analysis of the chicken [38], bovine [39], mouse [40], rat [41], and human MARCKS cDNA sequences [42] demonstrated that MARCKS is abundant in amino acids alanine and glutamic acid. Conversely, MARCKS is not found in agnaths and invertebrates [43]. The human MARCKS gene (*MARCKS*) maps to chromosome 6q21, while the *MARCKSL1* gene is located at chromosome 1p34 [44,45]. Both genes have multiple transcription factor binding sites within their promoters, lacking a TATA box in the case of MARCKS. Southern blot analysis in mice indicated that there is a single copy of the MARCKS gene per haploid genome [43]. Tumor necrosis factor alpha (TNF-α) stimulates the transcription of the human MACS gene in HL60 and U937 cells, with a 30-fold increase in mRNA levels seen after 2 h of stimulation [42]. The amino acid sequence analysis of the human MARCKS reveals the presence of a novel conserved domain in the C-terminal region compared to other species [35].

The MARCKS and MARCKSL1 mRNAs are present in the brain and spinal cord during the early embryonic development while mice heterozygous for MARCKS exhibit normal phenotype [6], with spatial learning being the only impaired function [46]. On the other hand, studies in genetic depletion murine models have revealed that MARCKS and MARCKSL1 are both necessary for embryogenesis [6,45,47–49].

The highly conserved effector domain is the phosphorylation site which can interact with both PKC and or Ca^{2+}/calmodulin [50] (Fig. 1) and contains four potential phosphorylation sites [50,51] and an actin-filament-binding site [52] (Table 1, Fig. 2). Characterization of the four MARCKS phosphorylation sites as the prominent cellular substrate for chicken and bovine PKC revealed that all harbor a highly conserved domain with 25 identical amino acid residues [53]. MARCKS phosphorylation has been demonstrated in oligodendroglial progenitors [54], HIR 3.5 cells [55], and Swiss 3 T3 fibroblasts [56].

MARCKS modulates the actin plasticity interaction with the plasma membrane thus participating in cytoskeleton regulation [57], and the anchoring of MARCKS to actin and calmodulin is finely modulated. The binding of MARCKS to actin filaments is disrupted by either phosphorylation, causing its translocation from the plasma membrane to the cytoplasm, or binding to calcium/calmodulin (Fig. 2). It has been hypothesized that MARCKS modulates the pathways involving downstream effector molecule, actin

[57], and the activation of this pathway leads to the unbinding of actin from MARCKS; the resulting increased plasticity with softening actin cytoskeleton, promotes actin-related cell bioactivities, such as chemotaxis and motility, cell adhesion, migration, secretion, phagocytosis, exocytosis.

During the quiescent status, MARCKS is anchored to the plasma membrane via a electrostatic interaction of the N-terminal domain [58]. MARCKS is highly expressed in the nervous tissue and spleen, moderately in the heart and kidney and, less in the liver [40]. Several mitogenic agents may reduce MARCKS expression, indicating that its downregulation might be essential for the cell cycle [59]. In macrophages, activated PKC phosphorylates the membrane MARCKS to support the cytoskeleton [60] while in response to inflammatory stimuli, such as bacterial lipopolysaccharide (LPS) and TNF-α, MARCKS influences neutrophil migration [61]. Murine macrophage express MARCKS which is regulated by LPS [40] while MARCKS negatively modulates the LPS signaling [28]. MARCKS is distributed primarily in the substrate-adherent surface of pseudopodia and filopodia in quiescent macrophage [60] where its phosphorylation by PKC prevents MARCKS from proteolytic degradation [62]. Membrane-bound or overexpressed MARCKS significantly impact the lateral mobility of B cell receptors (BCR) while the formed reduces mouse primary splenic B cells in vitro and in vivo [63] ultimately regulating BCR signaling [64]. Conversely, our group could not detect MARCKS expression in T cells (*unpublished data*).

A growing amount of evidence supports the role of the effector domain of MARCKS [65], the human MARCKS gene [42], and MARCKSL1 adducin [66]. MARCKS is phosphorylated by calmodulin [51], and its subcellular distribution is demonstrated in transformed 3 T3 cells [67] as well as in models of neurotransmitter release modulation [68].

MARCKSL1 is attached to the vesicular phospholipid membrane [69] while MARCKS modulates endothelial cell proliferation [70], influences dual-directional protein translocation [71] and there is a computational model for the electrostatic sequestration of PI(4,5)P2 by membrane- tethered MARCKS [72]. In the case of the liver and the biliary tract, choleretic and cholestatic agents activate the intracellular signaling kinases, including phosphoinositide-3-kinase (PI3K) and PKC which then regulate MARCKS [73] and secondary bile acids promote MARCKS translocation [74], while MARCKS phosphorylation modulates taurolithocholate (TLC)-induced cholestasis [75] and its hyperexpression is observed in the hepatic ischemia/reperfusion injury [76].

3. MARCKS domains

The comparison of the MARCKS proteins from different species (including bovine, mouse, chicken, and human) reveals three highly conserved domains. These three domains include an *N-terminal myristoylated domain* that binds to the membranes, a highly conserved Homology 2 (MH2) domain, and an effector domain acting as a phosphorylation site [57]. The N-terminal domain includes 14 amino acids with a consensus sequence of GAQFSKTA recognized by N-myristoyl transferase at the glycine residue [77], mediating the interaction

of MARCKS with the plasma membrane through the insertion of the N-terminal myristoyl moiety into the hydrophobic lipid bilayer [78]. N-terminal myristoylation takes place not only during translation but also at post-translation [79], with the myristoylation site undergoing a reversible co-translational attachment of myristic acid to its N-terminal glycine residue [80]. The *MH2 domain* can interact with actin or contains a potential dimerization motif [81].

Next to the N-terminal and the MH2 domains, the previously mentioned MARCKS *effector domain* (KKKKKRFSFKKSFKLSGF SFKKNKK) [42] has an overall charge of +13 where lysine residues are concentrated at both ends and likely contribute to an extended effector domain conformation via electrostatic repulsion. The multiple serine residues are susceptible to phosphorylation by protein kinase C (PKC), or other protein kinases, such as Rho kinase (ROCK) [82–86], and the Ser 166 is the most bioactive [87]. The MARCKS effector domain is also unusually enriched with 5 phenylalanine (F) residues and three out of 4 serines (positions 152, 156, and 163 in the mouse) known to be phosphorylated by classical and novel isoforms of PKC [87–89]. The highly positively charged effector domain is less than 10% α-helical and does not form any secondary structure, regardless of extensive phosphorylation [88]. The MARCKSL1 effector domain is very similar but contains a significant replacement of Ser-97 with a proline residue, which has important implications both for the conformation of the effector domain and its net charge upon phosphorylation. Besides PKC and Ca2+/calmodulin, the highly conserved effector domain also interacts with the PI3K-AKT signaling pathway (Fig. 3), BCR, and TLR signaling pathways. Moreover, the MARCKS effector domain could also induce polymerization of G-actin, via the N-terminus five continuous ranked lysine of the effector domain and could be reversed by calmodulin binding or PKC phosphorylation [90] (Fig. 4).

4. MARCKS membrane binding and functions

MARCKS has been identified as an essential modulator of plasma membrane phosphoinositide, nuclear localization regulator, and cytoplasm, such as phagosomes [91], and filamentous actin cytoskeleton [92]. Phosphatidylinositol 4,5-bisphosphate (PI (4,5) P2, also named PIP2), is a vital lipid second messenger, which include the diacylglycerol (DAG) and IP3 (which activate protein kinase C, promote intracellular calcium releasing, respectively), and other signaling poly phosphoinositides, like PI(3,4,5)P3 [93,94]. PIP2 acts as a docking site and as a direct activator of numerous membrane proteins. MARCKS proteins binding to membrane require both the hydrophobic insertion into the bilayer of the N-terminal myristate, and electrostatic interaction with lipids by the effector domain (Fig. 2). Immunofluorescence studies demonstrated that MARCKS and PIP2 colocalize in the nucleus indicating that the MARCKS effector domain is pivotal for regulation of PIP2 traffic to the nucleus and for downstream gene expression [95]. The MARCKS effector domain and the accumulating actins at PIP2- containing rafts are both essential for actin cytoskeleton construction. The overexpression of wild-type MARCKS (WT) and deletion of effector domain MARCKS in a glioblastoma multiforme (GBM) cell line demonstrate that MARCKS-WT localizes to the nucleus, while in the absence of the effector domain MARCKS remains in the cytoplasm. Intriguingly, the over-expression of MARCKS-WT is associated with an increase of total cellular PIP2, implying MARCKS modulates PIP2.

Both myristoylation of the N-terminus and phosphorylation of the phosphorylation site domain by PKC induces a change in affinity for Ca2+/calmodulin, indicating that it may play a role in calcium-dependent signaling by sequestering calmodulin in the plasma membrane until activation by PKC induces exocytosis of the secondary messenger (Fig. 2). The N-terminus of calmodulin interacts with the C-terminal lobe of the effector domain, and the binding of MARCKS to the plasma membrane locally sequesters the phosphatidylinositol-4,5-diphosphate (PIP2) pool in quiescent cells [96], MARCKS sequesters (PIP2) at lipid rafts in the plasma membrane, as proven by the electrostatic interactions between phosphorylation site domain of MARCKS and PIP2 [97]. The electrostatic sequestration of PIP2 could be reversed either by binding to the effector domain mediated with calcium/ calmodulin or by phosphorylation of the effector domain via PKC, which both decrease the positive electrostatic potential [98]. Bundling of MARCKS with PIP2 prevents phospholipase C (PLC)-δ or PLC-β hydrolyzing PIP2, likely via interaction with the catalytic site [99,100]. Subsequently, the release of sequestered PIP2 locating at the lipid rafts promotes cell motility [101,102], cell migration mediated by accumulated MARCKS. It has been hypothesized that the abundant membrane bundled bound MARCKS would enrich more PIP2 sequestered at plasma membrane while the PKC-dependent phosphorylation displaces membrane-attached MARCKS, and dephosphorylation leads to its relocalization to the membrane [52]. Nuclear magnetic resonance (NMR) and circular dichroism have elaborated that calmodulin bundling does not induce a secondary conformation in the effector domain [103,104]. A specific MARCKS peptide (N-KKKKKRFSFKKSFKLSGFSFKKNKK-C) at 10–100 nM can inhibit the hydrolysis of PIP2 mediated by PLC-β or -δ through strong binding of the peptide to PIP2 [100].

Several other proteins contain shared MARCKS homology region and could potentially interact with PIP2; these include MARCKSL1, diglyceride kinase-ζ (DKG-zeta), adducin, neuromodulin (GAP43), Drosophila A kinase anchor protein 200 (DAKAP200), phospholipase D (PLD), gelsolin, N-WASP, and the *N*-methyl-D-aspartate receptor [105]. The translocation of diacylglycerol kinase (DGK) - ζ (zeta) to the nucleus relies on the phosphorylation of the effector domain of MARCKS.

5. MARCKS in cancer

The ability of MARCKS to induce cellular migration underlies its effects on regeneration and cancer [106]. The hyperexpression of MARCKS facilitates angiogenesis and growth in vivo in a renal cell carcinoma (RCC) xenograft carcinoma while a reduced expression, both in RNA and protein levels, is observed in high-grade RCC cell lines ex vivo causing alleviation of proliferation and migration. The abrogation of MARCKS via MPS (MARCKS effect domain) peptide modulates the effects of regorafenib, an oral *multikinase* inhibitor, and influences the survival of kidney cancer cells through mitigating bioactivation of AKT and mTOR [18]. MARCKS promotes cell proliferation via the ErbB2- mediated signal pathway and cell polarity proteins [36,107]. ErbB2 overexpression induces astrocytes to dedifferentiate and revert to the progenitor status [108].

Data from translational research show the role of MARCKS in numerous cancers and it is potentially of prognostic value. In primary liver cancer, the transcription factor Zic family

member 2 (ZIC2) is highly expressed in liver tumor-initiating cells and proves essential for the disease perpetuation. The long noncoding RNA (lncRNA) lncZic2 locates in proximity to the ZIC2 locus and is upregulated in liver cancer and liver tumor-initiating cells, and promotes the production of MARCKS and (MARCKSL1. The LncZic2 then drives BRM/SWI2-related gene 1 (BRG1) transcriptional regulator to the promoters of the MARCKS and MARCKSL1 gene. Depletion of lncZic2 and BRG1 down regulates MARCKS and MARCKSL1. lncZic2 is essential for the self-renewal of liver T tumor-initiating cell ICs through up-regulating MARCKS and MARCKSL1 gene via BRG1 [24]. In the case of lung cancer, the phosphorylation status of the plasma membrane-anchored protein MARCKS is vital in the response to radiation; the unphosphorylated status of the effector domain is associated with a better response to ionizing radiation therapy. A549, H1792, and H1975 lung cancer cell lines treated with MARCKS effector domain peptide manifest reduced phosphorylation of MARCKS and Akt serine/threonine kinase 1, and MPS treatment could inhibit proliferation of lung cancer cell and increase radiation sensitivity. MARCKS effector domain peptide regulates MARCKS phosphorylation and modulates its function [109].

When comparing MARCKS protein expression between inflammatory and non-inflammatory breast cancer samples, inflammatory samples show an 18% rate of MARCKS-positivity (i.e. stained cells ≥1%), and MARCKS expression is also upregulated (36% vs. 11% in non- inflammatory samples). In inflammatory breast cancer, MARCKS over-expression is also associated with poor metastasis-free survival and prognosis [14]. In prostate cancer, MARCKS is upregulated in biochemically recurring cases (i.e. patients with at least a 0.2 mg/dl increase in prostate specific antigen levels), compared with non-recurrent ones; silencing of MARCKS attenuates the migration and invasion ability of prostate cancer cells, by downregulating the MMP9 mRNA expression, and cell spreading in contrast with the increased cell-cell adhesion [20]. Comparing MARCKS expression in normal versus epithelial ovarian cancer samples through immunohistochemistry, 75% of normal samples are positive for epithelial MARCKS staining versus 50% of tumor samples. Intriguingly, stromal MARCKS expression is upregulated in tumor samples (77%) compared to normal samples (22%), being correlated with shorter overall survival [26]. Studies on the expression of MARCKS in other types of cancer showed the association of glioma with the protein reduced phosphorylation inhibiting the PI3K/AKT pathway [110] or its phosphorylation associated with reduced cell proliferation in choroidal melanoma [111].

The impact of MARCKS in cancer is further supported by miRNA studies. In osteosarcoma, MARCKS is upregulated and inversely correlates with miR-34c-3p while being a direct target of miR-34c-3p, and when overexpressed, partly reversing the effects of this miRNA [112].

6. MARCKS and immune-mediated diseases

As previously discussed, MARCKS is highly expressed in macrophages and its inhibition blocks the LPS-induced expression of TNF-α while also affecting the macrophage transmigration [33,34]. The treatment of macrophages with phosphorylation site domain peptides, which prevent MARCKS phosphorylation at the N-terminus sequence, abolishes LPS-induced expression of TNF-α through inhibition of p38 and JNK MAPKs and NF-κB.

The same peptides in mice decrease the TNF-α and IL-6 serum levels and are associated with a 40% improved survival after a lethal dose of LPS, likely based on the decreased cytokine production in macrophages [32]. In the case of neutrophils, MARCKS influences the migration and adhesion [31] as well as the production of inflammatory cytokines [113,114]. Decreased binding of MARCKS to the plasma membrane leads to increased cell proliferation in both B-cell tumor and mouse primary splenic B cells in vitro, as also observed by transfer experiments in vivo. The importance of MARCKS in the modulation of the immune function is supported by both clinical and basic research in B-cell chronic lymphocytic leukemia (B-CLL) [115].

Similar to what has been discussed for cancer, there is a direct correlation between MARCKS immune effects and miRNA changes. During an acute infection, miR-21 regulates phagocytosis while increased MARCKS and the Rho–B, as in in miR-21(− /−) bone marrow-derived macrophages, correlate with augmented uptake of Listeria. The intra-peritoneal injection of *Listeria monocytogenes* causes higher bacterial invasiveness of the liver tissue in miR-21(− /−) mice compared to WT mice [116]. In the case of ulcerative colitis, dextran sulfate sodium DSS- induced colitis model compared to normal colon tissues points at the downregulation of miR-429. Among the 41 genes influenced by miR-429, MARCKS is a direct target gene, being downregulated both in mRNA and protein levels. The same miR-429 regulates the expression of MARCKS and MARCKS-associated mucin secretion both in DSS-induced colitis and colorectal cells, and targeting miR-429 upregulates MARCKS expression in colorectal cell lines. Further, miR-429 regulates mucin secretion in human colorectal cells and mouse colitis tissues through up- regulating MARCKS expression [25].

Data on the role of MARCKS in autoimmune diseases are limited to rheumatoid arthritis. Data from the collagen-induced arthritis model demonstrated that histone deacetylase 1 (HDAC1)is overexpressed and miR-124 and MARCKS poorly expressed in synovial tissues while the silencing of HDAC1 reduces synovial hyperplasia and inflammation by elevating MARCKS and miR-124 both in vitro and in vivo in a fashion with is hypothesized to be mediated by the JAK/STAT signaling pathway [117], a therapeutic target in rheumatoid arthritis [118]. As previously noted, the translocation of DGK-ζ to the nucleus relies on the phosphorylation of the effector domain of MARCKS and this pathway has also been proposed as a therapeutic target in numerous human diseases, including autoimmunity [119].

7. MARCKS and neurological disorders

MARCKS is highly expressed in neurological tissues, especially during embryogenesis and subsequent developmental phases as MARCKS is central to hemisphere fusion, neurulation, forebrain commissure formation, cortical development, and retinal laminations commissure formation [6,47]. The disruption of the MARCKS gene in mice leads to numerous neural tube defects [45] and perinatal death is invariably observed in MARCKS-deficient pups which also exhibit a characteristic lamination disorder of the cortex and retina. On the other hand, the MARCKSL1 gene deletion results in the suppression of the cranial neural tube closure with anencephaly [48,49].

In adult neurological diseases, phosphorylation of MARCKS is associated with neuron degeneration at the early stage of Alzheimer's disease, while extracellular Aβ aggregates are observed at histology. MARCKS is also a cross-talking modulator between lipid raft and endocytosis in the process of amyloid-β peptide production, which may represent a new therapeutic target for Alzheimer's disease treatment [120]. The expression of MARCKS is altered in dementia with Lewy bodies and Parkinson's disease both in mouse models and human tissues. The increase in the level of pSer46-MARCKS is observed before α-synuclein aggregate formation, while human α-Syn-BAC-Tg/GBA- hetero-KO mice demonstrate no abnormalities during aging, consistent with the pattern in human post mortem brains. Pre-aggregation neurite degeneration is present in Alzheimer's disease, dementia with Lewy bodies and Parkinson's disease [121]. Patients with microcephaly with pontine and cerebellar hypoplasia were studied by mutational search of CASK and screening of candidate genes via SNP array, targeted resequencing, and whole-exome sequencing [10]. Of note, MARCKS is one of the genes has been associated with microcephaly with pontine and cerebellar hypoplasia. MARCKS-related increase in free PIP2 is also reported to be involved in the endocytosis signaling, and mediates endocytosis through cross-talking with Neural Wiskott-Aldrich Syndrome Protein (N-WASP) [122].

8. Concluding remarks

Numerous unanswered questions remain from the available evidence on the MARCKS family, especially based on the limited understanding of the mechanisms by which MARCKS alterations produce specific phenotypes. As an example, we have only a partial understanding of the mechanisms by which knocking out the MARCKS genes results in perinatal and not intrauterine death. In the case of immunity, we observe that MARCKS expression is found primarily in macrophages and neutrophils while being virtually absent or found only marginally in T and B cells, two cell populations which are crucial for embryo development and aging. While some lines of evidence have been proposed linking MARCKS with autoimmune diseases, particularly rheumatoid arthritis, we may hypothesize that modulating this intracellular pathway may be beneficial to treat chronic inflammation, particularly in conditions where JAK inhibition is a viable therapeutic option [123]. We also envision that the role of MARCKS in cancer may be better defined, particularly by resolving the conflicting evidence from expression studies in specific cancers and elucidating the MARCKS signaling networks. In the neurological area, MARCKS influences *endo*- and exocytosis, neurite outgrowth, and synaptic plasticity with numerous clinical implications which should be better characterized mechanistically.

In conclusion, the current understanding of MARCKS well illustrates the common ground among complex areas such as immunology, oncology, and neurology and highlights the need for a multidisciplinary approach with the aim to translate basic science into clinical and therapeutic implications.

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Fig. 1.

The MARCKS protein includes three highly conserved domains: *i.* the N-Terminal domain, which can be myristoylated (Myr); *ii.* the MH2 domain (MH2D), and *iii.* The effector domain, highly positively charged with 4 potential phosphorylation sites.

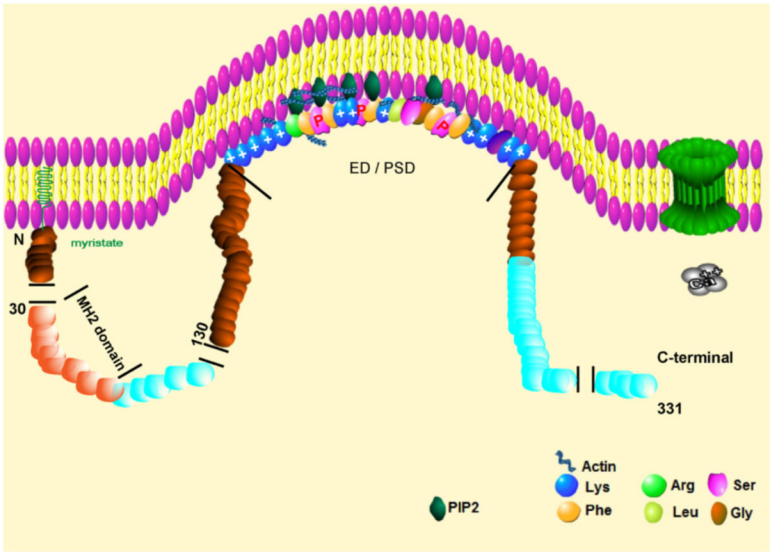

Fig. 2.

In the quiescent status and in the absence of calcium/calmodulin binding, MARCKS is tethered to the membrane by the N-terminal and effector domains.

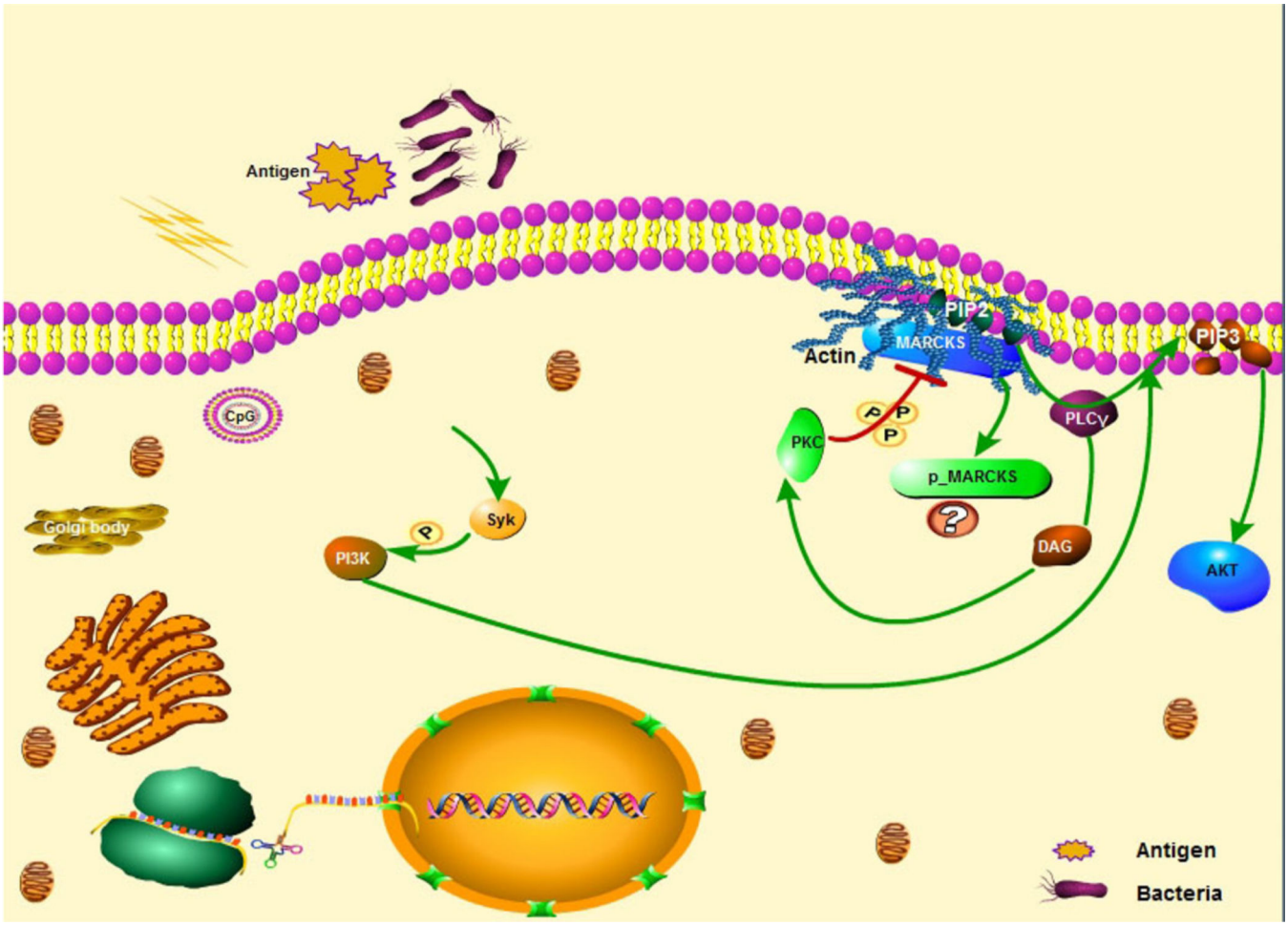

Fig. 3.

MARCKS and PIP2 colocalize in the nucleus indicating that the MARCKS effector domain is pivotal for regulation of PIP2 traffic to the nucleus and for downstream gene expression. The PI3K-AKT signaling and PIP2 mediated actin skeleton pathways of MARCK are illustrated, along with the positive feedback loop of PKC-MARCKS-PIP2-PLCγ-DAG.

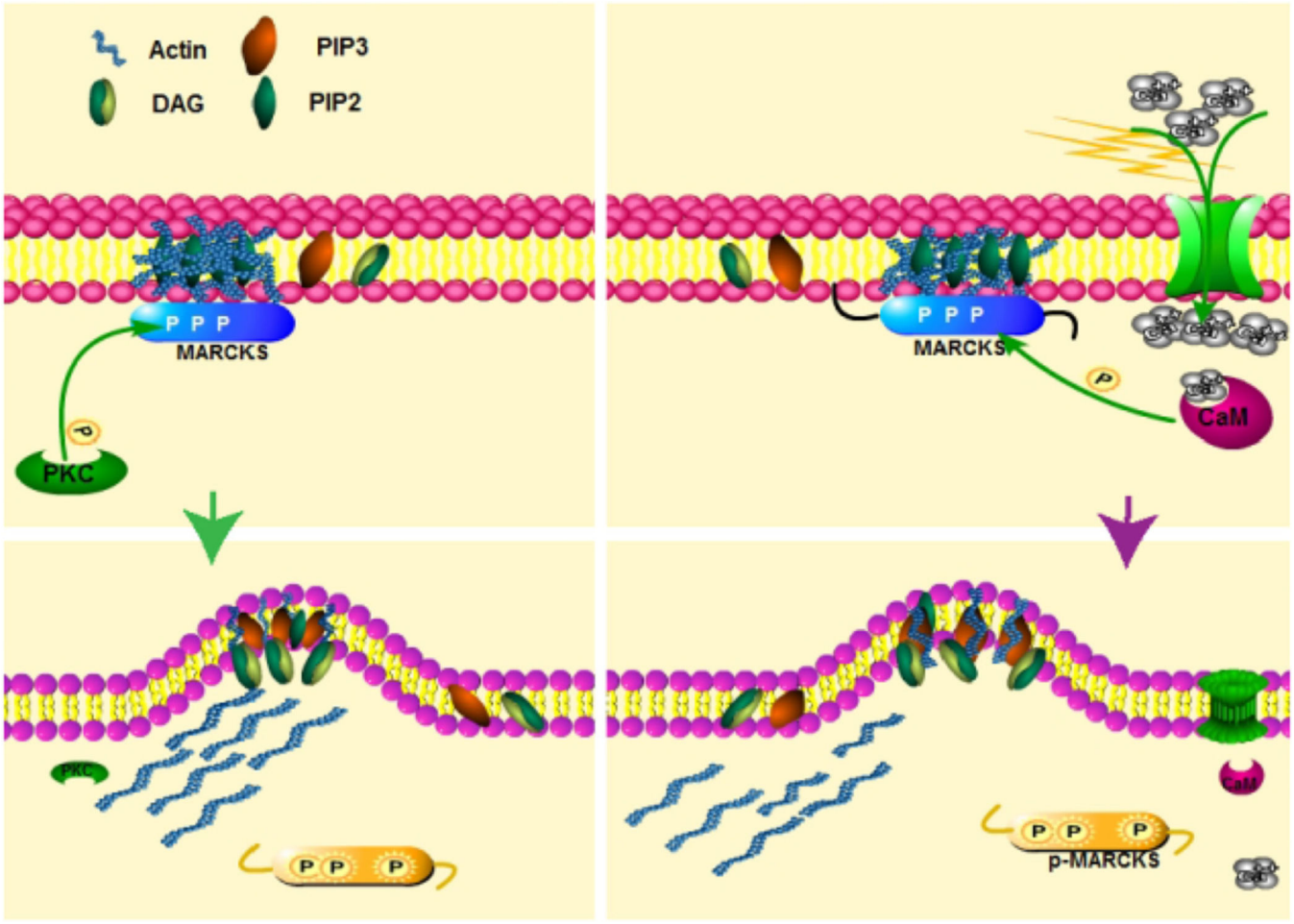

Fig. 4.

A proposed model for the modulated phosphorylation of MARCKS via activated PKC and/or $Ca2^{+}$-calmodulin (CaM). In this time-dependent mode, PKC activity predominates before $Ca2^{+}$ reaches its peak (Left panel) while once the $Ca2^{+}$ peak is reached, $Ca2^{+}$-CaM interacts with MARCKS and prevents its PKC-mediated phosphorylation (Right panel); in both cases leading to the release of PIP2 and actin.

Table 1

Amino acid sequences of the effector domains of MARCKS, MARCKS-like peptides, and the corresponding polybasic domains of potential cross-talking modular proteins. NCBI-protein (www.ncbi.nlm.nih.gov/protein) accession numbers are listed.

Protein (residues)	NCBI/Protein ID	Effector domain sequence	Reference
Human MARCKS (152–176)	P29966.4	KKKKKRFSFKKSFKLSGFSFKKNKK	[42]
Human MARCKSL1 (87–110)	P49006.2	KKKKKFSFKKPFKLSGLSFKRNRK	[45]
Bos taurus MARCKS (151–175)	AAI15990.1	KKKKKRFSFKKSFKLSGFSFKKNKK	[42]
Chicken MARCKS (117–141)	P16527.2	KKKKKRFSFKKSFKLSGFSFKKNKK	[42]
Mouse MARCKS (145–169)	P26645.2	KKKKKRFSFKKSFKLSGFSFKKSKK	[42]
DGK-ζ (259–273)	Q13574.4	KKKKRASFKRKSSKK	[124–127]
α-Adducin (717–734)	P35611.2	KKKKKFRTPSFLKKSKKK	[66,128,129]
DAKAP200 (119–141)	NP_001285753.1	KSKSKKDKVKKKWSFRSISFGKK	[130]
GAP43 (30–56)	P17677.1	KAHKAATKIQASFRGHITRKKLKGEKK	[131,132]
N-WASP (181–197)	BAA20128.1	NISHTKEKKKGKAKKKR	[133,134]
PLD2 (553–574)	O14939.2	RDLARHFIQRWNFTKTTKAKYK	[135]

Abbreviations: DKG-ζ, Diacylglycerol kinase zeta; DAKAP200: Drosophila A kinase anchor protein 200; GAP43, growth-associated protein of M_r 43,000; N-WASP, neuronal Wiskott-Aldrich Syndrome protein; PLD, phosphatidylcholinespecific phospholipase D.