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NON-THEMATIC REVIEW

Myristoylated alanine-rich C kinase substrate (MARCKS): a multirole signaling protein in cancers

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Abstract Emerging evidence implicates myristoylated alanine-rich C-kinase substrate (MARCKS), a major substrate of protein kinase C (PKC), in a critical role for cancer development and progression. MARCKS is tethered to the plasma membrane but can shuttle between the cytosol and plasma membrane *via* the myristoyl-electrostatic switch. Phosphorylation of MARCKS by PKC leads to its translocation from the plasma membrane to the cytosol where it functions in actin cytoskeletal remodeling, Ca^{2+} signaling through binding to calmodulin, and regulation of exocytic vesicle release in secretory cells such as neurons and airway goblet cells. Although the contribution of MARCKS to various cellular processes has been extensively studied, its roles in neoplastic disease have been conflicting. This review highlights the molecular and functional differences of MARCKS that exist between normal and tumor cells. We also discuss the recent advances in the potential roles of MARCKS in tumorigenesis, metastasis, and resistance to anti-cancer therapies, with a focus on addressing the inconsistent results regarding the function of MARCKS as a promoter or inhibitor of oncogenesis.

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1 Introduction

The myristoylated alanine-rich C-kinase substrate (MARCKS) is a membrane-associated protein originally identified as a major target of protein kinase C (PKC) [1]. Its roles in cellular processes such as cytoskeletal control, chemotaxis and motility, mediation of the inflammatory response, secretion and exocytosis, neurological function, and development have thus far been well-established. Given that some of these processes are often dysregulated in neoplastic disease and co-opted by tumor cells to support their growth, proliferation, and invasion, it is reasonable to expect that MARCKS would play a role in tumorigenesis and metastasis. While a growing body of work points to the role of MARCKS as an effector protein in the processes leading to neoplastic growth and subsequent metastasis, the nature of this role is yet to be elucidated. Due to intrinsic differences in the tissue origin and phenotype of the cancer cells, in certain malignancies, MARCKS appears to suppress the growth and invasion of cancer cells, while in others, it appears to promote these functions.

In this review, we discuss the current state of the body of knowledge on the role of MARCKS in tumorigenesis, metastasis, and resistance to anti-cancer therapies, focusing on the molecular mechanisms by which MARCKS causes the phenotypic changes driving these phenomena in order to possibly understand the discrepancies in the experimental results found in the literature. We also review the current advances made in the use of MARCKS as a druggable target for cancer. Finally, we briefly consider future directions for investigations of MARCKS based on recent studies, including its role in

antineoplastic resistance and its potential as a biomarker and therapeutic target.

2 Protein structure and localization

MARCKS is a 32kDa, acidic protein that adopts a non-helical, non-globular, unstructured shape both in solution and when complexed with calmodulin. It has three regions conserved by amino-acid sequence: an *N*-terminal domain, an MH2 domain, and a phosphorylation site domain (PSD, also known as the effector domain or ED) (Fig. 1) [2].

The *N*-terminal domain can be myristoylated; this modification is thought to be co-translational rather than post-translational in the vast majority of cases. The myristoyl group is embedded in the membrane. The PSD is highly basic and contains four serine residues on which it can be phosphorylated, although only three of these are commonly used as phosphorylation sites [1]. MARCKS protein phosphorylated at Ser159 and Ser163 (phospho-MARCKS) has been widely studied in inflammatory disease, and it is this form of phosphorylated MARCKS that will be most relevant to our discussion. Additionally, the PSD can associate with calmodulin in a calcium-dependent manner. Both myristoylation of the *N*-terminus and phosphorylation of the PSD by PKC induce a change in affinity for Ca^{2+}-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 [1, 3, 4].

When its PSD is unphosphorylated or unassociated with calmodulin, MARCKS is localized to the plasma membrane, where it is anchored to the phospholipid bilayer *via* the *N*-terminal domain. Phosphorylation of the PSD or association of the PSD with calmodulin causes MARCKS to dissociate from the plasma membrane and translocate to the cytoplasm [5]. Co-translational myristoylation of the *N*-terminus is thought to increase the affinity of the *N*-terminus through hydrophobic interactions between the myristoyl group and the hydrocarbon portion of the membrane, while phosphorylation of the PSD neutralizes the domain's positive charges and decreases the affinity between MARCKS and the phospholipid bilayer [1]. The mechanism by which the association of calmodulin with the PSD induces desorption of MARCKS from the plasma membrane is still not well-understood [6, 7].

Interactions between MARCKS and the plasma membrane are complex, likely involving a combination of independent electrostatic interactions between the phospholipid bilayer and both the *N*-terminal myristoyl group and the PSD motif. The dissociation of MARCKS from the plasma membrane is reversible; MARCKS has been shown to shuttle back and forth between the membrane and the cytoplasm [8, 9], and this shuttling has at least one known physiological function [10, 11].

3 Major Binding Partners in Cellular Functions

As its name suggests, MARCKS is a primary PKC substrate, although in certain situations, the MARCKS PSD is also phosphorylated by the Rho kinase (ROCK) [12–15]. MARCKS has been implicated as a downstream effector in various processes including cytoskeletal control, regulation of the cell cycle, chemotaxis and motility, mediation of the inflammatory response, secretion and exocytosis, muscle spreading, neurological function, and development. Under ordinary physiological conditions, these different processes are

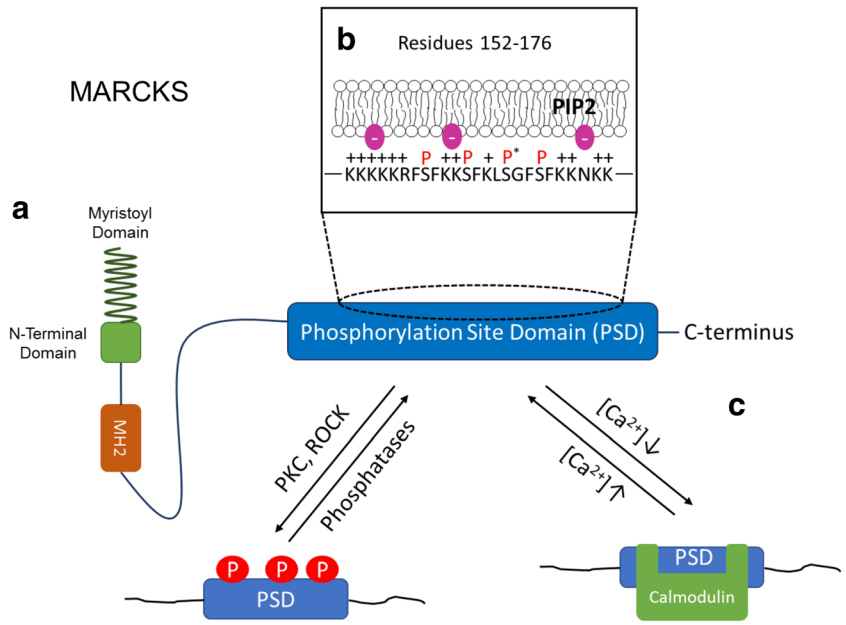

Fig. 1 **a** MARCKS protein, a 32-kDa protein, consists of three conserved regions: a myristoylated *N*-terminal domain, an MH2 domain, and a phosphorylation site domain (PSD). **b** MARCKS sequesters PIP_2 through electrostatic interactions. The PSD can be readily phosphorylated at four serine residues, although the third serine (*) is poorly phosphorylated. The domain can be phosphorylated by PKC and ROCK kinases. **c** The PSD plays important roles in cell signaling and can be regulated by PKC through phosphorylation and calmodulin through calcium dependent binding

controlled by different isoforms of PKC, which are differentially expressed and have varying affinities for MARCKS in different tissues [16–20].

Detailed molecular interactions of MARCKS with downstream signaling pathways have been characterized in a number of cases (Fig. 2). MARCKS sequesters phosphatidylinositol-4,5-diphosphate (PIP_2) in the plane of the cell membrane, *via* electrostatic interactions between PIP_2 and the MARCKS PSD [21]. Binding of MARCKS to PIP_2 inhibits the hydrolysis of PIP_2 by phospholipase C (PLC)-δ or PLC-β possibly by competitive inhibition of the catalytic site [22, 23]. This interaction allows PKC (and possibly calmodulin) [24] to control levels of PIP_2 and by extension the secondary messengers and products of PIP_2 hydrolysis, inositol triphosphate (IP_3) and diacylglycerol (DAG). This control of PIP_2 in turn allows MARCKS to regulate cell-signaling pathway effectors including phospholipase D (PLD) [25, 26] and phosphoinositide 3-kinase (PI3K) [27, 28], the latter of which activates AKT signaling *via* the phosphorylation of PIP_2 to PIP_3.

When localized to the plasma membrane, MARCKS cross-links F-actin in a PIP_2-dependent manner [26, 28]; when phosphorylated and released into the cytosol, no such interactions occur [29, 30], suggesting a role in control of the cytoskeleton. Although MARCKS is known to interact directly with F-actin *via* its effector domain, Laux et al.'s investigation of the mechanism, currently the most thorough, points to indirect regulation of cortical actin *via* sequestration of PIP_2, thereby interfering with its ability to sequester downstream effector proteins that do interact directly with cytoskeletal proteins [31]. Additionally, the studies on the binding of MARCKS to actin suggest a close relationship of MARCKS with the cytoskeletal machinery responsible for directed cell migration [32].

Several well-documented functions of MARCKS involve its association with and control of cytoskeletal actin. Among the best studied is its role in secretion, particularly mucin secretion in airway epithelial cells. The mechanism appears to involve the control of cytoskeletal actin by MARCKS, as shown in Fig. 2. Once in the cytoplasm, MARCKS is dephosphorylated by cGMP-dependent protein kinase (PKG), freeing its effector domain to interact with cytoskeletal actin. According to the proposed mechanism by Li et al., MARCKS links secretory granules to the actin and myosin of the cytoskeleton to allow transportation of the granules and subsequent exocytosis [33]. Control of cell motility and chemotaxis is another such function and has been established in fibroblasts [34], neutrophils [32], hepatic stellate cells [35], and macrophages [36], among others. The mechanism of action appears to involve the sequestration of PIP_2 at lipid rafts in the cell membrane. Two separate reports have both demonstrated that release of PIP_2 sequestered at the cell membrane increases cell motility [19, 37], a conclusion supported by Dedieu et al.'s study, which shows that myoblast migration is reduced by accumulation of MARCKS (which would presumably increase the amount of PIP_2 sequestered at the membrane) [38]. After activation of PKC by chemoattractant stimulation [9], MARCKS is phosphorylated and releases sequestered PIP_2, possibly allowing PIP_2 to itself sequester proteins regulating the formation of dynamic structures by the cortical cytoskeleton for protrusion and movement [31]. A study by Chen and Rotenberg on the role of MARCKS in melanoma metastasis presents another possible mechanism, likely

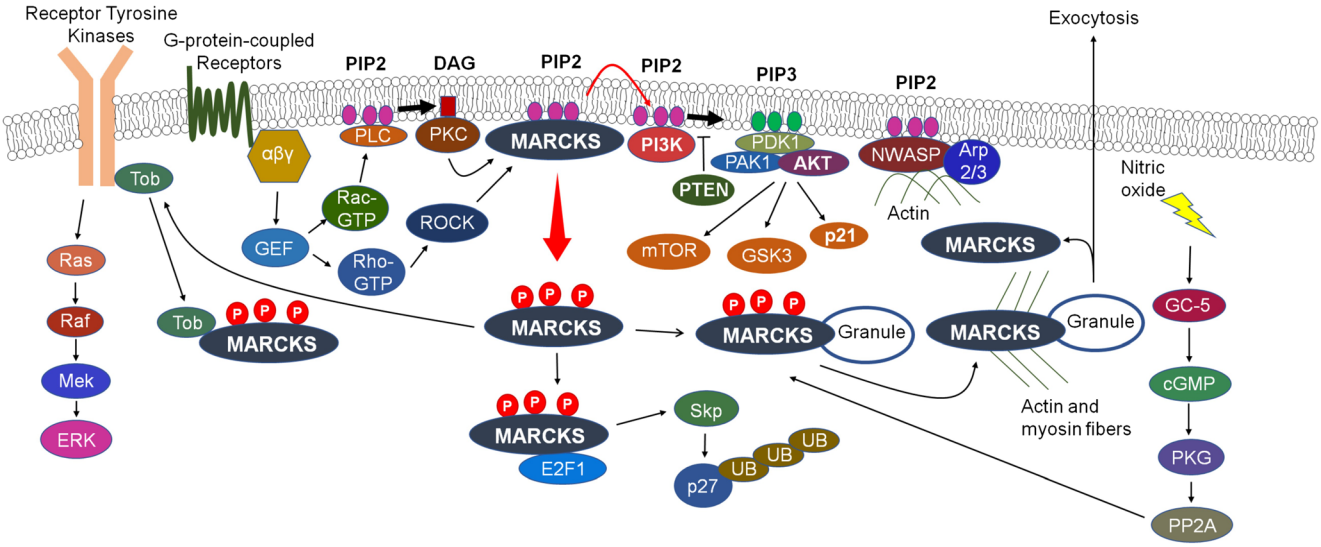

Fig. 2 MARCKS mediates various cellular processes and thereby plays important roles in cytoskeletal control, cell cycle regulation, inflammation, secretion/exocytosis, cell migration, and cell survival. The PI3K/AKT pathway in particular is dysregulated in response to MARCKS phosphorylation. Phosphorylation of MARCKS in cancer leads to prolonged PIP_3 signaling due to the loss of ability to sequester PIP_2 phospholipid pools. Exposure of PIP_2 on the membrane allows PI3K to phosphorylate it to PIP_3, which can activate AKT-mediated signaling

supplementing rather than opposing the one above: namely, after release from the plasma membrane, phosphorylated MARCKS also interacts with cytoplasmic proteins controlling cytoskeletal dynamics [39].

MARCKS also mediates the inflammatory response of both macrophages and neutrophils. This function is a result of the role of MARCKS in both chemotaxis and secretion, as MARCKS is involved in the migration of macrophages and neutrophils as well as secretion of inflammatory cytokines [40–43]. As MARCKS-mediated motility is induced by phosphorylation of the protein, it would be reasonable to assume that the MARCKS-mediated inflammatory response, affected by the motility of inflammatory leukocytes, would be dependent on phosphorylation of MARCKS. Indeed, experimental evidence indicates that the role of MARCKS in inflammation is associated with its phosphorylation [19, 40].

4 Contributions to tumorigenesis and metastasis

4.1 Growth and proliferation

Given that many of the cellular processes MARCKS is involved in are dysregulated in neoplasms, aberrant MARCKS signaling may participate in the malignant transformation, sustained growth, uncontrolled proliferation, motility, and invasion of tumor cells (Table 1). Studies have shown differentially expressed levels of MARCKS between healthy and tumor tissues, but different studies show inconsistent results as to the direction of the effect of MARCKS on cancer cell growth. MARCKS has been implicated in oncogenesis in lung cancer [58–62], cholangiocarcinoma [44], breast cancer [48–50], renal cell carcinoma (RCC) [56], pancreatic cancer [63], and hepatocellular carcinoma (HCC) [57]. In contrast, an early review connected MARCKS with suppression of proliferation; indeed, this has been the case in glioma [46], colorectal cancer [52], small-intestine adenocarcinoma [55], and melanoma [54].

The discrepancies among the different studies as to the role of MARCKS in tumorigenesis can be explained by the abundance of MARCKS phosphorylation, not just protein expression. In light of MARCKS' functionality in PIP_2 binding and accumulation [67, 68], higher levels of unphosphorylated MARCKS may result in the sequestration of PIP_2 and thereby suppress PIP_2-mediated signaling such as the PI3K/AKT and PLD pathways. A closer look at the results of these various investigations appears to support this possibility. Our studies of the role of MARCKS in lung, breast, and kidney cancers have shown that increased level of phospho-MARCKS is what contributes to cancer cell survival and proliferation [48, 56, 60], in agreement with a recent report [69]. Upregulation of MARCKS is able to inhibit the PI3K/AKT pathways as long as MARCKS is not phosphorylated and still binds to the membrane, where MARCKS sequesters PIP_2 and reduces the availability of PIP_2 to PI3K. Once MARCKS is phosphorylated at the PSD, PIP_2 pools gathered by unphosphorylated MARCKS are released and available for PI3K to activate AKT-mediated signaling, leading to cell growth and proliferation (Fig. 2). These dual roles of MARCKS may explain the differential effects of MARCKS (unphosphorylated *vs.* phosphorylated state) on PIP_2 and PIP_3 levels as well as on AKT activation.

Jarboe et al's study on the role of MARCKS in glioma provides further evidence for our proposition that MARCKS *per se*, not phospho-MARCKS, inhibits the PI3K/AKT pathway, although they tested only for gene expression of MARCKS [46]. Likewise, MARCKS expression, not levels of phospho-MARCKS, was found to be correlated with decreased cell proliferation in choroidal melanoma [54]. Furthermore, in Techasen *et al*'s study of cholangiocarcinoma, carcinogenesis was shown to be dependent on PKC, indicating that it is the phosphorylated form of MARCKS that is responsible [44]. We conclude that an upregulation of MARCKS would not necessarily contribute to tumorigenesis—but an increase in MARCKS phosphorylation would (Fig. 2).

Phosphorylation of MARCKS has been shown to contribute to proliferation of breast cancer cells by at least one additional mechanism. Ordinarily, the protein Tob suppresses proliferation by binding to and negatively regulating the effects of the receptor tyrosine kinase ErbB-2, part of the epidermal growth factor family. Phosphorylated MARCKS, however, binds to Tob, decreasing the protein's affinity for ErbB-2 and thus dampening its inhibitory effects [49]. Apart from its inhibition of Tob, a probable mechanism by which MARCKS induces tumorigenesis is activation of the neutrophil- and macrophage-mediated inflammatory response. MARCKS-induced carcinogenesis in cholangiocarcinoma is shown to be driven by inflammation [44, 70]. The MARCKS-mediated inflammatory response is activated by phosphorylation of MARCKS [41], further supporting our postulation that it is phospho-MARCKS that induces conditions favorable to cancer growth and proliferation, while unphosphorylated MARCKS inhibits these conditions.

4.2 Motility and invasion

The roles of MARCKS in chemotaxis and cytoskeletal control point to its possible function in metastasis. As with tumorigenesis, because it is the phosphorylated form of MARCKS that promotes cell motility and protrusion, there is a possibility that elevated levels of phospho-MARCKS compared to unphosphorylated MARCKS tend to potentiate cell motility and invasion. Upregulation of MARCKS expression without an accompanying increase in phosphorylation would attenuate the metastatic phenotype. Several studies lend credence to this

Table 1 Role of MARCKS phosphorylation forms in various cancers

Cancer	Expression	Phosphorylation	Proliferation/ tumorigenesis	Migration/ invasion/ metastasis	Angiogenesis	Drug/ radiation resistance	Prognosis	Comments	Ref.
Bile duct	Increased	Yes		+			Worse	Unphosphorylated MARCKS promotes cell attachment.	[44]
Bladder	Increased	Yes		−				Hyperphosphorylation of MARCKS	[45]
Brain	Deceased	No	−	+		−	Improved	Attenuation of MARCKS promotes proliferation and radiation resistance.	[46, 47]
	Increased	Yes						MARCKS increases glioma invasion.	
Breast	Increased	Yes	+	+		+	Worse	Phosphorylation of MARCKS increases binding to Tob; attenuation of MARCKS reduces angiogenesis, cell motility, and increases paclitaxel sensitivity.	[48–51]
Colon	Deceased	No					Worse	MARCKS overexpression increases sensitivity to apoptosis.	[52]
	Increased	Yes	+	+				MARCKS potentiates CRC metastasis.	[53]
Eye	Decreased	No	−					Unphosphorylated MARCKS reduces cell proliferation.	[54]
Intestine	Decreased	No						Reduced MARCKS expression promotes microsatellite instability.	[55]
Kidney	Increased	Yes	+	+	+	+	Worse	Attenuation of MARCKS decreases cell proliferation, migration, and enhances regorafenib sensitivity.	[56]
Liver	Increased	Yes						MARCKS overexpression in HCC	[57]
Lung	Increased	Yes	+	+		+	Worse	Increased levels of phospho-MARCKS associated with advanced disease and PI3K/AKT activity; attenuation of phospho-MARCKS reduces cell invasion, migration, metastasis, and erlotinib/radiation resistance.	[58–62]
Pancreas	Increased							MARCKS secretion in serum is significantly elevated in cancer.	[63]
Prostate	Increased	Yes		+			Worse	Attenuation of MARCKS decreases migration, invasion, and increased cell adhesions.	[64]
	Decreased	No		+				MARCKS is a direct target of miR-21.	[65]
Skin	Increased	Yes		+				Phospho-MARCKS drives cell motility.	[39]
	Increased	No		−				Overepxression of MARCKS stabilizes focal adhesions.	[66]

+ promote, − inhibit

notion, showing that phospho-MARCKS is associated with invasiveness in lung cancer [59, 60], glioma [47], breast cancer [48, 50, 51], cholangiocarcinoma [44], melanoma [39], colon cancer [53], and prostate cancer [64]. Further supporting the possibility of the opposing roles of unphosphorylated MARCKS and phospho-MARCKS in malignancy, some

studies have shown that phosphorylation of MARCKS promotes disassembly of dynamic adhesions and cell motility in melanoma [39, 66], and a report shows that inhibition of MARCKS expression by microRNA-21 promotes invasion in prostate cancer [65]. However, it should be noted that hyperphosphorylation of MARCKS was demonstrated to reduce invasiveness in bladder cancer [45].

Detailed mechanisms of MARCKS-mediated tumorigenesis and metastasis involving upstream regulators of MARCKS have not yet been elucidated, but our current knowledge of the role of PKC in oncogenesis and cancer progression provides hints as to the likely interactions. As mentioned earlier, the various isoforms of PKC are differentially expressed in different tissues under normal physiological conditions. Studies of PKC in colorectal and breast cancer have shown that expression of different PKC isoforms vary among normal, primary-tumor, and distant-metastasis tissue [71, 72], and we propose that upregulation of certain isoforms may lead to over-phosphorylation of MARCKS in these and possibly other types of cancer.

5 An emerging role of MARCKS in resistance to antineoplastic therapy

Although the role of MARCKS in various anti-cancer therapies is still sparsely documented, a growing body of work indicates that it may be involved in resistance to certain types of cytotoxic chemotherapeutic agents and targeted therapies as well as to radiotherapy. MARCKS phosphorylation status has been considered as a predictor for poor outcomes and for the response of cancer cells to tyrosine-kinase inhibitors (TKIs) such as erlotinib [60] and regorafenib [56]. In view of its role in the regulation of cellular PIP_2 and PIP_3 levels, MARCKS signaling may be an alternative pathway and act as a critical regulator for the crosstalk of signaling between receptor tyrosine kinases and the PI3K/AKT pathways [47, 56, 60]. In addition to resistance to TKIs, phospho-MARCKS is associated with reduced radiosensitivity of lung cancer cells [62, 69]. Studies on the role of MARCKS in resistance to radiotherapy and the mechanisms thereof have shown that increasing the levels of unphosphorylated MARCKS or blocking phosphorylation of MARCKS on its PSD decreases cell survival by inhibiting DNA-repair pathways [46, 62, 69]. Of note, targeting the PSD of MARCKS protein has been shown to increase sensitivity to both EGFR-TKIs and radiation in lung cancer [60, 62, 69].

The molecular interactions underlying resistance to radiotherapy and a few types of targeted therapy have been characterized, but those involved in resistance to cytotoxic chemotherapy have yet to be elucidated. In our study of the role of MARCKS in triple-negative breast cancer (TNBC), down-regulation of phospho-MARCKS was shown to attenuate resistance of TNBC to a specific class of mitotic inhibitors, taxanes, but not to chemotherapeutic agents that interact directly with DNA [48]. The mechanism by which phospho-MARCKS confers taxane resistance to TNBC cells is most likely mediated by both Src and PI3K/AKT signaling pathways, which are known to be involved in cell survival. Furthermore, MARCKS has also been shown to confer resistance to a type of targeted therapy in multiple myeloma (MM). Inhibition of phospho-MARCKS was reported to sensitize MM cells to the 20S proteasome inhibitor bortezomib by targeting Skp2 *via* E2F1 and subsequently inducing p27-mediated cell-cycle arrest and apoptosis [73, 74]. Given that AKT signaling has been linked to both increased tumor-cell survival by activating DNA-repair mechanisms [75], attenuating apoptosis mechanisms [76], and driving PD-L1–mediated immune resistance [77], these studies provide strong evidence for the hypothesis that phospho-MARCKS enhances therapeutic resistance by increased activation of the PI3K/AKT pathway.

6 Targeting MARCKS in anti-cancer therapy

The role of MARCKS in critical pathways in multiple cancer types makes it a rational and attractive target for cancer therapy; numerous studies have shown it to be essential for tumor-cell survival and differentially expressed between healthy and tumor tissues. Since phosphorylated MARCKS appears to be the form of the protein that exerts pro-tumor effects, the phosphorylation process or phospho-MARCKS itself is a logical target for therapy (Fig. 3). Despite the growing body of pre-clinical evidence demonstrating MARCKS to be a feasible therapeutic target, however, clinical studies on the inhibition of MARCKS or its phosphorylation are scarce and have not produced particularly noteworthy improvements in clinical cancer treatment. PKC, for example, the major kinase that phosphorylates MARCKS, would be a reasonable target for the inhibition of MARCKS phosphorylation, but clinical trials of PKC inhibitors as monotherapies have had lackluster results so far [78]. MARCKS itself may therefore be a more effective target in the clinic; furthermore, targeting MARCKS rather than PKC would reduce the chances of unwanted side effects from the inhibition of PKC's other substrates.

Although the crystal structure of its effector domain has not been experimentally determined or at least made publically available, MARCKS has no enzymatic activity and is therefore unlikely to contain a hydrophobic, substrate-binding pocket that would serve as a docking site for a small-molecule inhibitor. Currently, the most promising methods of targeting MARCKS are RNA-mediated inhibition (RNAi) and short peptides targeted to the MARCKS PSD, although all studies on such prospective therapies have been pre-clinical.

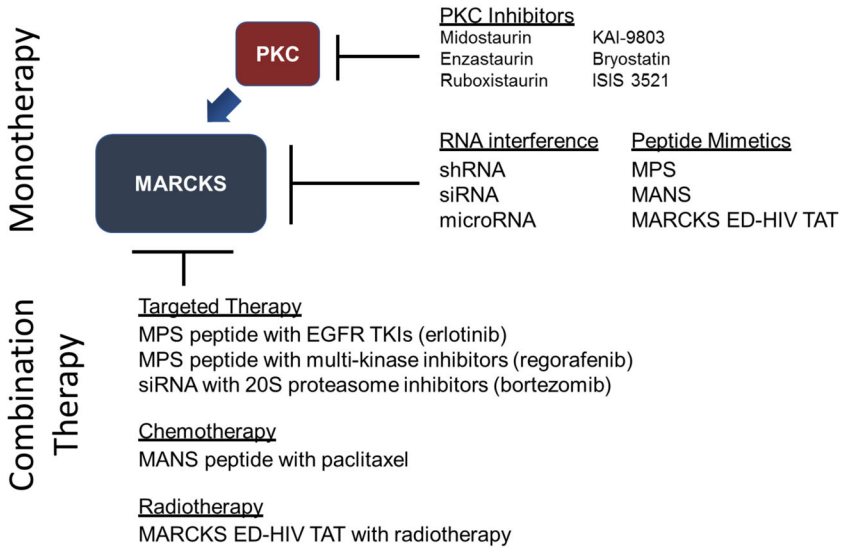

Fig. 3 MARCKS-targeting therapies in cancer. MARCKS inhibition by PKC inhibitors, RNA interference, and peptide mimetics suppresses tumor growth and metastasis and increases the efficacy of tyrosine kinase inhibitors, chemotherapy, and radiation therapy

Yang and colleagues' studies on phospho-MARCKS in MM and Jarboe and colleagues' work in glioma have demonstrated a potential for the use of siRNA or shRNA, respectively, in targeted therapy, while two separate groups have identified mimetics of the microRNA miR-34c-3p as a possible therapy for osteosarcoma and HCC [74, 79–81]. Three peptides targeting MARCKS have been developed so far, with one (the MANS peptide) targeting the myristoylated *N*-terminal domain and the others preventing phosphorylation of the PSD. One of the PSD peptidomimetics, MPS, was developed by our group and shown to suppress tumor growth and metastasis and increase sensitivity to erlotinib in non-small-cell lung cancer (NSCLC), decrease survival of RCC cells and increase their sensitivity to regorafenib, and overcome bortezomib resistance in MM [82]. The other PSD peptidomimetic, a MARCKS ED-HIV TAT peptide developed in the laboratory of Christopher Willey, was also shown to sensitize NSCLC cells to radiation therapy [69, 83]. As noted above, targeting of PKC as a monotherapy has had limited success in the clinic, and it is reasonable to expect that targeting of MARCKS will also be more effective when combined with other therapies. Our group's studies of NSCLC and RCC suggest that MARCKS inhibition may be particularly effective when combined with TKI therapy, although loss of MARCKS expression or function has been shown across several studies to sensitize different tumor types to not only targeted therapies but chemotherapy and radiation therapy as well.

Despite the favorable results seen in pre-clinical investigations, various difficulties may hinder the translation of these benchwork findings into clinically applicable treatments. In general, the major impediments to the clinical implementation of RNAi therapies include off-target effects, inherent instability of RNA molecules, difficulty of delivery, and adverse immunological reactions [84, 85]. One notable, MARCKS-specific example of the first is noticed in studies on the microRNA miR-34c-3p: although the results of Song et al. showed that miR-34c-3p reduced HCC proliferation and invasion by targeting MARCKS, Xiao and colleagues' experiments suggested that miR-34c-3p promotes those phenotypes in HCC by targeting NCKAP1 [86]. While these data present no challenge to and in fact support our own conclusions on the role of MARCKS in cancer, they present a possible situation in which use of that miRNA targets a contraindicated pathway, limiting the potential of that microRNA in the clinic. Indeed, the off-target effects of microRNAs are largely due to the fact that they often target multiple genes [87]. These limitations are not specific to miRNA; shRNA and siRNA also face the abovementioned challenges, and the difficulty of implementing RNAi therapeutics in the clinic is reflected in the fact that only six such therapies have secured FDA approval as of 2017 [88]. Peptides as well have their own challenges in making the bench-to-bedside transition. Perhaps most relevant for those targeting MARCKS, an intracellular protein, is the fact that native peptides do not readily cross the cell membrane [89]. Except for MPS, most MARCKS-targeting peptides require modifications such as myristoylation (MANS) or additional sequences (MARCKS ED-HIV TAT) to increase the peptides' membrane permeability or facilitate their transport into the cell. A major hurdle in bringing MARCKS-targeting cancer therapeutics into the clinic is the fact that to date, unmodified peptides tend to have short *in vivo* half-lives (2–30 min) and are typically limited to intravenous administration [89, 90]. Although the current body of research presents only a proof-of-concept validation of MARCKS as a therapeutic target, it is nonetheless solid evidence and strong support for conducting studies of the pharmacokinetics and pharmacodynamics of MARCKS inhibitors as the next step in delivering these therapies to the clinic.

7 Concluding remarks

Although observations connecting MARCKS to tumor malignancy are not novel, seemingly conflicting results and a dearth of detailed studies on the underlying mechanisms have made the exact role of MARCKS in neoplastic disease unclear. A growing body of work now indicates MARCKS as a phosphorylation-controlled negative regulator of major signaling pathways that are often dysregulated in cancer and points to its possible roles as a therapeutic target and prognostic biomarker in solid tumors and certain hematological malignancies. Targeting MARCKS appears to have potential in combination therapy as a method of attenuating chemo- and radiotherapeutic as well as targeted-therapy resistance. Studies on the role of MARCKS in resistance to other types of targeted therapies are still limited, presenting directions for future investigations. MARCKS also shows promise as a biomarker for therapeutic resistance and overall prognosis. This role, however, has not been thoroughly investigated, and continued research on this topic is needed. Furthermore, discovery of the specific molecular interactions driving MARCKS-mediated chemotherapeutic and targeted-therapy resistance is imperative for clinically applicable treatment strategies. While we have presented here a plausible mechanism based on a systematic review of the literature, our hypotheses have yet to be validated experimentally, and the specific components of the downstream pathways involved are yet to be identified. Additionally, the mechanisms contributing to MARCKS hyperphosphorylation are also poorly characterized and are another option for research on treatments for MARCKS-mediated pathophysiology in neoplastic disease.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interests.

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