TRANSLATIONAL REVIEW

MARCKS and Lung Disease

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Abstract

MARCKS (myristoylated alanine-rich C kinase substrate) is a prominent PKC substrate expressed in all eukaryotic cells. It is known to bind to and cross-link actin filaments, to serve as a bridge between Ca^{2+}/calmodulin and PKC signaling, and to sequester the signaling molecule phosphatidylinositol 4,5-bisphosphate in the plasma membrane. Since the mid-1980s, this evolutionarily conserved and ubiquitously expressed protein has been associated with regulating cellular events that require dynamic actin reorganization, including cellular adhesion, migration, and exocytosis. More recently, translational studies have implicated MARCKS in the pathophysiology of a number of airway diseases, including chronic obstructive pulmonary disease, asthma, lung cancer, and acute lung injury/acute respiratory distress syndrome. This article summarizes the structure and cellular function of MARCKS (also including MARCKS family proteins and MARCKSL1 [MARCKS-like protein 1]). Evidence for MARCKS's role in several lung diseases is discussed, as are the technological innovations that took MARCKS-targeting strategies from theoretical to therapeutic. Descriptions and updates derived from ongoing clinical trials that are investigating inhalation of a MARCKS-targeting peptide as therapy for patients with chronic bronchitis, lung cancer, and ARDS are provided.

Keywords: MARCKS; lung disease; inflammation; cancer

Properties of MARCKS Protein

Structure

MARCKS binds and cross-links actin, bridges calmodulin (CaM) and PKC signaling, and sequesters phosphatidylinositol 4,5-bisphosphate (PIP_2) in the plasma membrane (1–6). Associated with cellular events that require actin, including adhesion, migration and exocytosis (7–21), it is involved in pulmonary disorders such as chronic obstructive pulmonary disease (COPD), asthma, acute respiratory distress syndrome (ARDS) and lung cancer (12, 17, 20–23). In 1982, Wu and colleagues described an "87 kD" PKC substrate protein that is known today as "MARCKS" (6, 24). MARCKS has an actual molecular weight of 32 kD, but it was first described as 87 kD owing to its apparent molecular weight on SDS-PAGE gels (6). This anomalous migration behavior is due to its extended, nonglobular conformation and weak binding of SDS molecules to this highly acidic protein (2). MARCKS family proteins are comprised of MARCKS and MARCKSL1, also known as MacMARCKS, MRP (MARCKS-related protein), MLP (MARCKS-like protein), and F52 (brain protein F52) (25). MARCKS is ubiquitously expressed, whereas MARCKSL1 is expressed primarily in brain, reproductive tissue, and macrophages (1, 26).

MARCKS belongs to a class of "natively unfolded" proteins known for having very little secondary structure and no hydrophobic core (27). MARCKS and MARCKSL1 have three evolutionarily conserved amino acid domains. The first is a 24–amino acid sequence at the N-terminus, which includes an N-terminal myristoylation in which myristic acid, a C14 saturated fatty acid, is linked via an amide bond to the N-terminal glycine. The myristic acid moiety inserts hydrophobically into lipid bilayers to stabilize membrane binding of MARCKS (28). The second conserved region is the MH2 (multiple homology 2) domain. The function of the MH2 domain is currently unknown, but it contains the only known intron in the genes for MARCKS and MARCKS family proteins (29, 30). The final conserved region is located at the center of the protein and is known as the effector domain (ED; amino acid residues 151–175). The ED is lysine rich with four serine residues and a highly basic charge. The positively charged lysines play a key role in electrostatic interactions between the ED and negatively charged phospholipids in membranes, and they likely contribute to

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the "unfolded" conformation of the protein due to electrostatic repulsion (2). The four serine residues provide a site for phosphorylation by conventional and novel isoforms of PKC (31). The ED is also the site of CaM and actin binding (29, 32–34).

Binding Interactions

Myristoyl-electrostatic switch. In most resting cells, MARCKS is anchored to the plasma membrane through membrane interactions with both the N-terminal myristoyl moiety and the positively charged lysine residues of the ED. The myristate associates hydrophilically with the inner leaflet of the plasma membrane (28), and the basic ED associates electrostatically with negatively charged phospholipids, namely PIP_2 (35). Both the myristoyl moiety and the lysine-rich ED are required for MARCKS to optimally associate with the plasma membrane (36–38). When cells receive signals that activate conventional or novel PKCs, MARCKS is phosphorylated at the ED, and the electrostatic interaction with PIP_2 weakens, leading to MARCKS translocation from the plasma membrane to the cytosol (16, 18, 39–42). After dephosphorylation, MARCKS can return to the cell membrane (10, 37, 43) or target other intracellular membranes (44, 45), depending on the cell type. This fundamental framework relating cellular activation signals to reversible MARCKS membrane binding was first described by Graff and colleagues (6) and Hartwig and colleagues (3) and was later termed the "myristoyl-electrostatic switch" by McLaughlin and Aderem (36).

PIP_2. PIP_2 is continuously present in the plasma membrane of most mammalian cells at an effective concentration of approximately 10 μM, comprising about 1% of the total lipids (46). PIP_2 regulates numerous cellular functions by serving as a precursor for production of lipid second messengers, including diacylglycerol (DAG), inositol 1,4,5-trisphosphate (IP_3), and phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) (47); acting as a "lipid" anchor for actin-binding proteins (48); and activating membrane proteins, such as ion channels and transporters (49, 50).

In the resting state, MARCKS binds to PIP_2 in the plasma membrane, thereby sequestering it and keeping it unavailable for hydrolysis by phospholipase C (PLC). Upon cell stimulation and phosphorylation of MARCKS by PKC, or a rise in intracellular Ca^{2+}/CaM, MARCKS desorbs from the membrane, allowing PLC to hydrolyze PIP_2 and produce a measurable burst of IP_3 and DAG (51). Computational modeling, as well as research in large unilamellar vesicles and lipid monolayers, reveals that the MARCKS ED forms an electroneutral complex with approximately four PIP_2 molecules (52–54). The functional significance of MARCKS–PIP_2 binding is supported by several lines of evidence. First, MARCKS and PIP_2 are present at similar concentrations (~10 μM) within cells (26, 55). Second, overexpression of MARCKS increases the amount of PIP_2 present within cell membranes (44, 56). Third, similar to reports by Glaser and colleagues on MARCKS–PIP_2 binding and PLC, recent studies in both lipid bilayer systems and living cells show that MARCKS ED blocks PIP_3 production by the lipid kinase PI3Kα owing to sequestration of its substrate, PIP_2 (44, 56–58). In glioblastoma multiforme cells, the MARCKS ED is capable of regulating nuclear membrane PIP_2 with downstream effects on gene transcription (44). These findings, together with evidence from *in vitro* single-molecule and cell migration models, provide definitive evidence of MARCKS/ PIP_2 binding and support PKC and Ca^{2+}CaM regulation of MARCKS as an important mechanism used by cells to control the localization, availability, and downstream cell signaling of PIP_2 (46, 52, 53, 57–63).

Actin. Interest in MARCKS as an actin-binding protein first emerged when Hartwig and colleagues determined that both MARCKS and a synthetic peptide corresponding to the MARCKS ED directly bind to and cross-link F-actin and that this cross-linking is diminished when PKC phosphorylates, or when Ca^{2+}/CaM binds, MARCKS at the ED (3, 64, 65). MARCKS has two actin-binding sites in the ED (66), and both MARCKS and MARCKSL1 ED peptides induce rapid polymerization of G-actin and bundling of F-actin (64, 67). The current theory is that nonphosphorylated membrane-bound MARCKS stabilizes the cytoskeleton of quiescent cells by cross-linking F-actin, and cellular activation signals that cause PKC-mediated MARCKS phosphorylation or Ca^{2+}/CaM binding displace MARCKS to the cytosol while also decreasing actin cross-linking, leading to areas permissive of cytoskeletal reorganization (5, 56, 58, 68). Although significant evidence still points to MARCKS as an important actin-binding protein, recent theories regarding MARCKS regulation of actin-dependent processes such as cellular migration have shifted toward MARCKS regulation of PIP_2 availability as a mechanism for coordinating signals, including Ca^{2+}CaM, PKC, PI3K, and DAG, to generate dynamic cytoskeletal reorganization (58).

CaM. The same MARCKS ED that serves as the site for PKC phosphorylation also binds Ca^{2+}/CaM. Nonphosphorylated MARCKS binds CaM (when complexed with Ca^{2+}) with high affinity (6, 69–71). Although it was initially proposed that the MARCKS ED assumes an α-helix conformation consistent with many known CaM-binding domains (6, 72), later studies using circular dichroism, nuclear magnetic resonance, and X-ray crystallography confirmed that MARCKS binds Ca^{2+}/CaM in a nonhelical, extended conformation, making MARCKS family proteins among a novel class of CaM-binding proteins (73–76). Ca^{2+}/CaM binding to MARCKS is disrupted by PKC-dependent phosphorylation (2, 3, 5, 6, 14, 37, 69–71, 76–79).

Role in Cellular Function

Evidence for MARCKS's role in fundamental biological processes is extensive and ranges from events guiding embryogenesis, such as gastrulation (80) and neural tube closure (81); to reproductive functions, such as oocyte maturation (82); to neuronal functions, including neurite and axon outgrowth (56, 83), dendrite branching (84), and maintenance of memory (85). In addition to roles in health and homeostasis, MARCKS family proteins are also essential for growth and survival, because knockout of either MARCKS or MARCKSL1 in mice leads to embryonic fatality owing to abnormal brain and central nervous system development (86–88). Although a complete description of the numerous biological functions of MARCKS is beyond the scope of this review, evidence for MARCKS's role in cellular events relevant to the pathophysiology of inflammatory, allergic, and neoplastic airway diseases is presented.

Before reviewing the roles of MARCKS in respiratory/pulmonary disease, it is worthwhile to briefly describe technologies that have been developed to investigate MARCKS function in living cells and

Table 1. MARCKS Translational Research Summary: Model Types and Significant Findings

	Model/Cell Type	Investigational Strategy to Inhibit MARCKS Function	Significant Finding(s) for MARCKS Function	References
Acellular models	Computational models	ED peptide	MARCKS sequesters PIP_2 through electrostatic interactions	53
	Lipid models	ED peptide	MARCKS sequesters PIP_2/PIP_3, regulates PIP_2 availability as PI3K substrate	54, 60, 61
Cells *in vitro*	Neutrophils	N-terminal peptide (MANS), BIO-11006	MARCKS involved in directed migration, adhesion, respiratory burst, cytokine production, and degranulation	8, 21, 93, 94, 100
	Macrophages	MANS, siRNA	MARCKS involved in directed migration and cytokine production	13, 96
	Mast cells	ED peptide	MARCKS involved in degranulation	130
	Fibroblasts	MANS, siRNA	MARCKS involved in directed migration	15, 16
	Stem cells	MANS	MARCKS involved in directed migration	95
	Airway goblet cells	MANS, BIO-11006	MARCKS involved in mucin secretion	18, 92, 97, 100, 108–111, 113–115
	Vascular smooth muscle cells	siRNA, mutated protein expression	MARCKS involved in adhesion and $\alpha_5\beta_1$ integrin function	138
	Endothelial cells	siRNA	MARCKS involved in directed migration	63, 139
	Lung cancer cells	MANS, siRNA, BIO-11006, ED peptide, mutated protein expression	NSCLC cells: elevated p-MARCKS expression, MARCKS involved in cancer cell migration, MARCKS enhances radiation sensitivity	12, 104, 141–143, 163
In vivo, animal models	Mouse mucus hypersecretion/allergic inflammation models	MANS, BIO-11006	MARCKS involved in airway mucin secretion and inflammation	17, 20, 98, 107
	Mouse ALI/ARDS models	BIO-11006, MANS	MARCKS pulmonary inflammation and ALI	22, 23
	Mouse lung cancer models	MANS, BIO-11006, ED peptide	MARCKS involved in metastasis and tumor growth	12, 99, 164
Human clinical trials	Healthy volunteers	Inhaled BIO-11006	Safe pulmonary and systemic profile	Clinical Trials BIM-CL-001, BIM-CL-002
	Chronic bronchitis	Inhaled BIO-11006: 172 subjects with COPD, double-blind, placebo-controlled, multicenter dose escalation study for 21 d	75 mg twice daily increased proportion of clinically significant FEV_1 response	136, Clinical Trial BIM-CL-003
	ARDS	Inhaled BIO-11006: 40 ventilated ICU patients, randomized, placebo-controlled trial	Expected 2019	Clinical Trial BIM-CL-005
	NSCLC	Inhaled BIO-11006: randomized, SOC controlled trial	Expected 2019	Clinical Trial BIM-NSCLC-001

Definition of abbreviations: ALI = acute lung injury; ARDS = acute respiratory distress syndrome; COPD = chronic obstructive pulmonary disease; ED = effector domain; ICU = intensive care unit; MANS = myristoylated N-terminal sequence; MARCKS = myristoylated alanine-rich C kinase substrate; NSCLC = non–small cell lung cancer; PIP_2 = phosphatidylinositol 4,5-bisphosphate; PIP_3 = phosphatidylinositol (3,4,5)-trisphosphate; p-MARCKS = phosphorylated MARCKS; SOC = standard of care.

organisms. As previously mentioned, knockout of MARCKS results in embryonic lethality in mice; therefore, adult knockout mice are unavailable for MARCKS investigations. Deletion studies have been limited to cells such as macrophages and megakaryocytes collected from bone marrow or liver of MARCKS-knockout mouse embryos (89, 90). Recently, investigators have used newer technologies such as CRISPR/Cas9 to conduct MARCKSL1 deletion studies in epithelial cells (91). In addition to these approaches, structural/functional analysis, targeted mutation, siRNA knockdown, cell fractionation, fluorescence microscopy, and other newer analytic techniques have been applied to studies of MARCKS.

However, before 2000, inhibition or alteration of MARCKS function relied on silencing MARCKS, mutating MARCKS, or altering MARCKS regulation by targeting phosphatases or PKC. In a breakthrough in 2001, Li and colleagues described a synthetic, myristoylated peptide identical to the first 24 amino acids of the N-terminus of MARCKS that inhibited mucin granule secretion in human airway epithelial cells (92). The sequence for this peptide, known as MANS (myristoylated N-terminal sequence) is MA-GAQFSKTAAKGEAAAERPGEAAVA (MA denotes myristic acid). The control peptide (random N-terminal Sequence [RNS]) contains the same amino acid composition as MANS but arranged in random order (MA- GTAPAAEGAGAEVK RASAEAKQAF). Because the N-terminus of these peptides contains a hydrophobic myristate moiety, these peptides are readily taken up by cells (8, 92). Since its discovery in 2001, the MARCKS-specific MANS has been used in numerous investigations of MARCKS function *in vitro* and *in vivo* (8, 12, 13, 15, 17, 20–22, 92–96). This method of MARCKS inhibition marked a turning point for investigators looking for ways to target this omnipresent protein and opened the door to development of a new class of airway therapeutics. The MANS peptide is not suitable as a drug because of its limited solubility, large size, and rapid hydrolysis by lung-lining fluid and blood, so attention was turned toward creating a smaller and more soluble peptide that encompasses the active site on MANS. After screening numerous peptides with amino acid and myristic acid substitutions, a peptide with a sequence encompassing the first 10 amino acids of the MARCKS N-terminus was developed. The solubility of this peptide was greatly enhanced by substituting an acetyl group for the myristic acid moiety. This peptide, called BIO-11006, is resistant to hydrolysis in airway lining fluid and has been shown to be equally as effective as MANS in attenuating mucus secretion, inflammation, and cell motility in several *in vitro* and *in vivo* systems (22, 23, 97–100). BIO- 11006 was developed as an inhaled aerosol drug by BioMarck Pharmaceuticals and is the basis for several clinical trials in different lung diseases, discussed below.

MARCKS inhibition has also been achieved with an ED (amino acids 151–175) peptide, known as the ED peptide, which has been described in numerous biophysical studies of MARCKS interactions with lipids (51, 52, 54). Solubility of this peptide, however, can be problematic (96, 101–104), consistent with its extremely basic nature, although this peptide can be modified to enhance cell permeability, such as by C-terminal addition of a tyrosine aminotransferase (105).

Reports on mechanisms of MARCKS inhibition afforded by these two different peptide inhibitors have varied, likely owing to differences in cell type and function. The MANS peptide seems to displace MARCKS from normal membrane associations, alter cytoskeletal dynamics, and decrease MARCKS phosphorylation (8, 12, 92). The ED peptide has been reported to decrease MARCKS phosphorylation, to directly bind and sequester lipopolysaccharide (LPS), and to act as a nuclear localization signal and regulator of nuclear membrane PIP_2, with downstream effects on gene expression (44, 105, 106).

MARCKS and Lung Disease

Mucin Secretion

Excess mucus secretion is a common lesion in patients with inflammatory airway diseases, predisposing to obstruction, infection, and even destruction of airway walls and contiguous tissues. As a result, cellular regulators of airway mucus production and secretion are attractive therapeutic targets. To date, both *in vitro* and *in vivo* evidence strongly supports a role for MARCKS in regulation of airway mucin (the solid component of mucus) secretion. Using an *in vitro* model system of well-differentiated normal human bronchial epithelial (NHBE) cells, Li and colleagues (92) showed that, consistent with other cell types, PKC activation led to MARCKS phosphorylation and translocation to the cytosol. They further showed that subsequent PKG (protein kinase G) activation resulted in protein phosphatase 2A–mediated MARCKS dephosphorylation and that downregulation of MARCKS protein, or inhibition of MARCKS phosphorylation/dephosphorylation, significantly inhibited mucin secretion. In addition, these investigators showed for the first time that treatment of NHBE cells with the MANS peptide significantly attenuated mucin hypersecretion in response to PKC activation. Using radiolabeled immunoprecipitation, these investigators determined that dephosphorylated cytoplasmic MARCKS associated with myosin and actin, and they hypothesized a role for MARCKS in actin-dependent mucin granule exocytosis (92). Further evidence for MARCKS's role in mucin granule exocytosis comes from an *in vivo* study in mice. Using ovalbumin-sensitized mice as a model for allergic inflammation and goblet cell metaplasia, Singer and colleagues showed that intratracheal MANS peptide pretreatment attenuated methacholine-induced mucin secretion into the airway lumen (20). Subsequent *in vivo* studies using intranasal administration in the same mouse model showed that MANS blocked mucin release and subsequently improved physiological measurements of airway obstruction, as determined by plethysmographic measures of airway conductance (17). In a model of mucin hypersecretion provoked by exposure of rats to the occupational pollutant and cigarette smoke component, acrolein, Chen and colleagues found that intratracheal administration of MANS blocked the secretory response (107). In each of the above-described studies, the RNS control peptide was without effect.

Although it is clear that MARCKS plays a significant role in airway mucin secretion, mechanisms of regulation are not fully understood. When NHBE cells were exposed to human neutrophil elastase (a possible model of mucin hypersecretion, as seen in chronic bronchitis), human neutrophil elastase–induced mucin release was shown to be dependent on a PKC-mediated mechanism, specifically the delta isoform of PKC (PKCδ) (108), which acted by phosphorylating MARCKS (18). Additional studies in NHBE cells showed

that two chaperone proteins—HSP70 (heat shock protein 70) and CSP (cysteine string protein)—were integral to MARCKS regulation of mucin secretion. Both of these chaperones appeared to form a complex with MARCKS and with mucin granule membranes. Inhibition of either chaperone attenuated mucin release by these cells (109). Additional morphological studies using fluorescent markers of MARCKS and HSP70 revealed tight physical associations between these two proteins that were enhanced when mucin secretion was stimulated with PMA (110). The aforementioned MARCKS–HSP70–CSP complex was shown to bind to the cytoskeleton, specifically an unconventional myosin isoform, myosin V (111), which is associated with moving intracellular vesicles and granules along actin filaments (112). When mucin granule membranes from NHBE cells were isolated via immune isolation techniques, the mucin granule membrane marker hCLCA1 (human calcium-activated chloride channel accessory 1), together with MARCKS, HSP70, CSP, myosin V, and protein phosphatases, was associated with mucin granule membranes, supporting the above-described paradigm (113). These data are consistent with a mechanism whereby MARCKS is released from its membrane attachment after it is phosphorylated by PKC and then goes into the cytoplasm, where, in concert with the chaperones HSP70 and CSP, it binds to both the cytoskeleton and mucin granule membranes and thereby regulates granule release (92, 109, 110).

Another explanation of MARCKS's role in mucin secretion points to actin as a barrier of mucin granule exocytosis and to MARCKS phosphorylation as a key step regulating the removal of that barrier upon cell stimulation. This hypothesis is based on observations reported by Ehre and colleagues (114) using SPOC1 cells, an immortalized rat tracheal epithelial cell line. Unstimulated SPOC1 cells have a subplasmalemmal sheet of β- and γ-actin between granules and the apical membrane, and the actin sheet disappeared when the cells were exposed to secretory agonists. In contrast, stabilization of the actin sheet inhibited mucin secretion. These investigators also showed, similarly to Li and colleagues, that MARCKS is phosphorylated and displaced from the membrane to the cytosol upon stimulation of goblet cells. They concluded that MARCKS phosphorylation/translocation is a key step in dissolving the apical actin that otherwise presents a barrier to mucus secretion in these cells (114).

There are numerous other proteins that could be involved in mucin exocytosis, such as SNAREs (SNAP receptor proteins), SNAPs (soluble *N*-ethylmaleimide-sensitive factor attachment proteins), and VAMPs (vesicle-associated membrane proteins) (115, 116). Exactly how MARCKS and these related proteins interact to regulate exocytotic release of mucin in airways has not been fully established. However, it seems probable that the highly complex secretory mechanisms used by airway mucin-secreting cells, or similar mechanisms, would also be involved in other secretory pathways. To this point, MARCKS has been implicated in secretion of colonic mucin (117, 118), insulin (119), apolipoprotein E (120), cathepsin K (121), amylase (122), neurotensin (123), adrenocorticotropin (124), and acrosomal exocytosis (125). It is conceivable that other cell types have similar secretory mechanisms involving MARCKS in a central role.

Inflammation

Exocytosis/degranulation. With a demonstrated role for MARCKS in the release of secretory granules from respiratory epithelial cells (described above), the possibility that MARCKS could also be involved in regulating release of granules from other cell types was addressed. Attention was turned to release of inflammation-associated granules from inflammatory cells. Several cell types feature prominently in the pathophysiology of airway inflammation, including leukocytes such as neutrophils and mast cells, and a role for MARCKS in the secretion/exocytosis of inflammatory mediators by these cells was investigated.

Neutrophils are required for normal host defense, but when dysregulated in their response, they contribute to pathological airway inflammation in patients with chronic bronchitis, severe forms of neutrophilic asthma, cystic fibrosis, and acute lung injury (ALI)/ARDS (126). As a normal mechanism for host defense, neutrophils synthesize and store degradative enzymes in membrane-bound granules within their cytoplasm. Inflammatory signaling during airway disease can stimulate exocytosis of these granules, the contents of which can damage host tissue. Inhibition of MARCKS with the MANS peptide attenuated PMA-stimulated release of myeloperoxidase from isolated human peripheral blood neutrophils in a concentration-dependent manner (21). MANS also attenuated secretion of eosinophil peroxidase from the eosinophil-like cell line HL-60 clone 15, lysozyme from the monocytic leukemia cell line U937, and granzyme from the lymphocyte natural killer cell line NK-9222 (21).

Pretreatment of canine neutrophils with MANS significantly reduced both mRNA and protein expression of several proinflammatory cytokines, including IL-8 and TNF-α. The observed reduction in cytokine secretion was associated with reduced transcript synthesis (93). Treatment of isolated macrophages with MANS or a peptide identical to the MARCKS ED attenuated LPS-induced expression of TNF-α through suppression of p38 and JNK (c-Jun N-terminal kinase) mitogen-activated protein kinases, as well as NF-κB (96). Treatment of mice *in vivo* with the MANS peptide reduced serum TNF-α and IL-6 concentrations and resulted in 40% survival of mice after the administration of a lethal dose of LPS (96).

Mast cells also contribute to the pathophysiology of inflammatory airway diseases, including asthma, COPD, and idiopathic pulmonary fibrosis, primarily through exocytosis of granules containing mediators such as histamine and tryptase capable of eliciting a programmed inflammatory response (127). Exocytosis of mast cell granules depends on elevated intracellular Ca^{2+} (128). In an *in vitro* model using antigen-stimulated rat basophilic leukemia (RBL-2H3) cells, Gadi and colleagues showed that MARCKS dissociates from the membrane at the same time that activated PKC-β1 translocates to the membrane (129). Furthermore, by expressing a mutated, nonphosphorylatable MARCKS-ED, these investigators showed that MARCKS sequestration of membrane PIP_2 delayed intracellular Ca^{2+} mobilization and inhibited granule exocytosis. They concluded that MARCKS regulates mast cell granule exocytosis in a PKC-dependent manner by regulating availability of membrane phosphoinositides required for granule fusion with the plasma membrane (129).

Inflammatory cell migration. MARCKS has been shown to bind to and to cross-link actin in different cell types, as well as to colocalize with actin in the leading edge of migrating cells (10). Interestingly, MARCKS constitutes 90% of all proteins synthesized in neutrophils in response to exposure to TNF-α or LPS (130). Given the known relationship between MARCKS, the actin cytoskeleton, and inflammatory signals, it was hypothesized that MARCKS could be involved in inflammatory cell motility and that MANS could have additional antiinflammatory effects related to inhibiting leukocyte and inflammatory cell migration into the lung. Treatment of isolated human neutrophils with MANS, but not RNS, dramatically inhibited migration toward the chemotactic agents formyl-methionyl-leucyl-phenylalanine peptide, IL-8, and LTB4 *in vitro*. MANS treatment also inhibited β_2-integrin–dependent adhesion of these cells (8), as well as platelet-activating factor– and LTB4 (leukotriene B4)-induced chemotaxis and chemokinesis of equine neutrophils (94). Treatment of primary human neutrophils with the PKCδ inhibitor rottlerin blocked MARCKS phosphorylation and attenuated fMLP-, LTB4-, and IL-8–induced chemotaxis in a concentration-dependent manner (16).

Treatment with MANS, but not RNS, also inhibited migration of macrophages in response to the chemoattractant MCP-1 (monocyte chemoattractant protein 1), and visual and biochemical evidence of a transient interaction between MARCKS and actin during the process of migration was observed (13). MANS treatment also blocked *in vitro* migration of mesenchymal stem cells in response to injury-associated chemokines, such as complement component 5a, stromal cell–derived factor 1α, and MCP-1 (95).

As described above, MARCKS binds to and sequesters PIP_2 in membranes and subsequently releases PIP_2 after phosphorylation by PKC. This putative mechanism is related to MARCKS regulation of leukocyte migration and trafficking. Indeed, directed migration of neutrophils involves PIP_2 and downstream signaling molecules, especially PI3K (131). The leading edge of migrating neutrophils and macrophages contains many PIP_2-related signaling molecules, including MARCKS, PIP_3, PI3K, and numerous actin-binding and regulatory proteins (58, 132, 133). A series of recent publications by Ziemba and colleagues offered significant insight into the MARCKS-PIP_2 role in cellular migration. These investigators first used single-molecule fluorescence and total internal reflection fluorescence microscopy of supported lipid bilayers to measure the surface densities and activities of proteins and phospholipids involved in a hypothesized Ca^{2+}-PKC-MARCKS- PIP_2-PI3K-PIP_3 amplification module (57, 58, 62, 134, 135). These studies confirmed that MARCKS sequesters PIP_2 at the lipid membrane and prevents PI3Kα-mediated generation of PIP_3, until Ca^{2+}-activated PKCα phosphorylates MARCKS (57). A second study also using single-molecule fluorescence microscopy and supported lipid bilayers showed that Ca^{2+}/CaM binding to MARCKS ED also causes MARCKS to release sequestered PIP_2, leading to increased production of PIP_3 by PI3Kα (62). Interestingly, the kinetics of PIP_2 release from MARCKS is much more rapid with Ca^{2+}/CaM binding than with PKCα phosphorylation, taking seconds rather than minutes, suggesting two different pathways for regulation of membrane PIP_2 availability by MARCKS. Finally, studies with live cell fluorescence of polarized macrophages confirmed that the pathway activators PDGF-BB (platelet-derived growth factor BB) and ATP promote PIP_3-enriched leading edge expansion that is regulated by a Ca^{2+}–PKC–MARCKS–PIP_2 signaling feedback module (58). These authors proposed that this signaling network is relevant not only to cells with amoeboid-like movement (i.e., neutrophils and macrophages) but also for mesenchymal cell chemotaxis and, perhaps, cancer cell migration.

Chronic Bronchitis

Based on *in vitro* and *in vivo* results showing a relationship between MARCKS, inflammation, and mucin secretion, a potential therapy related to chronic bronchitis, which is characterized by both mucus hypersecretion and inflammation in the airways, was considered. As indicated above, the biotech company BioMarck Pharmaceuticals developed the MANS derivative BIO-11006 as an inhaled aerosolized anti-MARCKS drug. Preclinical studies in dogs and rats for as long as 90 days indicated an absence of toxicity of inhaled BIO-11006, including no adverse effects on body weight; food consumption; respiratory function; neurological function; cardiovascular function; or laboratory, microscopic, or macroscopic parameters. BIO-11006 showed no evidence of mutagenicity or clastogenicity. The U.S. Food and Drug Administration (FDA) then was approved BIO-11006 as an investigational new drug for a clinical trial in patients with chronic bronchitis. Two phase I clinical studies were performed in healthy volunteers ($n = 67$). A single 75-mg dose of inhaled BIO-11006 was well tolerated by eight normal adult volunteers in the first study (BIM-CL-001). In the second study (BIM-CL-002), a single inhaled dose of up to 1,000 mg and multiple inhaled doses of up to 250 mg/d for 14 days were well tolerated by 59 healthy adult volunteers. Headache, pyrexia, and cough were the most common adverse events seen with multiple inhaled doses.

On the basis of the foregoing, the FDA approved the phase IIa study (BIM-CL-003), BREATH 1 (BIO-11006: Respiratory Effectiveness of a Targeted Therapeutic), consisting of a double-blind, placebo (half-normal saline)-controlled, multicenter dose escalation study in subjects with chronic bronchitis. The primary objective was to evaluate the safety and tolerability of BIO-11006 when administered once (75 and 150 mg) or twice (75 and 125 mg) daily for 21 days by inhaled aerosol to subjects with COPD experiencing chronic bronchitis by assessing adverse events, physical examinations and vital signs, pulmonary function (including FEV_1), 12-lead electrocardiogram, clinical blood chemistries, hematology, and urinalysis. The secondary objective of the study was to evaluate the efficacy of BIO-11006 by assessing the change from baseline in pulmonary function as measured by FEV_1. A total of 172 subjects with stable COPD (mostly Global Initiative for Chronic Obstructive Lung Disease stages 2 and 3) were randomized in the double-blind study. Trough FEV_1 (primary endpoint) was measured at baseline and on Days 3, 7, 14, 21, 28, and 49. Secondary endpoints of Breathlessness, Cough and Sputum Scale and St. George's Respiratory Questionnaire were measured regularly.

Although the primary and secondary efficacy endpoints did not meet statistical significance at Day 21, the conclusion of the study researchers was that the 75-mg twice-daily dose of BIO-11006 proved to be

efficacious by increasing the proportion of FEV_1 responders statistically significantly as compared with half-normal saline on Days 14 and 28 of the study. In addition, bronchitic symptoms associated with COPD decreased (136).

ARDS

Given the above-described *in vitro* effects of MANS and BIO-11006 on leukocyte trafficking and cytokine/chemokine secretion, and given the antiinflammatory effects in lungs of mice exposed to ozone (22), it was hypothesized that inhibition of MARCKS with inhaled peptides might be beneficial for patients with the lung disease ARDS. The United States has about 200,000 cases of ARDS annually. It is a disease associated with sepsis, pneumonia, smoke inhalation, near drowning, among others, and is characterized by a massive influx of neutrophils into the lung. Mortality is approximately 40%, and the current standard-of-care treatment is mechanical ventilation. There are no drugs proven effective in this disease.

The possibility of an inhaled anti-MARCKS peptide affecting disease progression was looked at, using intratracheal instillation of bacterial LPS as a murine model of ALI/ARDS. BIO-11006 was delivered via inhalation either just before intratracheal instillation of 5 μg of LPS into BALB/c mice or 4, 12, 24, or 36 hours after LPS instillation. Treatment with aerosolized BIO-11006 at 0, 4, 12, 24, and even 36 hours after LPS instillation reversed the disease process: Mouse behavior returned to normal after two treatments 12 hours apart with the inhaled peptide after LPS injury, whereas control LPS-instilled animals treated with PBS only remained moribund. Histological appearance of inflammation, BAL fluid protein concentrations, leukocyte and neutrophil numbers, KC (keratinocyte chemoattractant; CXCL1, mouse IL-8 equivalent) and TNF-α gene and protein expression, and NF-κB activation were all significantly attenuated by inhaled BIO-11006 at all time points (23). The fact that the treatment was effective as long as 36 hours after administration of LPS to the mice suggests that inhaled MARCKS inhibitors could be an effective treatment for patients who present with already-active ARDS. In another related study, treatment of mice with the MANS peptide administered intravenously reduced serum TNF-α and IL-6 concentrations and resulted in 40% survival of mice after the administration of a lethal dose of LPS (96).

On the basis of the data reported above, the FDA approved a phase IIa clinical study in ARDS using inhaled BIO-11006, which already had been shown to have a good safety profile in human volunteers and patients with chronic bronchitis in a previous clinical trial (*see above*). This clinical study of ARDS (BIM-CL-005) commenced in the spring of 2018. The title of the study is "A Phase 2 Multicenter Study to Evaluate the Safety and Efficacy of BIO-11006 Inhalational Solution in Patients with Acute Respiratory Distress Syndrome" (NCT03202394). This study is placebo controlled and funded by BioMarck Pharmaceuticals. All patients are receiving ventilation in an intensive care unit, with half randomized to receive BIO-11006 and half to receive placebo. The primary endpoint is survival at Day 28. Other factors such as days on ventilator and percentage arterial oxygen are secondary endpoints.

Lung Cancer

Clearly, MARCKS plays an integral and probably critical role in the motility and migration of different cell types, including inflammatory cells, fibroblasts, endothelial cells, stem cells, and epithelial cells (8, 13, 15, 16, 95, 137–140). The potential role of MARCKS in migration of lung cancer cells, and possibly in lung cancer metastasis, was investigated. Results of studies looking at effects of MARCKS-inhibitory peptides on migration of cancer cells in chambers showed that both MANS (12) and BIO-11006 (99, 141–143) blocked *in vitro* migration of non–small cell lung cancer (NSCLC) cells, suggesting that MARCKS could potentially be an effective target for attenuating metastasis of lung cancer *in vivo*.

Certainly, MARCKS appears to be a valid therapeutic target in lung cancer. There are many published reports indicating that the presence of MARCKS, especially the phosphorylated, activated form, correlates with aggressiveness and poor prognosis in numerous neoplasias, including melanoma (9, 11), cholangiosarcoma (144), bladder (145), glioma (146, 147), breast (148, 149), colorectal (150, 151), intestinal (152), kidney (153), liver (154), pancreas (155), prostate (156, 157), multiple myeloma (158), osteosarcoma (159), ovarian (160, 161), and, indeed, lung (105, 162). On the basis of these reports, Chen and colleagues looked retrospectively at lung cancer archived tissues (163). Samples from a cohort of over 100 patients with lung cancer were analyzed by immunohistochemical staining with a monoclonal antibody recognizing phosphorylated MARCKS (p-MARCKS). High concentrations of p-MARCKS were found in tumor tissues compared with normal lung tissues, and, importantly, the concentrations of MARCKS phosphorylation correlated directly and significantly with the stage of the cancer; samples from stages III and IV had the highest concentrations. This also correlated with lymph node metastatic status (163).

Studies were then performed to determine the potential role of MARCKS and phosphorylated MARCKS in metastasis *in vivo*. An orthotopic model of lung cancer in which PC-9 cells, an aggressive human lung cancer cell line, were injected into the left lobe of immunocompromised mice was used. Treatment with the MANS peptide, injected intraperitoneally into these mice every 3 days, dramatically inhibited the metastasis of these cells to other regions of the lung and to distal organs (12). Another study with the same orthotopic model used a 25-mer peptide targeting the MARCKS phosphorylation site domain (ED motif). This peptide suppressed tumor growth and metastasis *in vivo* and reduced concentrations of p-MARCKS, PIP_3, and Akt activity. The peptide also enhanced the sensitivity of lung cancer cells to erlotinib treatment, especially those cells with sustained activation of PI3K/Akt signaling (164). These inhibitory effects on metastasis in models of lung cancer also held true for treatment *in vivo* with inhaled BIO-11006 (99, 141).

A third phase II clinical trial is currently ongoing, again under the auspices of BioMarck Pharmaceuticals, for NSCLC (A Randomized Study of the Safety and Efficacy of BIO-11006 in Treatment of Advanced Non-Small Cell Lung Cancer in Patients Who Are Not Candidates for Curative Surgery and/or Radiation and Who Are Receiving Pemetrexed and Carboplatin; BIO-NSCLC-001 [NCT03472053]). This study is controlled by standard-of-care therapy (pemetrexed and carboplatin). All patients receive standard of care,

and half are randomized to receive nebulized BIO-11006 in addition for a 3-month period. The primary endpoint is progression-free survival at 3 months, but patients will be followed for a total of 12 months to assess overall survival and weight. Patients will be monitored for adverse events and serious adverse events during the 12-month study. The study commenced in early 2018.

Conclusions

A summary of relevant translational research involving MARCKS is provided in Table 1. From the time that MARCKS was identified as a molecule that plays a role in secretion of mucin in the airways, its role as a potential therapeutic target in the airways and lungs has expanded from COPD to include airway inflammation and ARDS, and even NSCLC. Means of inhibiting MARCKS pharmacologically include the MANS peptide, which is identical to the 24–amino acid stretch encompassing the MARCKS amino terminus, and BIO-11006, which contains the active portion of MANS but is smaller (10 vs. 24 amino acids) and more soluble (acetate substituted for myristic acid on N-terminal amino acid). Peptides containing the ED of MARCKS, including the MRP peptide, also have been shown to be effective in inhibiting MARCKS and p-MARCKS actions in preclinical models. At this point, BIO-11006, under FDA investigational new drug status, has been tested in phase I studies in human volunteers, as well as in 172 patients with COPD, and it has shown a good safety profile. Currently, there are ongoing phase IIa clinical trials with BIO-11006 in patients with ARDS and NSCLC, with potentially other trials to come. The fact that the same therapeutic target might occur in three different respiratory/pulmonary diseases goes back to the fact that MARCKS, found ubiquitously in all eukaryotic cells and conserved evolutionarily for such a long time, clearly has an important and basic function in cells, and it is therefore not surprising that its inhibition or attenuation could have diverse effects. Whatever the outcome(s) of these trials, it is clear that MARCKS and p-MARCKS represent "druggable" targets in several lung diseases. Pending results of current investigations, specific MARCKS-inhibitory peptides could be available as airway therapeutics in the near future. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

References

1. Aderem A. The MARCKS brothers: a family of protein kinase C substrates. *Cell* 1992;71:713–716.
2. Arbuzova A, Schmitz AA, Vergères G. Cross-talk unfolded: MARCKS proteins. *Biochem J* 2002;362:1–12.
3. Hartwig JH, Thelen M, Rosen A, Janmey PA, Nairn AC, Aderem A. MARCKS is an actin filament crosslinking protein regulated by protein kinase C and calcium-calmodulin. *Nature* 1992;356: 618–622.
4. Yamaguchi H, Shiraishi M, Fukami K, Tanabe A, Ikeda-Matsuo Y, Naito Y, *et al*. MARCKS regulates lamellipodia formation induced by IGF-I via association with PIP2 and beta-actin at membrane microdomains. *J Cell Physiol* 2009;220:748–755.
5. Nairn AC, Aderem A. Calmodulin and protein kinase C cross-talk: the MARCKS protein is an actin filament and plasma membrane cross-linking protein regulated by protein kinase C phosphorylation and by calmodulin. *Ciba Found Symp* 1992;164:145–154, discussion 154–161.
6. Graff JM, Young TN, Johnson JD, Blackshear PJ. Phosphorylation-regulated calmodulin binding to a prominent cellular substrate for protein kinase C. *J Biol Chem* 1989;264:21818–21823.
7. Disatnik MH, Boutet SC, Pacio W, Chan AY, Ross LB, Lee CH, *et al*. The bi-directional translocation of MARCKS between membrane and cytosol regulates integrin-mediated muscle cell spreading. *J Cell Sci* 2004;117:4469–4479.
8. Eckert RE, Neuder LE, Park J, Adler KB, Jones SL. Myristoylated alanine-rich C-kinase substrate (MARCKS) protein regulation of human neutrophil migration. *Am J Respir Cell Mol Biol* 2010;42: 586–594.
9. Estrada-Bernal A, Gatlin JC, Sunpaweravong S, Pfenninger KH. Dynamic adhesions and MARCKS in melanoma cells. *J Cell Sci* 2009; 122:2300–2310.
10. Myat MM, Anderson S, Allen LA, Aderem A. MARCKS regulates membrane ruffling and cell spreading. *Curr Biol* 1997;7:611–614.
11. Chen X, Rotenberg SA. PhosphoMARCKS drives motility of mouse melanoma cells. *Cell Signal* 2010;22:1097–1103.
12. Chen CH, Thai P, Yoneda K, Adler KB, Yang PC, Wu R. A peptide that inhibits function of myristoylated alanine-rich C kinase substrate (MARCKS) reduces lung cancer metastasis. *Oncogene* 2014;33: 3696–3706.
13. Green TD, Park J, Yin Q, Fang S, Crews AL, Jones SL, *et al*. Directed migration of mouse macrophages *in vitro* involves myristoylated alanine-rich C-kinase substrate (MARCKS) protein. *J Leukoc Biol* 2012;92:633–639.
14. Larsson C. Protein kinase C and the regulation of the actin cytoskeleton. *Cell Signal* 2006;18:276–284.
15. Ott LE, Sung EJ, Melvin AT, Sheats MK, Haugh JM, Adler KB, *et al*. Fibroblast migration is regulated by myristoylated alanine-rich C-kinase substrate (MARCKS) protein. *PLoS One* 2013;8:e66512.
16. Sheats MK, Sung EJ, Adler KB, Jones SL. *In vitro* neutrophil migration requires protein kinase C-delta (δ-PKC)-mediated myristoylated alanine-rich C-kinase substrate (MARCKS) phosphorylation. *Inflammation* 2015;38:1126–1141.
17. Agrawal A, Rengarajan S, Adler KB, Ram A, Ghosh B, Fahim M, *et al*. Inhibition of mucin secretion with MARCKS-related peptide improves airway obstruction in a mouse model of asthma. *J Appl Physiol (1985)* 2007;102:399–405.
18. Park JA, Crews AL, Lampe WR, Fang S, Park J, Adler KB. Protein kinase Cδ regulates airway mucin secretion via phosphorylation of MARCKS protein. *Am J Pathol* 2007;171:1822–1830.
19. Salli U, Saito N, Stormshak F. Spatiotemporal interactions of myristoylated alanine-rich C kinase substrate (MARCKS) protein with the actin cytoskeleton and exocytosis of oxytocin upon prostaglandin F2α stimulation of bovine luteal cells. *Biol Reprod* 2003;69:2053–2058.
20. Singer M, Martin LD, Vargaftig BB, Park J, Gruber AD, Li Y, *et al*. A MARCKS-related peptide blocks mucus hypersecretion in a mouse model of asthma. *Nat Med* 2004;10:193–196.
21. Takashi S, Park J, Fang S, Koyama S, Parikh I, Adler KB. A peptide against the N-terminus of myristoylated alanine-rich C kinase substrate inhibits degranulation of human leukocytes *in vitro*. *Am J Respir Cell Mol Biol* 2006;34:647–652.
22. Damera G, Jester WF, Jiang M, Zhao H, Fogle HW, Mittelman M, *et al*. Inhibition of myristoylated alanine-rich C kinase substrate (MARCKS) protein inhibits ozone-induced airway neutrophilia and inflammation. *Exp Lung Res* 2010;36:75–84. [Published erratum appears in *Exp Lung Res* 2010;36:321.]
23. Yin Q, Fang S, Park J, Crews AL, Parikh I, Adler KB. An inhaled inhibitor of myristoylated alanine-rich C kinase substrate reverses LPS-induced acute lung injury in mice. *Am J Respir Cell Mol Biol* 2016; 55:617–622.

24. Wu WC, Walaas SI, Nairn AC, Greengard P. Calcium/phospholipid regulates phosphorylation of a Mr "87k" substrate protein in brain synaptosomes. *Proc Natl Acad Sci USA* 1982;79:5249–5253.
25. Li J, Aderem A. MacMARCKS, a novel member of the MARCKS family of protein kinase C substrates. *Cell* 1992;70:791–801.
26. Blackshear PJ. The MARCKS family of cellular protein kinase C substrates. *J Biol Chem* 1993;268:1501–1504.
27. Weinreb PH, Zhen W, Poon AW, Conway KA, Lansbury PT Jr. NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. *Biochemistry* 1996;35:13709–13715.
28. Vergères G, Manenti S, Weber T, Stürzinger C. The myristoyl moiety of myristoylated alanine-rich C kinase substrate (MARCKS) and MARCKS-related protein is embedded in the membrane. *J Biol Chem* 1995;270:19879–19887.
29. Stumpo DJ, Graff JM, Albert KA, Greengard P, Blackshear PJ. Molecular cloning, characterization, and expression of a cDNA encoding the "80- to 87-kDa" myristoylated alanine-rich C kinase substrate: a major cellular substrate for protein kinase C. *Proc Natl Acad Sci USA* 1989;86:4012–4016.
30. Stumpo DJ, Eddy RL Jr, Haley LL, Sait S, Shows TB, Lai WS, *et al*. Promoter sequence, expression, and fine chromosomal mapping of the human gene (MLP) encoding the MARCKS-like protein: identification of neighboring and linked polymorphic loci for MLP and MACS and use in the evaluation of human neural tube defects. *Genomics* 1998;49:253–264.
31. Herget T, Oehrlein SA, Pappin DJ, Rozengurt E, Parker PJ. The myristoylated alanine-rich C-kinase substrate (MARCKS) is sequentially phosphorylated by conventional, novel and atypical isotypes of protein kinase C. *Eur J Biochem* 1995;233:448–457.
32. Harlan DM, Graff JM, Stumpo DJ, Eddy RL Jr, Shows TB, Boyle JM, *et al*. The human myristoylated alanine-rich C kinase substrate (MARCKS) gene (MACS): analysis of its gene product, promoter, and chromosomal localization. *J Biol Chem* 1991;266:14399–14405.
33. Seykora JT, Ravetch JV, Aderem A. Cloning and molecular characterization of the murine macrophage "68-kDa" protein kinase C substrate and its regulation by bacterial lipopolysaccharide. *Proc Natl Acad Sci USA* 1991;88:2505–2509.
34. Erusalimsky JD, Brooks SF, Herget T, Morris C, Rozengurt E. Molecular cloning and characterization of the acidic 80-kDa protein kinase C substrate from rat brain: identification as a glycoprotein. *J Biol Chem* 1991;266:7073–7080.
35. Dietrich U, Krüger P, Gutberlet T, Käs JA. Interaction of the MARCKS peptide with PIP_2 in phospholipid monolayers. *Biochim Biophys Acta* 2009;1788:1474–1481.
36. McLaughlin S, Aderem A. The myristoyl-electrostatic switch: a modulator of reversible protein-membrane interactions. *Trends Biochem Sci* 1995;20:272–276.
37. Seykora JT, Myat MM, Allen LA, Ravetch JV, Aderem A. Molecular determinants of the myristoyl-electrostatic switch of MARCKS. *J Biol Chem* 1996;271:18797–18802.
38. Swierczynski SL, Blackshear PJ. Myristoylation-dependent and electrostatic interactions exert independent effects on the membrane association of the myristoylated alanine-rich protein kinase C substrate protein in intact cells. *J Biol Chem* 1996;271:23424–23430.
39. Kim SS, Kim JH, Kim HS, Park DE, Chung CH. Involvement of the theta-type protein kinase C in translocation of myristoylated alanine-rich C kinase substrate (MARCKS) during myogenesis of chick embryonic myoblasts. *Biochem J* 2000;347:139–146.
40. Ohmori S, Sakai N, Shirai Y, Yamamoto H, Miyamoto E, Shimizu N, *et al*. Importance of protein kinase C targeting for the phosphorylation of its substrate, myristoylated alanine-rich C-kinase substrate. *J Biol Chem* 2000;275:26449–26457.
41. Rosen A, Keenan KF, Thelen M, Nairn AC, Aderem A. Activation of protein kinase C results in the displacement of its myristoylated, alanine-rich substrate from punctate structures in macrophage filopodia. *J Exp Med* 1990;172:1211–1215.
42. Uberall F, Giselbrecht S, Hellbert K, Fresser F, Bauer B, Gschwendt M, *et al*. Conventional PKC-α, novel PKC-ε and PKC-θ, but not atypical PKC-λ are MARCKS kinases in intact NIH 3T3 fibroblasts. *J Biol Chem* 1997;272:4072–4078.
43. Thelen M, Rosen A, Nairn AC, Aderem A. Regulation by phosphorylation of reversible association of a myristoylated protein kinase C substrate with the plasma membrane. *Nature* 1991;351:320–322.
44. Rohrbach TD, Shah N, Jackson WP, Feeney EV, Scanlon S, Gish R, *et al*. The effector domain of MARCKS is a nuclear localization signal that regulates cellular PIP2 levels and nuclear PIP2 localization. *PLoS One* 2015;10:e0140870.
45. Allen LA, Aderem A. Protein kinase C regulates MARCKS cycling between the plasma membrane and lysosomes in fibroblasts. *EMBO J* 1995;14:1109–1121.
46. McLaughlin S, Wang J, Gambhir A, Murray D. PIP_2 and proteins: interactions, organization, and information flow. *Annu Rev Biophys Biomol Struct* 2002;31:151–175.
47. Berridge MJ. Inositol trisphosphate and diacylglycerol as second messengers. *Biochem J* 1984;220:345–360.
48. Insall RH, Weiner OD. PIP3, PIP2, and cell movement—similar messages, different meanings? *Dev Cell* 2001;1:743–747.
49. Hille B, Dickson EJ, Kruse M, Vivas O, Suh BC. Phosphoinositides regulate ion channels. *Biochim Biophys Acta* 2015;1851:844–856.
50. Suh BC, Leal K, Hille B. Modulation of high-voltage activated Ca^{2+} channels by membrane phosphatidylinositol 4,5-bisphosphate. *Neuron* 2010;67:224–238.
51. Glaser M, Wanaski S, Buser CA, Boguslavsky V, Rashidzada W, Morris A, *et al*. Myristoylated alanine-rich C kinase substrate (MARCKS) produces reversible inhibition of phospholipase C by sequestering phosphatidylinositol 4,5-bisphosphate in lateral domains. *J Biol Chem* 1996;271:26187–26193.
52. Wang J, Arbuzova A, Hangyás-Mihályné G, McLaughlin S. The effector domain of myristoylated alanine-rich C kinase substrate binds strongly to phosphatidylinositol 4,5-bisphosphate. *J Biol Chem* 2001;276:5012–5019.
53. Wang J, Gambhir A, McLaughlin S, Murray D. A computational model for the electrostatic sequestration of $PI(4,5)P_2$ by membrane-adsorbed basic peptides. *Biophys J* 2004;86:1969–1986.
54. Rauch ME, Ferguson CG, Prestwich GD, Cafiso DS. Myristoylated alanine-rich C kinase substrate (MARCKS) sequesters spin-labeled phosphatidylinositol 4,5-bisphosphate in lipid bilayers. *J Biol Chem* 2002;277:14068–14076.
55. Albert KA, Nairn AC, Greengard P. The 87-kDa protein, a major specific substrate for protein kinase C: purification from bovine brain and characterization. *Proc Natl Acad Sci USA* 1987;84:7046–7050.
56. Laux T, Fukami K, Thelen M, Golub T, Frey D, Caroni P. GAP43, MARCKS, and CAP23 modulate $PI(4,5)P_2$ at plasmalemmal rafts, and regulate cell cortex actin dynamics through a common mechanism. *J Cell Biol* 2000;149:1455–1472.
57. Ziemba BP, Burke JE, Masson G, Williams RL, Falke JJ. Regulation of PI3K by PKC and MARCKS: single-molecule analysis of a reconstituted signaling pathway. *Biophys J* 2016;110:1811–1825.
58. Ziemba BP, Falke JJA. A PKC-MARCKS-PI3K regulatory module links Ca^{2+} and PIP3 signals at the leading edge of polarized macrophages. *PLoS One* 2018;13:e0196678.
59. Wang J, Gambhir A, Hangyás-Mihályné G, Murray D, Golebiewska U, McLaughlin S. Lateral sequestration of phosphatidylinositol 4,5-bisphosphate by the basic effector domain of myristoylated alanine-rich C kinase substrate is due to nonspecific electrostatic interactions. *J Biol Chem* 2002;277:34401–34412.
60. Zhang W, Crocker E, McLaughlin S, Smith SO. Binding of peptides with basic and aromatic residues to bilayer membranes: phenylalanine in the myristoylated alanine-rich C kinase substrate effector domain penetrates into the hydrophobic core of the bilayer. *J Biol Chem* 2003;278:21459–21466.
61. Gambhir A, Hangyás-Mihályné G, Zaitseva I, Cafiso DS, Wang J, Murray D, *et al*. Electrostatic sequestration of PIP_2 on phospholipid membranes by basic/aromatic regions of proteins. *Biophys J* 2004; 86:2188–2207.
62. Ziemba BP, Swisher GH, Masson G, Burke JE, Williams RL, Falke JJ. Regulation of a coupled MARCKS-PI3K lipid kinase circuit by calmodulin: single-molecule analysis of a membrane-bound signaling module. *Biochemistry* 2016;55:6395–6405.
63. van den Bout I, van Rheenen J, van Angelen AA, de Rooij J, Wilhelmsen K, Jalink K, *et al*. Investigation into the mechanism regulating MRP localization. *Exp Cell Res* 2008;314:330–341.

64. Wohnsland F, Schmitz AA, Steinmetz MO, Aebi U, Vergeres G. Influence of the effector peptide of MARCKS-related protein on actin polymerization: a kinetic analysis. *Biophys Chem* 2000;85: 169–177.
65. Wohnsland F, Schmitz AA, Steinmetz MO, Aebi U, Vergéres G. Interaction between actin and the effector peptide of MARCKS-related protein: identification of functional amino acid segments. *J Biol Chem* 2000;275:20873–20879.
66. Yarmola EG, Edison AS, Lenox RH, Bubb MR. Actin filament cross-linking by MARCKS: characterization of two actin-binding sites within the phosphorylation site domain. *J Biol Chem* 2001;276: 22351–22358.
67. Wohnsland F, Steinmetz MO, Aebi U, Vergères G. MARCKS-related protein binds to actin without significantly affecting actin polymerization or network structure: myristoylated alanine-rich C kinase substrate. *J Struct Biol* 2000;131:217–224.
68. Aderem A. The MARCKS family of protein kinase-C substrates. *Biochem Soc Trans* 1995;23:587–591.
69. Blackshear PJ, Verghese GM, Johnson JD, Haupt DM, Stumpo DJ. Characteristics of the F52 protein, a MARCKS homologue. *J Biol Chem* 1992;267:13540–13546.
70. McIlroy BK, Walters JD, Blackshear PJ, Johnson JD. Phosphorylation-dependent binding of a synthetic MARCKS peptide to calmodulin. *J Biol Chem* 1991;266:4959–4964.
71. Verghese GM, Johnson JD, Vasulka C, Haupt DM, Stumpo DJ, Blackshear PJ. Protein kinase C-mediated phosphorylation and calmodulin binding of recombinant myristoylated alanine-rich C kinase substrate (MARCKS) and MARCKS-related protein. *J Biol Chem* 1994;269:9361–9367.
72. Ulrich A, Schmitz AA, Braun T, Yuan T, Vogel HJ, Vergères G. Mapping the interface between calmodulin and MARCKS-related protein by fluorescence spectroscopy. *Proc Natl Acad Sci USA* 2000;97: 5191–5196.
73. Matsubara M, Yamauchi E, Hayashi N, Taniguchi H. MARCKS, a major protein kinase C substrate, assumes non-helical conformations both in solution and in complex with Ca^{2+}-calmodulin. *FEBS Lett* 1998; 421:203–207.
74. Matsubara M, Titani K, Taniguchi H, Hayashi N. Direct involvement of protein myristoylation in myristoylated alanine-rich C kinase substrate (MARCKS)-calmodulin interaction. *J Biol Chem* 2003;278: 48898–48902.
75. Yamauchi E, Nakatsu T, Matsubara M, Kato H, Taniguchi H. Crystal structure of a MARCKS peptide containing the calmodulin-binding domain in complex with Ca^{2+}-calmodulin. *Nat Struct Biol* 2003;10: 226–231.
76. Porumb T, Crivici A, Blackshear PJ, Ikura M. Calcium binding and conformational properties of calmodulin complexed with peptides derived from myristoylated alanine-rich C kinase substrate (MARCKS) and MARCKS-related protein (MRP). *Eur Biophys J* 1997;25:239–247.
77. Kim J, Shishido T, Jiang X, Aderem A, McLaughlin S. Phosphorylation, high ionic strength, and calmodulin reverse the binding of MARCKS to phospholipid vesicles. *J Biol Chem* 1994;269:28214–28219.
78. McLaughlin S, Hangyás-Mihályné G, Zaitseva I, Golebiewska U. Reversible - through calmodulin - electrostatic interactions between basic residues on proteins and acidic lipids in the plasma membrane. *Biochem Soc Symp* 2005;(72):189–198.
79. Sundaram M, Cook HW, Byers DM. The MARCKS family of phospholipid binding proteins: regulation of phospholipase D and other cellular components. *Biochem Cell Biol* 2004;82:191–200.
80. Iioka H, Ueno N, Kinoshita N. Essential role of MARCKS in cortical actin dynamics during gastrulation movements. *J Cell Biol* 2004;164: 169–174.
81. Aparicio G, Arruti C, Zolessi FR. MARCKS phosphorylation by PKC strongly impairs cell polarity in the chick neural plate. *Genesis* 2018; 56:e23104.
82. Michaut MA, Williams CJ, Schultz RM. Phosphorylated MARCKS: a novel centrosome component that also defines a peripheral subdomain of the cortical actin cap in mouse eggs. *Dev Biol* 2005; 280:26–37.
83. Shiraishi M, Tanabe A, Saito N, Sasaki Y. Unphosphorylated MARCKS is involved in neurite initiation induced by insulin-like growth factor-I in SH-SY5Y cells. *J Cell Physiol* 2006;209:1029–1038.
84. Garrett AM, Schreiner D, Lobas MA, Weiner JA. γ-protocadherins control cortical dendrite arborization by regulating the activity of a FAK/PKC/MARCKS signaling pathway. *Neuron* 2012;74:269–276.
85. Trovò L, Ahmed T, Callaerts-Vegh Z, Buzzi A, Bagni C, Chuah M, *et al*. Low hippocampal $PI(4,5)P_2$ contributes to reduced cognition in old mice as a result of loss of MARCKS. *Nat Neurosci* 2013;16:449–455.
86. Stumpo DJ, Bock CB, Tuttle JS, Blackshear PJ. MARCKS deficiency in mice leads to abnormal brain development and perinatal death. *Proc Natl Acad Sci USA* 1995;92:944–948.
87. Chen J, Chang S, Duncan SA, Okano HJ, Fishell G, Aderem A. Disruption of the MacMARCKS gene prevents cranial neural tube closure and results in anencephaly. *Proc Natl Acad Sci USA* 1996; 93:6275–6279.
88. Wu M, Chen DF, Sasaoka T, Tonegawa S. Neural tube defects and abnormal brain development in F52-deficient mice. *Proc Natl Acad Sci USA* 1996;93:2110–2115.
89. Carballo E, Pitterle DM, Stumpo DJ, Sperling RT, Blackshear PJ. Phagocytic and macropinocytic activity in MARCKS-deficient macrophages and fibroblasts. *Am J Physiol* 1999;277:C163–C173.
90. Machlus KR, Wu SK, Stumpo DJ, Soussou TS, Paul DS, Campbell RA, *et al*. Synthesis and dephosphorylation of MARCKS in the late stages of megakaryocyte maturation drive proplatelet formation. *Blood* 2016;127:1468–1480.
91. Van Itallie CM, Tietgens AJ, Aponte A, Gucek M, Cartagena-Rivera AX, Chadwick RS, *et al*. MARCKS-related protein regulates cytoskeletal organization at cell-cell and cell-substrate contacts in epithelial cells. *J Cell Sci* 2018;131:jcs210237.
92. Li Y, Martin LD, Spizz G, Adler KB. MARCKS protein is a key molecule regulating mucin secretion by human airway epithelial cells *in vitro*. *J Biol Chem* 2001;276:40982–40990.
93. Li J, D'Annibale-Tolhurst MA, Adler KB, Fang S, Yin Q, Birkenheuer AJ, *et al*. A myristoylated alanine-rich C kinase substrate-related peptide suppresses cytokine mRNA and protein expression in LPS-activated canine neutrophils. *Am J Respir Cell Mol Biol* 2013;48:314–321.
94. Sheats MK, Pescosolido KC, Hefner EM, Sung EJ, Adler KB, Jones SL. Myristoylated alanine rich C kinase substrate (MARCKS) is essential to β_2-integrin dependent responses of equine neutrophils. *Vet Immunol Immunopathol* 2014;160:167–176.
95. Miller JD, Lankford SM, Adler KB, Brody AR. Mesenchymal stem cells require MARCKS protein for directed chemotaxis *in vitro*. *Am J Respir Cell Mol Biol* 2010;43:253–258.
96. Lee SM, Suk K, Lee WH. Myristoylated alanine-rich C kinase substrate (MARCKS) regulates the expression of proinflammatory cytokines in macrophages through activation of p38/JNK MAPK and NF-κB. *Cell Immunol* 2015;296:115–121.
97. Ha EV, Rogers DF. Novel therapies to inhibit mucus synthesis and secretion in airway hypersecretory diseases. *Pharmacology* 2016;97:84–100.
98. Agrawal A, Murphy EC III, Park J, Adler KB, Parikh I. Aerosolized BIO-11006, a novel MARCKS-related peptide, improves airway obstruction in a mouse model of mucus hypersecretion. *J Epithel Biol Pharmacol* 2011;4:1–6.
99. Yin Q, Crews A, Fang S, Park J, Parikh I, Long W, *et al*. Inhibition of lung cancer metastasis in mice by inhaled aerosolized anti-MARCKS peptides [abstract]. *Am J Respir Crit Care Med* 2015;191:A2429.
100. Fang S, Parikh I, Takashi S, Crews AL, Adler KB. BIO−11006, a truncated form of a MARCKS−related peptide, inhibits secretion of mucin and degranulation of leukocytes *in vitro* [abstract]. *Am J Respir Crit Care Med* 2009;179:A6310.
101. Kastelowitz N, Tamura R, Onasoga A, Stalker TJ, White OR, Brown PN, *et al*. Peptides derived from MARCKS block coagulation complex assembly on phosphatidylserine. *Sci Rep* 2017;7: 4275.
102. Elzagallaai A, Rosé SD, Trifaró JM. Platelet secretion induced by phorbol esters stimulation is mediated through phosphorylation of MARCKS: a MARCKS-derived peptide blocks MARCKS phosphorylation and serotonin release without affecting pleckstrin phosphorylation. *Blood* 2000;95:894–902.
103. Elzagallaai A, Rosé SD, Brandan NC, Trifaró JM. Myristoylated alanine-rich C kinase substrate phosphorylation is involved in thrombin-induced serotonin release from platelets. *Br J Haematol* 2001;112:593–602.

104. Graff JM, Rajan RR, Randall RR, Nairn AC, Blackshear PJ. Protein kinase C substrate and inhibitor characteristics of peptides derived from the myristoylated alanine-rich C kinase substrate (MARCKS) protein phosphorylation site domain. *J Biol Chem* 1991;266: 14390–14398.
105. Rohrbach TD, Jones RB, Hicks PH, Weaver AN, Cooper TS, Eustace NJ, *et al*. MARCKS phosphorylation is modulated by a peptide mimetic of MARCKS effector domain leading to increased radiation sensitivity in lung cancer cell lines. *Oncol Lett* 2017;13: 1216–1222.
106. Mancek-Keber M, Bencina M, Japelj B, Panter G, Andrä J, Brandenburg K, *et al*. MARCKS as a negative regulator of lipopolysaccharide signaling. *J Immunol* 2012;188:3893–3902.
107. Chen P, Deng Z, Wang T, Chen L, Li J, Feng Y, *et al*. The potential interaction of MARCKS-related peptide and diltiazem on acrolein-induced airway mucus hypersecretion in rats. *Int Immunopharmacol* 2013;17:625–632.
108. Park JA, He F, Martin LD, Li Y, Chorley BN, Adler KB. Human neutrophil elastase induces hypersecretion of mucin from well-differentiated human bronchial epithelial cells *in vitro* via a protein kinase Cδ-mediated mechanism. *Am J Pathol* 2005;167: 651–661.
109. Park J, Fang S, Crews AL, Lin KW, Adler KB. MARCKS regulation of mucin secretion by airway epithelium *in vitro*: interaction with chaperones. *Am J Respir Cell Mol Biol* 2008;39:68–76.
110. Fang S, Crews AL, Chen W, Park J, Yin Q, Ren XR, *et al*. MARCKS and HSP70 interactions regulate mucin secretion by human airway epithelial cells *in vitro*. *Am J Physiol Lung Cell Mol Physiol* 2013; 304:L511–L518.
111. Lin KW, Fang S, Park J, Crews AL, Adler KB. MARCKS and related chaperones bind to unconventional myosin V isoforms in airway epithelial cells. *Am J Respir Cell Mol Biol* 2010;43:131–136.
112. Trybus KM. Myosin V from head to tail. *Cell Mol Life Sci* 2008;65: 1378–1389.
113. Raiford KL, Park J, Lin KW, Fang S, Crews AL, Adler KB. Mucin granule-associated proteins in human bronchial epithelial cells: the airway goblet cell "granulome". *Respir Res* 2011;12:118.
114. Ehre C, Rossi AH, Abdullah LH, De Pestel K, Hill S, Olsen JC, *et al*. Barrier role of actin filaments in regulated mucin secretion from airway goblet cells. *Am J Physiol Cell Physiol* 2005;288: C46–C56.
115. Adler KB, Tuvim MJ, Dickey BF. Regulated mucin secretion from airway epithelial cells. *Front Endocrinol (Lausanne)* 2013;4:129.
116. Jones LC, Moussa L, Fulcher ML, Zhu Y, Hudson EJ, O'Neal WK, *et al*. VAMP8 is a vesicle SNARE that regulates mucin secretion in airway goblet cells. *J Physiol* 2012;590:545–562.
117. Cornick S, Moreau F, Chadee K. *Entamoeba histolytica* cysteine proteinase 5 evokes mucin exocytosis from colonic goblet cells via αvβ3 integrin. *PLoS Pathog* 2016;12:e1005579.
118. Mo JS, Alam KJ, Kim HS, Lee YM, Yun KJ, Chae SC. MicroRNA 429 regulates mucin gene expression and secretion in murine model of colitis. *J Crohns Colitis* 2016;10:837–849.
119. Wuttke A, Idevall-Hagren O, Tengholm A. $P2Y_1$ receptor-dependent diacylglycerol signaling microdomains in β cells promote insulin secretion. *FASEB J* 2013;27:1610–1620.
120. Karunakaran D, Kockx M, Owen DM, Burnett JR, Jessup W, Kritharides L. Protein kinase C controls vesicular transport and secretion of apolipoprotein E from primary human macrophages. *J Biol Chem* 2013;288:5186–5197.
121. Cremasco V, Decker CE, Stumpo D, Blackshear PJ, Nakayama KI, Nakayama K, *et al*. Protein kinase C-δ deficiency perturbs bone homeostasis by selective uncoupling of cathepsin K secretion and ruffled border formation in osteoclasts. *J Bone Miner Res* 2012;27: 2452–2463.
122. Satoh K, Matsuki-Fukushima M, Qi B, Guo MY, Narita T, Fujita-Yoshigaki J, *et al*. Phosphorylation of myristoylated alanine-rich C kinase substrate is involved in the cAMP-dependent amylase release in parotid acinar cells. *Am J Physiol Gastrointest Liver Physiol* 2009;296:G1382–G1390.
123. Li J, O'Connor KL, Greeley GH Jr, Blackshear PJ, Townsend CM Jr, Evers BM. Myristoylated alanine-rich C kinase substrate-mediated neurotensin release via protein kinase C-δ downstream of the Rho/ROK pathway. *J Biol Chem* 2005;280: 8351–8357.
124. Liu JP, Engler D, Funder JW, Robinson PJ. Arginine vasopressin (AVP) causes the reversible phosphorylation of the myristoylated alanine-rich C kinase substrate (MARCKS) protein in the ovine anterior pituitary: evidence that MARCKS phosphorylation is associated with adrenocorticotropin (ACTH) secretion. *Mol Cell Endocrinol* 1994;105:217–226.
125. Rodriguez Peña MJ, Castillo Bennett JV, Soler OM, Mayorga LS, Michaut MA. MARCKS protein is phosphorylated and regulates calcium mobilization during human acrosomal exocytosis. *PLoS One* 2013;8:e64551.
126. Liu J, Pang Z, Wang G, Guan X, Fang K, Wang Z, *et al*. Advanced role of neutrophils in common respiratory diseases. *J Immunol Res* 2017;2017:6710278.
127. Cruse G, Bradding P. Mast cells in airway diseases and interstitial lung disease. *Eur J Pharmacol* 2016;778:125–138.
128. Bai J, Tucker WC, Chapman ER. PIP_2 increases the speed of response of synaptotagmin and steers its membrane-penetration activity toward the plasma membrane. *Nat Struct Mol Biol* 2004;11: 36–44.
129. Gadi D, Wagenknecht-Wiesner A, Holowka D, Baird B. Sequestration of phosphoinositides by mutated MARCKS effector domain inhibits stimulated Ca^{2+} mobilization and degranulation in mast cells. *Mol Biol Cell* 2011;22:4908–4917.
130. Thelen M, Rosen A, Nairn AC, Aderem A. Tumor necrosis factor alpha modifies agonist-dependent responses in human neutrophils by inducing the synthesis and myristoylation of a specific protein kinase C substrate. *Proc Natl Acad Sci USA* 1990;87: 5603–5607.
131. Tuosto L, Capuano C, Muscolini M, Santoni A, Galandrini R. The multifaceted role of PIP_2 in leukocyte biology. *Cell Mol Life Sci* 2015;72:4461–4474.
132. Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G, *et al*. Cell migration: integrating signals from front to back. *Science* 2003;302:1704–1709.
133. Mañes S, Gómez-Moutón C, Lacalle RA, Jiménez-Baranda S, Mira E, Martínez-A C. Mastering time and space: immune cell polarization and chemotaxis. *Semin Immunol* 2005;17:77–86.
134. Evans JH, Falke JJ. Ca^{2+} influx is an essential component of the positive-feedback loop that maintains leading-edge structure and activity in macrophages. *Proc Natl Acad Sci USA* 2007;104: 16176–16181.
135. Falke JJ, Ziemba BP. Interplay between phosphoinositide lipids and calcium signals at the leading edge of chemotaxing ameboid cells. *Chem Phys Lipids* 2014;182:73–79.
136. Van As A, Kraft M, Hanania NA, Parikh I, Murphy EC. Inhibition of airway mucus hypersecretion and inflammation with BIO-11006, a novel dual action drug, results in improvement of indices of bronchitis and lung function in chronic obstructive pulmonary disease (COPD) [abstract]. *Am J Respir Crit Care Med* 2011;183: A4092.
137. Boitano S, Adker KB. MARCKS protein is involved in airway epithelial cell wound repair [abstract]. *Am J Respir Crit Care Med* 2010;181: A1447.
138. Yu D, Makkar G, Strickland DK, Blanpied TA, Stumpo DJ, Blackshear PJ, *et al*. Myristoylated alanine-rich protein kinase substrate (MARCKS) regulates small GTPase Rac1 and Cdc42 activity and is a critical mediator of vascular smooth muscle cell migration in intimal hyperplasia formation. *J Am Heart Assoc* 2015;4:e002255.
139. Kalwa H, Michel T. The MARCKS protein plays a critical role in phosphatidylinositol 4,5-bisphosphate metabolism and directed cell movement in vascular endothelial cells. *J Biol Chem* 2011;286: 2320–2330.
140. Rombouts K, Lottini B, Caligiuri A, Liotta F, Mello T, Carloni V, *et al*. MARCKS is a downstream effector in platelet-derived growth factor-induced cell motility in activated human hepatic stellate cells. *Exp Cell Res* 2008;314:1444–1454.

141. Yin Q, Fang S, Park J, Crews A, Dickson B, Parikh I, *et al*. An inhibitor Of MARCKS protein attenuates migration of human lung cancer cells; role of protein phosphatase type 2A (PP2A). *Am J Respir Crit Care Med* 2017;195:A2357.
142. Fang S, Yin Q, Park J, Crews A, Dickson B, Adler K. A peptide inhibitor of MARCKS protein attenuates migration of human lung cancer cells; cytoskeletal rearrangement upon treatment indicates a shift from motile to structural cells. *Am J Respir Crit Care Med* 2017;195: A2358.
143. Park J, Fang S, Yin Q, Crews A, Adler K. Modulation of FAK activity by MARCKS is involved in migration of lung cancer cells. *Am J Respir Crit Care Med* 2016;193:A2565.
144. Techasen A, Loilome W, Namwat N, Takahashi E, Sugihara E, Puapairoj A, *et al*. Myristoylated alanine-rich C kinase substrate phosphorylation promotes cholangiocarcinoma cell migration and metastasis via the protein kinase C-dependent pathway. *Cancer Sci* 2010;101:658–665.
145. Yokoyama Y, Ito T, Hanson V, Schwartz GK, Aderem AA, Holland JF, *et al*. PMA-induced reduction in invasiveness is associated with hyperphosphorylation of MARCKS and talin in invasive bladder cancer cells. *Int J Cancer* 1998;75:774–779.
146. Micallef J, Taccone M, Mukherjee J, Croul S, Busby J, Moran MF, *et al*. Epidermal growth factor receptor variant III-induced glioma invasion is mediated through myristoylated alanine-rich protein kinase C substrate overexpression. *Cancer Res* 2009;69:7548–7556.
147. Jarboe JS, Anderson JC, Duarte CW, Mehta T, Nowsheen S, Hicks PH, *et al*. MARCKS regulates growth and radiation sensitivity and is a novel prognostic factor for glioma. *Clin Cancer Res* 2012;18: 3030–3041.
148. Chen CH, Cheng CT, Yuan Y, Zhai J, Arif M, Fong LWR, *et al*. Elevated MARCKS phosphorylation contributes to unresponsiveness of breast cancer to paclitaxel treatment. *Oncotarget* 2015;6:15194–15208.
149. Manai M, Thomassin-Piana J, Gamoudi A, Finetti P, Lopez M, Eghozzi R, *et al*. MARCKS protein overexpression in inflammatory breast cancer. *Oncotarget* 2017;8:6246–6257.
150. Rombouts K, Carloni V, Mello T, Omenetti S, Galastri S, Madiai S, *et al*. Myristoylated alanine-rich protein kinase C substrate (MARCKS) expression modulates the metastatic phenotype in human and murine colon carcinoma *in vitro* and *in vivo*. *Cancer Lett* 2013;333:244–252.
151. Kuranami M, Powell CT, Hug H, Zeng Z, Cohen AM, Guillem JG. Differential expression of protein kinase C isoforms in human colorectal cancers. *J Surg Res* 1995;58:233–239.
152. Michel S, Kloor M, Singh S, Gdynia G, Roth W, von Knebel Doeberitz M, *et al*. Coding microsatellite instability analysis in microsatellite unstable small intestinal adenocarcinomas identifies MARCKS as a common target of inactivation. *Mol Carcinog* 2010;49:175–182.
153. Chen CH, Fong LWR, Yu E, Wu R, Trott JF, Weiss RH. Upregulation of MARCKS in kidney cancer and its potential as a therapeutic target. *Oncogene* 2017;36:3588–3598.
154. Song J, Wang Q, Luo Y, Yuan P, Tang C, Hui Y, *et al*. miR-34c-3p inhibits cell proliferation, migration and invasion of hepatocellular carcinoma by targeting MARCKS. *Int J Clin Exp Pathol* 2015;8: 12728–12737.
155. Brandi J, Dalla Pozza E, Dando I, Biondani G, Robotti E, Jenkins R, *et al*. Secretome protein signature of human pancreatic cancer stem-like cells. *J Proteomics* 2016;136:1–12.
156. Dorris E, O'Neill A, Hanrahan K, Treacy A, Watson RW. MARCKS promotes invasion and is associated with biochemical recurrence in prostate cancer. *Oncotarget* 2017;8:72021–72030.
157. Li T, Li D, Sha J, Sun P, Huang Y. MicroRNA-21 directly targets MARCKS and promotes apoptosis resistance and invasion in prostate cancer cells. *Biochem Biophys Res Commun* 2009;383: 280–285.
158. Yang Y, Chen Y, Saha MN, Chen J, Evans K, Qiu L, *et al*. Targeting phospho-MARCKS overcomes drug-resistance and induces antitumor activity in preclinical models of multiple myeloma. *Leukemia* 2015;29:715–726.
159. Liu H, Su P, Zhi L, Zhao K. miR-34c-3p acts as a tumor suppressor gene in osteosarcoma by targeting MARCKS. *Mol Med Rep* 2017; 15:1204–1210.
160. Doghri R, Manai M, Finetti P, Driss M, Agavnian E, Lopez M, *et al*. Stromal expression of MARCKS protein in ovarian carcinomas has unfavorable prognostic value. *Int J Mol Sci* 2017;19:E41.
161. Yang Z, Xu S, Jin P, Yang X, Li X, Wan D, *et al*. MARCKS contributes to stromal cancer-associated fibroblast activation and facilitates ovarian cancer metastasis. *Oncotarget* 2016;7:37649–37663.
162. Hanada S, Kakehashi A, Nishiyama N, Wei M, Yamano S, Chung K, *et al*. Myristoylated alanine-rich C-kinase substrate as a prognostic biomarker in human primary lung squamous cell carcinoma. *Cancer Biomark* 2013;13:289–298.
163. Chen CH, Chiu CL, Adler KB, Wu R. A novel predictor of cancer malignancy: up-regulation of myristoylated alanine-rich C kinase substrate phosphorylation in lung cancer. *Am J Respir Crit Care Med* 2014;189:1002–1004.
164. Chen CH, Statt S, Chiu CL, Thai P, Arif M, Adler KB, *et al*. Targeting myristoylated alanine-rich C kinase substrate phosphorylation site domain in lung cancer: mechanisms and therapeutic implications. *Am J Respir Crit Care Med* 2014;190:1127–1138.