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A Novel Predictor of Cancer Malignancy: Up-regulation of Myristoylated Alanine-Rich C Kinase Substrate Phosphorylation in Lung Cancer

To the Editor:

Lung cancer currently remains the leading cause of cancer-related deaths because of its aggressive nature. The 5-year survival rates for localized and regional disease are 54 and 26%, respectively, but only 4% for patients with late-stage (stage IV) disease (1). Thus, development of biomarkers to identify patients at high risk for aggressive progression is of urgent need. Recently, we have reported myristoylated alanine-rich C kinase substrate (MARCKS), predominantly its phosphorylated state, as a risk factor associated with lung cancer invasiveness and metastasis (2). MARCKS is a substrate of protein kinase C, and also a membrane-associated protein. Upon phosphorylation at Ser159 and Ser163 within its phosphorylation site domain, phosphorylated MARCKS (phospho-MARCKS) is detached from the plasma membrane and is able to regulate various cellular processes, including cell migration and exocytic vesicle release (2–4). In the lungs, MARCKS has been extensively studied because of its role in regulating mucus secretion and inflammation. Inhibition of MARCKS activity not only reduces airway mucus hypersecretion both *in vitro* and *in vivo* (3, 5), but also represses inflammatory leukocyte migration and degranulation (6, 7). There have been limited studies on MARCKS in cancer metastasis, but the results have been conflicting (8–13). This is because MARCKS expression is ubiquitous in various normal and tumor tissues. Despite this, there is a consensus that phospho-MARCKS, a post-translational modification, is associated with cell motility, and has a role in the regulation of cancer cell invasiveness and metastasis (2, 4, 14, 15). Of note, our laboratory discovered that inhibition of MARCKS phosphorylation was able to reduce lung cancer metastasis in murine models (2). However, the clinical significance of phospho-MARCKS in different cancers remains to be determined. In particular, there is limited information regarding its relevance in cancer progression, especially lung cancer.

Based on 18 pairs of normal and malignant lung cancer tissue sections, we previously reported that elevated phospho-MARCKS was seen in malignant lung cancer tissue sections, but not in their adjacent normal counterparts (2), suggesting a potential association between MARCKS phosphorylation and more aggressive lung cancer histological grades. To investigate more fully this previous finding, we analyzed samples from a cohort of 110 human patients with lung cancer using immunohistochemical staining with an anti-pSer159/163 MARCKS monoclonal antibody (*see* the online supplement). The clinical characteristics of these patients are summarized in Table 1. Consistent with our previous reports (2), high levels of MARCKS phosphorylation were found in tumor tissues compared with normal lung tissues (Figures 1A–1F). Weak phospho-MARCKS staining was observed in the cytoplasm of lung cancer tissue samples from patients in stage I (Figure 1C). In contrast, strong MARCKS phosphorylation occurred in advanced-stage lung cancer tissue samples (Figures 1D–1F). The levels of MARCKS phosphorylation correlated significantly with advanced stages of disease (Figure 1G, Pearson's chi-square test).

To quantitatively investigate these impressions, bivariate logistic regression models to predict the likelihood of high phospho-MARCKS levels from advanced tumor stages were estimated, and the probabilities of high phospho-MARCKS with stage I to III are shown in Figure 1H. The analyses demonstrated that, for a one-unit increase in stages II and III, the log odds of high expression of phospho-MARCKS levels increased by 1.00 and 2.46 compared with stage I. There were significant differences in the logistic probabilities of high phospho-MARCKS levels between stages I and II ($P = 0.039$), as well as stages I and III ($P < 0.001$), respectively. These results suggest that phospho-MARCKS may be a promising clinical predictor of tumor stages in patients with lung cancer.

Moreover, we also investigated the significance of phospho-MARCKS in lymph node status and found that higher levels of MARCKS phosphorylation correlated with lymph node metastasis (Figure 1I, N0 versus N1–2). Notably, MARCKS phosphorylation was lower in a subtype of adenocarcinoma, bronchoalveolar carcinoma, which shows a less invasive phenotype than adenocarcinoma (Figure 1J, AC versus bronchoalveolar carcinoma). Because tumor necrosis is a common event in aggressive cancers, we further checked phospho-MARCKS levels in the 10 tumor tissues with necrosis in this set of tissue arrays. Interestingly, we found higher staining intensity and increased numbers of cells stained with anti–phospho-MARCKS antibody in these tumors. These data raise the possibility that high phospho-MARCKS levels may contribute to cancer progression in non–small

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Table 1: Phosphorylated Myristoylated Alanine-Rich C Kinase Substrate Levels in Relation to Clinicopathologic Characteristics of Patients with Non–Small Cell Lung Cancer*

Characteristic	Total Patients	High [*No. of Patients (%)*]	Low [*No. of Patients (%)*]	*P* Value
Number of patients, n	110	65	45	
Age, yr (mean ± SD)	56.3 ± 9.5	56.5 ± 9.8	56.0 ± 9.1	0.768†
Sex, n				0.213‡
Male	75	41 (37.3)	34 (30.9)	
Female	35	24 (21.8)	11 (10.0)	
Stage, n				<0.001‡
I	48	18 (16.4)	30 (27.3)	
II	29	18 (16.4)	11 (10.0)	
III	32	28 (25.5)	4 (3.6)	
IV	1	1 (0.9)	0 (0.0)	
Cell type, n				0.045‡
Adenocarcinoma	48	33 (30.0)	15 (13.6)	
Squamous cell carcinoma	41	23 (20.9)	18 (16.4)	
Bronchioloalveolar carcinoma	4	0 (0.0)	4 (3.6)	
Other	17	9 (8.2)	8 (7.3)	
Regional lymph node, n				<0.001‡
N0	54	22 (20.0)	32 (29.1)	
N1	44	32 (29.1)	12 (10.9)	
N2	12	11 (10.0)	1 (0.9)	

*Patients were grouped by high levels (score = 2 and 3) and low levels (score = 0 and 1) of myristoylated alanine-rich C kinase substrate phosphorylation.
†*t* test.
‡Fisher's exact test.

cell lung cancers, and the detection of phospho-MARCKS could potentially be used as a prognostic biomarker for the disease.

In this correspondence, we demonstrate that higher MARCKS phosphorylation is correlated with lung cancer in advanced stages (stage II–IV), lymph node metastatic status, and malignant phenotypes. In addition to our previously published results (2), the current work further confirms the importance of phospho-MARCKS in driving the progression of lung cancer toward more malignancy, suggesting that phospho-MARCKS levels may determine the progression of localized lung cancer toward late stage. Taken together, high phospho-MARCKS levels appear to confer cancer malignancy, and may serve as a novel biomarker. Inhibition of MARCKS phosphorylation, the post-translational step, may be an effective strategy for controlling lung cancer progression. ■

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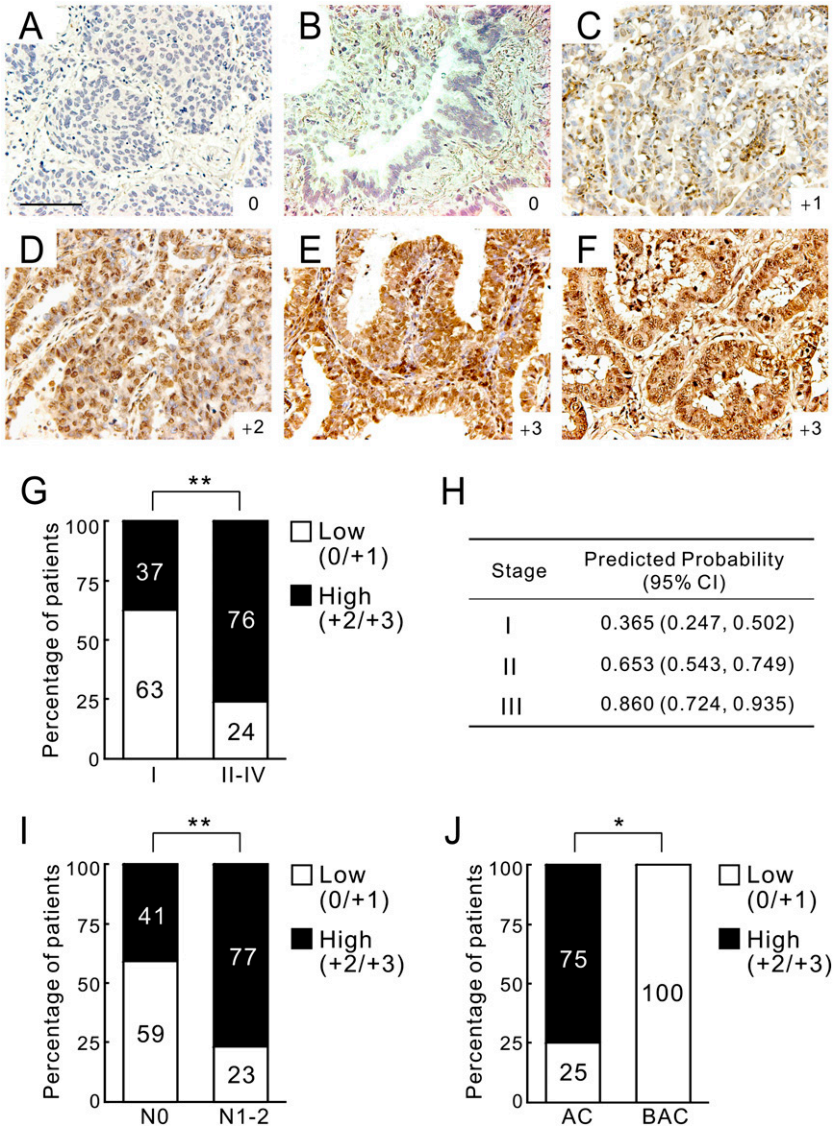

Figure 1. High phosphorylated myristoylated alanine-rich C kinase substrate (phospho-MARCKS) levels correlate with advanced stages, lymph node metastasis, and invasion of lung cancer. (*A–F*) Representative images of immunohistochemical staining using anti-pSer159/163 MARCKS monoclonal antibody in normal lung tissue and lung cancer specimens with low levels and high levels of phospho-MARCKS. *Scale bar* = 100 μm. (*A*) Staining with normal mouse IgG as negative control (score = 0). (*B*) Negative staining of phospho-MARCKS in normal lung bronchi. (*C*) Weak staining of phospho-MARCKS in lung cancer with stage I (score = +1). (*D*) Moderate staining of phospho-MARCKS in lung cancer with stage II (score = +2). (*E* and *F*) Strong staining of phospho-MARCKS in lung cancer with stage III (*E*) and IV (*F*) (score = +3). (*G*) Percentage of patients with high levels (score = +2 and +3) and low levels (score = 0 and +1) of MARCKS phosphorylation according to tumor stage. (*H*) Predicted probability of high phospho-MARCKS levels with different stages of lung cancer. (*I* and *J*) Percentage of patients with high and low levels of MARCKS phosphorylation according to lymph node status (*I*) and cancer types (*J*). Numbers in *bars* represent the percentage of patients for each condition. AC = invasive adenocarcinoma; BAC = bronchoalveolar carcinoma; CI = confidence interval. $^{*}P = 0.014$, $^{**}P < 0.001$, n = 110 (*G* and *I*); n = 52 (*J*).

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