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The expression and clinical effects of alpha-methylacyl-CoA racemase (AMACR/ P504S) as an immunohistochemical marker in malign pleural mesothelioma

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Background/aim: Alpha-methylacyl-CoA racemase (AMACR), an intracellular enzyme involved in lipid metabolism, has emerged as an immunohistochemical marker for many types of cancer. Recent studies about the role of lipid metabolism in pathogenesis of mesothelioma have brought up some positive results. This study was conducted to investigate AMACR expression in the diagnosis of malignant pleural mesothelioma (MPM) and the correlation of this marker with clinical characteristics and survival.

Materials and methods: The clinicopathologic characteristics and resection materials of 71 patients were reviewed retrospectively. AMACR expression was evaluated immunohistochemically. The correlations among AMACR expression, clinicopathologic factors, and survival were investigated.

Results: AMACR expression was detected in 42.3% of the study group. The specificity and sensitivity of AMACR immunostaining in detecting mesothelioma were 41.1% and 42.3%, respectively. AMACR-positive and negative groups were similar for age, sex, smoking history, tumor diameter, lymph node involvement, differentiation, T–N factor, and stage. Overall survival was not significantly different between the groups, either.

Conclusion: The sensitivity of immunostaining was not high enough to use AMACR as a diagnostic tool in MPM. AMACR expression did not have a prognostic value in MPM, either.

Key words: AMACR, carcinogenesis, mesothelioma

1. Introduction

The most common primary malignant tumor of the pleura is malignant mesothelioma (MPM). It has four major histological subtypes: epithelioid, sarcomatoid, desmoplastic, and biphasic (1).

Mesothelioma appears to have a complex etiology in which environmental carcinogens (asbestos and erionite), ionizing radiation, dietary factors, viruses, and genetic factors act alone or in concert to cause malignancy (2,3). Current studies showed that lipid metabolism may have an important role in pathogenesis suggesting new targets for treatment of MPM (4–6).

Alpha-methylacyl-CoA racemase (AMACR) is an intracellular enzyme that is involved in the beta-oxidation of branched fatty acids (7,8). There is increasing evidence showing that AMACR is a useful marker for many cancers of the prostate, liver, kidney, and colon (9–11). However, the expression and clinical effects of AMACR in mesothelioma have not been researched yet. This study investigated AMACR expression and its correlation with clinical characteristics and survival of patients with MPM.

2. Materials and methods

2.1. Patients

The clinicopathologic characteristics of 71 patients who underwent a surgical resection for therapeutic and/or diagnostic approaches between June 2000 and June 2009 in our institute were reviewed retrospectively. In the preoperative evaluation, the results of a biochemistry panel including renal and liver function tests, alkaline phosphatase and serum calcium levels, complete blood count, posteroanterior chest radiographs, computed tomographic scans of the thorax including the upper abdomen, and bronchoscopy were obtained for all of the patients. The relationships between AMACR immunoreactivity and clinicopathologic factors, including

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age, sex, smoking history, histological type of tumor, pathologic T–N status, and stage were evaluated.

For correlating pathological findings with survival, patients who died due to postoperative mortality (1 patient) and patients whose date of death was unknown (17 patients) were all excluded. The remaining group comprised 53 patients.

The stage of each patient was evaluated according to the staging system of the International Mesothelioma Interest Group (IMIG), revised in 1995 (12).

2.2. Surgical procedure and treatment modality

The surgical procedures consisted of 36 video-assisted thoracic surgeries (VATS) (50.7%), 14 decortications (19.7%), 5 pleuropneumonectomies (7%), and 16 chest wall/pleural biopsies (22.5%). VATS and biopsies were performed for diagnostic approaches, whereas decortications and pleuropneumonectomies were for therapeutic approaches. The patient's performance status, stage of the disease, and the patient's own choices were considered in the choice of treatment modality. Treatment modalities applied to this group of patients were local radiotherapy (4 patients, 5.7%), chemotherapy with cisplatin and gemcitabine or pemetrexed (7 patients, 10%), local radiotherapy and chemotherapy (6 patients, 8.6%), pleuropneumonectomy (5 patients, 7.1%), decortication (7 patients, 10%), and decortication and radiotherapy and/or chemotherapy (6 patients, 8.6%). After diagnostic procedures, 35 patients (50%) did not receive any of these treatments due to their poor performance status or refusal of treatment.

2.3. Pathologic evaluation

All specimens were histologically reviewed by one pathologist and the most representative slides of tumors were selected. Immunohistochemistry was carried out on 5-µm-thick paraffin sections. Sections were dewaxed in a xylene substitute (Thermo Scientific, USA) and hydrated with a graded series of ethanol concentrations and water. Subsequently, antigen retrieval was obtained by boiling in 0.01 mol/L citrate buffer (pH 6.0) for 20 min in a microwave oven. Sections were incubated with primary antibody solution for rabbit monoclonal AMACR/p504S (clone 13H4, Thermo Scientific) at a dilution of 1:100 for 30 min at room temperature. Immunostaining was performed with a streptavidin–biotin complex kit (Thermo Scientific). Diaminobenzidine was used as a chromogen. After incubation, chromogen specimens were counterstained with Harris hematoxylin and cover-slipped. Samples of prostate carcinoma were used as a positive control. Negative controls omitting the primary antibodies were also included.

The intensity of AMACR immunostaining was evaluated by light microscopy (Labophot 2; Nikon, Japan). Only cytoplasmic staining was considered. Intensity of staining was graded as 1+ weak, 2+ moderate, 3+ strong. Tumors without any staining were considered AMACR-negative (13) (Figure 1).

2.4. Statistical analyses

Pearson's chi-squared test, Yates' chi-squared test, or Fisher's exact test were used to compare differences among groups for categorical variables. The survival curves were estimated using the Kaplan–Meier method. Patient survival was expressed by using time zero as the date of pathologic diagnosis and death as the end point. The log-rank test was used for comparison of the survival curves in univariate analysis. In all the statistical analyses, $P < 0.05$ was considered statistically significant. All statistical analyses were performed by SPSS for Windows, version 15.0.

3. Results

The clinical and pathological characteristics of the patients are summarized in Table 1. Positive staining was detected in 42.3% of the cases. The expression of AMACR according to histological subtypes is expressed in Table 2.

Forty-two percent of 50 patients with epithelioid mesothelioma and 52.9% of 17 patients with biphasic mesothelioma were positive for AMACR immunostaining. Epitheloid sections were found to be AMACR-positive in four patients with biphasic mesothelioma; in five patients, only sarcomatoid sections showed positive staining. The remaining 9 patients with biphasic mesothelioma were totally AMACR-negative. However, the patients with pure sarcomatoid (2 cases) and desmoplastic mesothelioma (2 cases) were AMACR-negative.

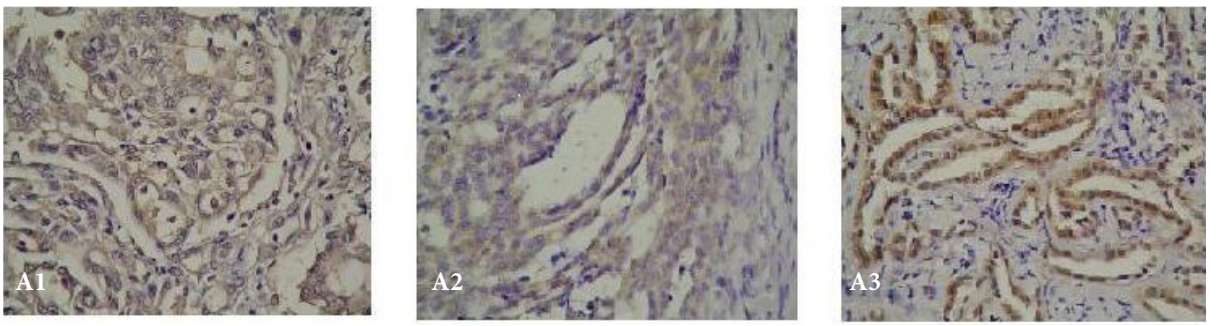

Figure 1. Samples of slides with MPM showing cytoplasmic AMACR immunostaining with weak (A1), moderate (A2), and strong (A3) intensity (AMACRX200. H&E).

Table 1. Patient characteristics.

	n = 71	
	n	%
Age (years)	56.17 ± 11.39	
<60 (median)	40	56.30
≥60	31	43.70
Sex		
Female	26	36.60
Male	45	63.40
History of smoking		
Ever smoked	35	49.3
Nonsmokers	36	50.7
Median package/year smoking	22 (10–100)	
T factor		
T1		
T1a	3	4.2
T1b	0	0
T2	39	54.9
T3	17	23.9
T4	12	16.9
N factor		
N0	63	88.7
N2	7	9.9
N3	1	1.4
Stage		
I	2	2.80
II	35	49.30
III	19	26.80
IV	15	21.10
Histological subtypes		
Epithelioid	50	70.40
Sarcomatoid	2	2.80
Biphasic	17	23.90
Desmoplastic	2	2.8
Type of operation		
VATS	36	50.7
Decortication	14	19.70
Pleuropneumonectomy	5	7.00
Chest wall/pleural biopsy	16	22.50

VATS: Video-assisted thoracic surgery.

Table 2. AMACR immunoreactivity according to histological subtypes.

AMACR	Positive staining		Intensity of staining			
	n	%	0 (%)	1+ (%)	2+ (%)	3+ (%)
MPM	30	42.3	41 (57.7)	14 (19.7)	8 (11.3)	8 (11.3)
Epitheloid			29 (58.0)	9 (18.0)	6 (12.0)	6 (12.0)
Sarcomatoid			2 (100.0)			
Biphasic			8 (47.1)	5(29.4)	2(11.8)	2(11.8)
Desmoplastic			2 (100.0)			

AMACR: Alpha-methylacyl-CoA racemase, MPM: malignant pleural mesothelioma.

As shown in Table 3, AMACR expression and clinicopathologic factors, including age, sex, smoking history, pathologic T–N status, and stage, did not show a statistically significant correlation ($P > 0.05$).

In a previous study of our clinic we had explored AMACR immunoreactivity in adenocarcinoma and squamous cell carcinoma of the lung (14). When we compared our results with the results of the adenocarcinoma group (73 cases) in the previous study, we found that AMACR immunoreactivity was observed more frequently in adenocarcinoma group than in the MPM group ($P = 0.046$). The specificity and sensitivity of AMACR immunostaining in detecting MPM were 42.3% and 41.1%, respectively.

After the patients were excluded according to the criteria mentioned above, data from remaining 53 patients were evaluated for survival analysis. The median survival for the AMACR-negative group was 13 ± 3.65 months whereas it was 16 ± 5.96 months for the AMACR-positive group. Although survival lines on the graph seem to be parallel, we could not show a statistically significant effect of AMACR on survival ($P = 0.190$, log-rank). In a larger group of patients the results may be statistically significant.

We performed Cox regression analysis to reveal the effect of age, sex, history of smoking, N status, stage, therapy modality, histological subtype, and AMACR immunoreactivity on overall survival (Table 4).

N status was proven to have a statistically significant effect on overall survival of patients with MPM ($P = 0.001$). However, we could not show prognostic value of the other variables ($P > 0.05$).

4. Discussion

MPM is an important threat to public health, especially in regions with environmental exposure to asbestos. The pathologic differential diagnosis of MPM depends on electron microscopic, histochemical, and immunohistochemical studies. However, none of these methods are 100% specific (1). Therefore, new methods, especially new immunohistochemical markers, for diagnosis and prognostic estimation are of interest for medical research.

AMACR is an intracellular enzyme that is bimodally distributed to both the peroxisome and the mitochondrion. It has an important role in the beta-oxidation of branched fatty acids, bile acid intermediates, and metabolism of ibuprofen (7,8).

This enzyme takes a part in beta-oxidation of phytanic acid and pristanic acid. These branched chain fatty acids are naturally occurring ligands for the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-α). PPAR-α is one of the nuclear receptor proteins that has an important role in cellular differentiation, evolution, and metabolism (8). The enhancement of PPAR-γ activity with its ligands, and the suppression of PPAR-α with its inhibitors, may prevent the formation of lung tumors, as well as accelerate lung cancer therapy (9). Therefore, it is hypothesized that AMACR, which plays a role in the metabolism of the ligands of these nuclear receptors, may affect the pathogenesis and prognosis of cancer. Reactive oxygen radicals formed by beta-oxidation of branched chain fatty acids may also contribute to DNA damage in carcinogenesis. An alternative hypothesis would suggest that AMACR is overexpressed in the development of cancer, perhaps playing an important role in providing energy for the neoplastic cells. As the tumors become dedifferentiated, they no longer require these sources of energy.

AMACR overexpression was first proven to be an important diagnostic marker for prostate carcinoma, but recent studies showed that it is overexpressed in several cancers, including colorectal carcinomas, papillary renal cell carcinomas, hepatocellular carcinomas, melanoma, lymphoma, and endometrium, lung, breast, bladder, and sebaceous neoplasms (9–11,15–21). AMACR is also overexpressed in precursor lesions like high-grade

Table 3. AMACR expression according to clinicopathologic factors.

	AMACR(+)		AMACR (-)		P-value
	n	%	n	%	
Median age (years)					
<60	19	63.30	21	51.20	0.439
≥60	11	36.70	20	48.80	
Sex					
Female	13	43.30	13	31.70	0.45
Male	17	56.70	28	68.30	
History of smoking					
Ever smoked	15	50.00	20	48.8	1
Nonsmokers	15	50	21	51.2	
T factor					
T1	3	10.00	0	0.00	0.146
T2	17	56.70	22	53.70	
T3	7	23.30	10	24.40	
T4	3	10.00	9	22.00	
N factor					
N0	26	86.70	37	90.20	0.714
N2–N3	4	13.30	4	9.80	
p-Stage (pTNM)					
I	2	6.70	0	0.00	0.445
II	15	50.00	20	48.80	
III	8	26.70	11	26.80	
IV	5	16.70	10	24.40	

AMACR: alpha-methylacyl-CoA racemase.

prostatic intraepithelial neoplasia and colonic adenomas (18), but there is a limited number of studies reporting on AMACR expression in lung cancer (9,11,13,18). Shilo et al. reported that 47% of 477 pulmonary carcinomas were positive for AMACR; among tumor types, 22% of squamous cell carcinoma and 56% of adenocarcinoma were AMACR-positive (13). Zhou et al. found that AMACR was overexpressed in lung cancer while Jiang et al. concluded that lung cancers were negative or rarely positive for AMACR (11,18). The main cause of this contradiction is the type of antibody used in these studies. In the study by Zhou et al., a polyclonal antibody was used but Jiang et al. used a monoclonal antibody. Nassar et al. found that only 14.3% of twenty-eight specimens with lung cancer were positive for AMACR, where only moderate and strong staining was considered as positive (9).

However, this is the first study about the expression and clinical effects of AMACR in mesothelioma. In our study, we used a monoclonal antibody and considered weak staining as positive, which resulted in 42.3% positive staining in all patients. However, we observed that the expression of AMACR differs according to histological subtypes. Epithelioid and biphasic mesothelioma had AMACR immunoreactivity rates of 42% and 52.9%,

Table 4. Prognostic factors in MPM.

Variables	HR	95.0% CI		P-value
Sex (male/female)	1.508	0.550	4.130	0.425
Age (>60/≤60 years)	1.426	0.639	3.186	0.386
History of smoking (ever/none)	1.793	0.616	5.215	0.284
N factor				
N2–N3/N0	6.585	2.117	20.483	0.001
Stage				
Stages III–IV/Stages I–II	1.053	0.433	2.561	0.909
Histological subtypes				
Epithelioid/others	1.976	0.738	5.290	0.175
Therapy modality				
RT/none	0.789	0.096	6.451	0.825
CT/none	0.38	0.100	1.440	0.155
RT + CT/none	0.552	0.168	1.818	0.328
PP/none	0.843	0.163	4.366	0.839
Decortication/none	0.605	0.164	2.226	0.449
Decortication + RT and/or CT/none	0.467	0.122	1.782	0.265
AMACR (–/+)	2.141	0.91	5.039	0.081

AMACR: Alpha-methylacyl-CoA racemase. MPM: malignant pleural mesothelioma.
HR: hazard ratio. CI: confidence interval. RT: radiotherapy. CT: chemotherapy.
PP: pleuropneumonectomy. None: the patients who did not receive any treatment.

respectively, whereas the patients with pure sarcomatoid and desmoplastic mesothelioma were totally AMACR-negative. Additionally, sarcomatoid sections showed positive staining in only five of the patients with biphasic histology. We recommend that AMACR immunoreactivity in the differential diagnosis of sarcomatoid and desmoplastic mesothelioma must be further researched.

The differential diagnosis of MPM from metastatic neoplasms of pleura, especially adenocarcinoma of lung, is one of the most common dilemmas of thoracic pathology. A definitive diagnosis of MPM requires a pathologic workup including immunohistochemistry. Positive carcinoma markers recommended for adenocarcinoma are thyroid transcription factor 1, carcinoembryonic antigen, LeuM1(CD15), Ber-Ep4, B72.3, HMFG-2, MOC31, and BG8 (Lewis^{Y}). There is an increasing interest in defining positive mesothelioma markers. Positive mesothelioma markers recommended recently include WT-1 protein, keratin 5/6, podoplanin (D2-40), HBM1, thrombomodulin, and calretinin (22,23).

In a previous study, which included 73 patients with adenocarcinoma and 69 patients with squamous cell carcinoma of the lung, the positive AMACR-immunoreactivity for each group was 59% and 42%, respectively (14). When we compared our results with the results of the adenocarcinoma group in the previous study, we found that AMACR immunoreactivity was observed more frequently in the adenocarcinoma group than in the MPM group (P = 0.046). The specificity and sensitivity of AMACR immunostaining in detecting MPM were 42.3% and 41.1%, respectively. The minimum sensitivity of an immunohistochemical marker recommended for clinical use must be at least 80% (23). The specificity and sensitivity of AMACR immunostaining in detecting MPM were not sufficient enough to recommend AMACR alone as a diagnostic tool in differential diagnosis.

The pattern of immunohistochemical staining is also important with certain antibodies; calretinin requires both cytoplasmic and nuclear staining to support a diagnosis of mesothelioma, while WT-1 should be only nuclear (23). The pattern of immunohistochemical staining for AMACR is characterized by fine granular cytoplasmic staining due to its localization in the peroxisome and the mitochondrion. This pattern is easy to recognize but in some studies about prostate carcinoma weak staining was accepted as negative because some benign prostate lesions show weak staining with AMACR (13). This is logical, especially if a polyclonal antibody was used for minimizing false positive results. However, due to studies showing that AMACR immunoreactivity does not exist

in alveolar parenchyma (9,11,13) and the monoclonal antibody used in this study, we considered a weak staining pattern to be positive.

Immunohistochemical markers also serve as prognostic indicators. A tissue-specific effect of AMACR immunoreactivity on degree of differentiation in colon, breast, and bladder carcinomas was revealed by some studies (24,25). The results of our study did not indicate any correlations with the clinicopathologic factors including age, sex, pathologic T status, therapy modality, or histological subtype or stage ($P > 0.05$). However, in Cox regression analysis, N status was proven to have a statistically significant effect on overall survival of patients with MPM ($P = 0.001$). This result emphasizes the prognostic significance of lymph node involvement in the IMIG staging system for MPM. It was found that treatment modalities used for this group of patients did not affect the overall survival of patients. We think this may be a good clue when it comes to researching new therapy modalities for this challenging disease. Recent efforts in multimodality treatment include treatment of a symptomatic malignant pleural effusion through an indwelling pleural catheter, systemic treatment with targeted agents, addition of monoclonal antibodies to a standard chemotherapy backbone, new techniques in radiation therapy, pleural intensity-modulated radiotherapy, helical tomography, and proton therapy (26). We may support these efforts by encouraging our patients to participate in these clinical trials.

In a study by Shilo et al., low-intensity staining in small-cell carcinoma of lung in stages I and II was associated with worse patient outcome (13). AMACR immunoreactivity may be more intense in metabolically active cells. Therefore, AMACR-positive groups might show better response to chemotherapy, which may result in better prognosis for AMACR-positive groups. Similar to these studies, the median survival of the AMACR-positive group in our study was better than that of the AMACR-negative group (16 and 13 months, respectively), but this correlation could not be proven statistically through overall survival analysis ($P = 0.190$). We think that the relatively small number of patients included in this analysis and the heterogeneity of our group in terms of performance status and other prognostic factors might cause these statistically nonsignificant results in survival analysis.

In conclusion, AMACR alone cannot be used as a diagnostic tool in the differential diagnosis of MPM, but we recommend that the effect of AMACR expression on survival and response to chemotherapy be further investigated in large-scale studies with a more homogeneous study population.

References

1. Sterman DH, Litzky LA, Albelda SM. Malignant mesothelioma and other primary pleural tumors. In: Fishman AP, Elias JA, Fishman JA, Grippi MA, Senior RM, Pack AI, editors. Fishman's Pulmonary Diseases and Disorders. 4th ed. New York, NY, USA: McGraw-Hill; 2008. pp. 1535–1552.
2. Carbone M, Kratzke RA, Testa JR. The pathogenesis of mesothelioma. Semin Oncol 2002; 29: 2–17.
3. Huncharek M. Non-asbestos related diffuse malignant mesothelioma. Tumori 2002; 88: 1–9.
4. Gabrielson EW, Pinn ML, Testa JR, Kuhajda FP. Increased fatty acid synthase is a therapeutic target in mesothelioma. Clin Cancer Res 2001; 7: 153–157.
5. Niu K, Asada M, Okazaki T, Yamanda S, Ebihara T, Guo H, Zhang D, Nagatomi R, Arai H, Kohzuki M et al. Adiponectin pathway attenuates malignant mesothelioma cell growth. Am J Respir Cell Mol Biol 2012; 46: 515–523.
6. Cardillo I, Spugnini EP, Verdina A, Galati R, Citro G, Baldi A. Cox and mesothelioma: an overview. Histol Histopathol 2005; 20: 1267–1274.
7. Ferdinandusse S, Denis S, IJlst L, Dacremont G, Waterham HR, Wanders RJA. Subcellular localization and physiological role of a-methylacyl-CoA racemase. J. Lipid Res 2000; 41: 1890–1896.
8. Zomer AW, van Der Burg B, Jansen GA, Wanders RJ, Poll-The BT, van Der Saag PT. Pristanic acid and phytanic acid: naturally occurring ligands for the nuclear receptor peroxisome proliferator-activated receptor. J Lipid Res 2000; 41: 1801–1807.
9. Nassar A, Amin MB, Sexton DG, Cohen C. Utility of alpha-methylacyl coenzyme A racemase (p504s antibody) as a diagnostic immunohistochemical marker for cancer. Appl Immunohistochem Mol Morphol 2005; 13: 252–255.
10. Luo J, Zha S, Gage WR, Dunn TA, Hicks JL, Bennett CJ, Ewing CM, Platz EA, Ferdinandusse S, Wanders RJ et al. Alpha-methylacyl-CoA racemase: a new molecular marker for prostate cancer. Cancer Research 2002; 62: 2220–2226.
11. Jiang Z, Fanger GR, Woda BA, Banner BF, Algate P, Dresser K, Xu J, Chu PG. Expression of alpha-methylacyl-CoA racemase (P504s) in various malignant neoplasms and normal tissues: a study of 761 cases. Hum Pathol 2003; 34: 792–796.
12. Rusch VW. A proposed new international TNM staging system for malignant pleural mesothelioma. From the International Mesothelioma Interest Group. Chest 1995; 108: 1122–1128.
13. Shilo K, Dracheva T, Mani H, Fukuoka J, Sesterhenn IA, Chu WS, Shih JH, Jen J, Travis WD, Franks TJ. Alpha-methylacyl CoA racemase in pulmonary adenocarcinoma, squamous cell carcinoma, and neuroendocrine tumors: expression and survival analysis. Arch Pathol Lab Med 2007; 131: 1555–1560.

14. Erdogan Y, Demirag F, Duyar SS, Yilmaz A, Yazıcı Ü, Aydoğdu K. The expression and clinical effects of alpha-methylacyl-CoA racemase (AMACR/ P504S) in squamous cell carcinoma and adenocarcinoma of lung. Acta Chir Belg 2011; 113: 263–269.

15. Li MY, Yuan H, Ma LT, Kong AW, Hsin MK, Yip JH, Underwood MJ, Chen GG. Roles of PPAR{alpha} and PPAR {gamma} in the development of non-small cell lung cancer. Am J Respir Cell Mol Biol 2010; doi:10.1165/rcmb.2009-0349OC.

16. Xu J, Stolk JA, Zhang X, Silva SJ, Houghton RL, Matsumura M, Vedvick TS, Leslie KB, Badaro R, Reed SG. Identification of differentially expressed genes in human prostate cancer using subtraction and microarray. Cancer Res 2000; 60: 1677–1682.

17. Daugherty SE, Platz EA, Shugart YY, Fallin MD, Isaacs WB, Chatterjee N, Welch R, Huang WY, Hayes RB. Variants in the alpha-methylacyl-CoA racemase gene and the association with advanced distal colorectal adenoma. Cancer Epidemiol Biomarkers Prev 2007; 16: 1536–1542.

18. Zhou M, Chinnaiyan AM, Kleer CG, Lucas PC, Rubin MA. Alpha-methylacyl-CoA racemase: a novel tumor marker overexpressed in several human cancers and their precursor lesions. Am J Surg Pathol 2002; 26: 926–931.

19. Tretiakova MS, Sahoo S, Takahashi M, Turkyilmaz M, Vogelzang NJ, Lin F, Krausz T, Teh BT, Yang XJ. Expression of alpha-methylacyl-CoA racemase in papillary renal cell carcinoma. Am J Surg Pathol 2004; 28: 69–76.

20. Went PT, Sauter G, Oberholzer M, Bubendorf L. Abundant expression of AMACR in many distinct tumour types. Pathology 2006; 38: 426–432.

21. Halsey MA, Calder KB, Mathew R, Schlauder S, Morgan MB. Expression of alpha-methylacyl-CoA racemase (P504S) in sebaceous neoplasms. J Cutan Pathol 2010; 37: 446–451.

22. Comin CE, Novelli L, Boddi V, Paglierani M, Dini S. Calretinin, thrombomodulin, CEA, and CD15: a useful combination of immunohistochemical markers for differentiating mesothelioma from pulmonary adenocarcinoma. Hum Pathol 2001; 32: 529–532.

23. Husain AN, Colby T, Ordonez N, Krausz T, Attanoos R, Beasley MB, Borczuk AC, Butnor K, Cagle PT, Chirieac LR et al. Guidelines for pathologic diagnosis of malignant mesothelioma: a consensus statement from the International Mesothelioma Interest Group. Arch Pathol Lab Med 2009; 133: 1317–1331.

24. Witkiewicz AK, Varambally S, Shen R, Mehra R, Sabel MS, Ghosh D, Chinnaiyan AM, Rubin MA, Kleer CG. Alpha-methylacyl-CoA racemase protein expression is associated with the degree of differentiation in breast cancer using quantitative image analysis. Cancer Epidemiol Biomarkers Prev 2005; 14: 1418–1423.

25. Kuefer R, Varambally S, Zhou M, Lucas PC, Loeffler M, Wolter H, Mattfeldt T, Hautmann RE, Gschwend JE, Barrette TR et al. α-Methylacyl-CoA racemase: expression levels of this novel cancer biomarker depend on tumor differentiation. Am J Pathol 2002; 161: 841–848.

26. Hiddinga BI, Surmont VF, Van Meerbeeck JP. Management of malignant pleural mesothelioma: have we made any progress? Panminerva Med 2013; 55: 157–173.