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ORIGINAL ARTICLE

Monoclonal Caveolin 1 Expression in the Differential Diagnosis of Malignant Pleural Mesothelioma and Pulmonary Adenocarcinoma: Is it Useful?

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Abstract
In this study we aim to demonstrate the value of monoclonal Caveolin 1 expression in distinguishing between malignant pleural mesothelioma and pulmonary adenocarcinoma. Total of 129 cases, consisting of 68 cases of malignant pleural mesothelioma (51 epitheloid, 12 biphasic, and 5 sarcomatoid type) and 61 cases of pulmonary adenocarcinoma were examined and stained with monoclonal Caveolin-1. Caveolin 1 expression with a membranous and /or cytoplasmic pattern was detected only in 32.35% (n:22/68) of malignant pleural mesothelioma and 6.5% (n:4/61) of pulmonary adenocarcinoma cases. This finding suggests that the choice of poly/monoclonal antibody for Caveolin 1 in the differential diagnosis of malignant pleural mesothelioma and pulmonary adenocarcinoma is important.

Keywords Caveolin 1 · Mesothelioma · Pulmonary adenocarcinoma

Introduction

Malignant pleural mesothelioma (MPM) is a rare and aggressive malignant tumor arising from the mesothelial cells lining the serosal surfaces [1]. Even though asbestos exposure is the main risk factor for the disease, synthetic materials such as ceramics and nanoparticles, ionizing radiation, and the SV-40 virus, are other potential cofactors [1, 2]. The global incidence of MPM has risen steadily over the past decade, and is predicted to continue to an estimated peak in 2020 [1]. The prognosis of MPM is poor and median survival ranges from 8 to 14 months from diagnosis [2, 3]. As the tumor has a heterogeneous morphologic phenotype and similar clinical presentation, it overlaps with benign mesothelial proliferations or nonmesothelial tumors involving the serosal membranes [4].

This study was presented as a poster presentation in the part of the 31th International Congress of the International Academy of Pathology and 28th Congress of the European Society of Pathology, Cologne, Germany, 2016.

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After excluding benign entities and metastatic tumors, pulmonary adenocarcinoma (PA) is the main dilemma for the differential diagnosis. Hence, as MPM and PA patients are treated differently and have different prognoses, it is very important that they are diagnosed correctly and differentiated from other malignancies. Immunohistochemical markers are mainly used to differentiate MPM from benign and malignant conditions [5, 6]. In the past few decades, a large number of immunohistochemical markers that are frequently expressed in carcinomas and mesotheliomas have been investigated. However, the sensitivity and the specificity of these markers are limited. There is still no unique immunohistochemical marker for the diagnosis. The use of a panel of immunohistochemistry markers is now an indispensable routine for MPM diagnosis. However, despite the use of several well-known markers, the diagnosis is still challenging in some cases. Therefore, many studies are being conducted to investigate new markers that can improve the diagnosis [4, 7, 8].

Caveolin-1 (Cav-1), is a member of the caveolin protein family, which is a structural protein of the endocyticcaveolae plasma membrane. It is highly expressed in adipocytes, endothelial cells, Type I pneumocytes, fibroblasts and these terminal differentiation cells. Cav-1 regulates multiple cancer-associated processes, including cellular transformation, tumor growth, cell migration, metastasis, cell death and survival [9]. It has also been presented as a new marker for MPM [10]. In

this study we aim to demonstrate the value of monoclonal Cav-1 staining in distinguishing between MPM and PA.

Materials and Methods

Patients and Sample Collection

The samples included in this study were obtained from the archives of Gaziantep University's Pathology Department. The study group consisted of 68 cases of MPM (51 epitheloid, 12 biphasic, and 5 sarcomatoid type) and 61 cases of PA of mixed subtypes. PA showed different combinations of histological subtypes: papillary, acinar, solid growth with and without mucin. All of the specimens were derived from patients who had undergone surgical resection. All cases were diagnosed by an expert pulmonary pathologist (KB) based on at least two positive (mesothelial) and two negative (epithelial) immunohistochemical markers. The most frequently chosen markers were pCEA, MOC 31, CK5/6 and calretinin. WT1, TTF-1, D2–40 were also used if needed. The small biopsies were excluded to see the adequate tumoral area for immunohistochemical staining.

The clinical data was retrieved from the hospital records and the approval of the Ethics Committee was obtained (16/236).

Immunohistochemical Staining

The hematoxylin and eosin (H&E) and immunohistochemistry-stained slides of each case were reevaluated under light microscopy by two pathologists (ZB and OFD). Four-micron sections were taken from appropriate formalin-fixed-paraffin-embedded tissue blocks. The immunohistochemical antibody of Cav-1 (NCL-L-Caveolin-1, Leica, mouse monoclonal antibody, 1/200) was studied using an automated immunohistochemistry-staining device (Ventana Ultra Auto-Stainer). The cytoplasmic and/or membranous staining of Cav-1 were accepted as positive and alveolar or endothelial cells around the tumor or inside the tumor were also stained for Cav-1 used as a positive internal control.

The immunoreactivity of the cases for Cav-1 were graded based on both the percentage of positive cells and the intensity of immunopositivity (at ×100 magnification). The percentage of immunopositivity was given a numerical score: 0; negative, 1; positivity in 1–10% of tumor cells, 2; positivity in 11–50% of tumor cells, and 3; positivity in >50% of tumor cells. On the other hand, the intensity of immunoreactivity was scored as none (0), mild (1+), moderate (2+) and strong (3+) by comparing the positive internal control similar with the published criteria (10).

As a result, the cases were considered to be positive when >1% tumor cells showed cytoplasmic and/or membranous Cav-1 expression with any intensity.

Statistical Analysis

The categorical variables were analyzed using the Pearson Chi-Square Test with IBM SPSS® Version 23.0 for Window 7. *P* values less than 0.05 were accepted as significant.

Results

In this study, we investigated staining intensity in a total of 129 cases consisting of 68 cases of MPM (51 epitheloid, 12 biphasic, and 5 sarcomatoid type) and 61 cases of PA. Patient characteristics are summarized in Tables 1 and 2.

Cav-1 expression with a membranous and /or cytoplasmic pattern was detected only in 32.35% (n:22/68) of MPM and 6.5% (n:4/61) of PA cases. The internal positive control which was detected in all cases were the alveolar or endothelial cells around and inside the tumor. The distribution of positive MPM cases according to their histologic subtypes were as following; 15 (29.41%, n:15/51) epitheloid, 5 (41.66%, n:5/12) biphasic and 2 (40.0%, n:2/5) sarcomatoid type MPM showed Cav-1 expression. Two of the positive epitheloid MPM cases had a history of neoadjuvant chemotherapy, wheras 2 other cases with a history of chemotherapy showed

Table 1 Clinicopathologic characteristics of 68 patients with malignant pleural mesothelioma

Characteristics	No. (%) of cases
Total	68 (100)
Sex	
Female	26 (38)
Male	42 (62)
Age	
<65	48 (71)
≥65	20 (29)
Histology	
Epitheloid	51 (75)
Biphasic	12 (18)
Sarcomatoid	5 (7)
Neoadjuvant chemotherapy	
Yes	4 (6)
No	64 (94)
Status	
Alive with disease	5 (7)
Dead	63 (93)
Survival month	
<24	47 (69)
>24	16(24)

Table 2 Clinicopathologic characteristics of 61 patients with pulmonary adenocarcinoma

Characteristics	No. (%) of cases
Total	61 (100)
Sex	
Female	13 (21)
Male	48 (79)
Age	
<65	34 (56)
≥65	27 (44)
Histology	
Aciner	27 (44)
Solid	26 (43)
Lepidic	5 (8)
Micropapillary	2 (3)
Mucinous	1 (2)
pT classification	
T1	14 (23)
T2	26 (43)
T3	9 (18)
T4	12 (20)
pN classification	
No	38 (62)
Yes	23 (38)
Status	
Alive with disease	22 (36)
Dead	39 (64)
Survival month	
<36	36 (59)
>36	25 (41)

no positivity. Four cases of positive PA were solid types (Fig. 1).

The distribution of positive MPM cases according to their percentage was as following; 12 showed 1+ (1–10%), 7 showed 2+ (11–50%), and 3 showed 3+ (>50%), positivity with Cav-1. Four cases of PA showed 1+ (1–10%) positivity. The data is summarized in the Table 3.

Statistically, there was no significant difference between the two groups.

Discussion

Due to MPM's heterogeneous morphology, it may exhibit similar characteristics to reactive benign mesothelial proliferations and various nonmesothelial malignant tumors [4]. PA cases demonstrating pleural involvement in particular and epithelial MPM cases may constitute serious diagnostic obstacles for pathologists. The increased global incidence of MPM, especially in the last 30 years, has led to a search for new immunohistochemical markers that can be beneficial in differential diagnosis in this area [4, 7, 8]. However, still no marker that can be sufficient in diagnosis on its own has been found. Therefore, the IHC panel containing 2 positive and 2 negative mesothelioma markers should be used in line with the recommendation of the International Mesothelioma Interest Group [11, 12]. No definite consensus is available on the markers to be selected in MPM. The variety of positive markers expressed in mesothelioma and negative markers expressed in carcinomas but not in MPM and the fact that none of them are 100% sensitive and specific on their own is a motivating factor for investigators to find new markers.

Although there are different opinions, while the 4 positive markers frequently used in mesothelioma are calretinin (nuclear staining), cytokeratin 5/6 (cytoplasmic staining), WT-1 (nuclear staining) and D2–40 (cytoplasmic staining), the 5 positive markers in carcinoma are polyclonal carcinoembryonic antigen ((pCEA), cytoplasmic staining), CD15 (LeuM1, nuclear staining), Ber-EP4 (membranous staining), MOC 31 (nuclear staining) and TTF1 (nuclear staining) [13, 14]. However, there are isolated case presentations reporting that the markers with known positivity in primary PA such as TTF-1 are positive in MPM [15]. In recent times, the markers claimed to be beneficial with their positivity in PA have included Claudin 3, Claudin 4 (CL-3, CL-4) and BAP 1, while those claimed to be beneficial with their positivity in MPM include Cav-1 [10, 16, 17]. In addition to these, there are markers such as CD90, GLUT 1, MUC 4 and Fibulin 3 with different sensitivity and specificity in different series [18–21]. In our study, we assessed the expressions of Cav-1, which was reported as a new marker in the diagnosis of MPM by Amatya et al. for the first time, to determine its value in the differential diagnosis of PA and MM in our series [10].

Caveolae are the invaginations of the plasma membrane, which have important roles in signal transmission. On the other hand, caveolins known to have three types, namely Cav-1, Cav-2 and Cav-3, are the structural proteins of caveola. Cav-1 and Cav-2 are expressed in adipocytes, endothelial cells, pneumocytes, and fibroblasts. Cav-1 is known to be effective in many stages related with cancer, such as cellular transformation, tumor growth, cell migration, metastasis, cell death and angiogenesis [9].

There are studies on the functions of Cav-1 in esophagus, lungs, breast, liver, stomach, pancreas, colon, kidney and prostate adenocarcinoma and its effects on the prognosis of these cancer types [22–30].

Christopher J et al. demonstrated that Cav-1 is expressed in human and mice mesothelial cells [31]. Later, studies were conducted on its differential diagnosis and prognostic significance in mesothelioma [32, 33]. In a study by Amatya et al. suggesting it as a new marker in the differential diagnosis of epithelioid mesothelioma and pulmonary adenocarcinoma, Cav-1 expression was detected in all of the 80 malignant mesothelioma (MM) cases. According to this study, the

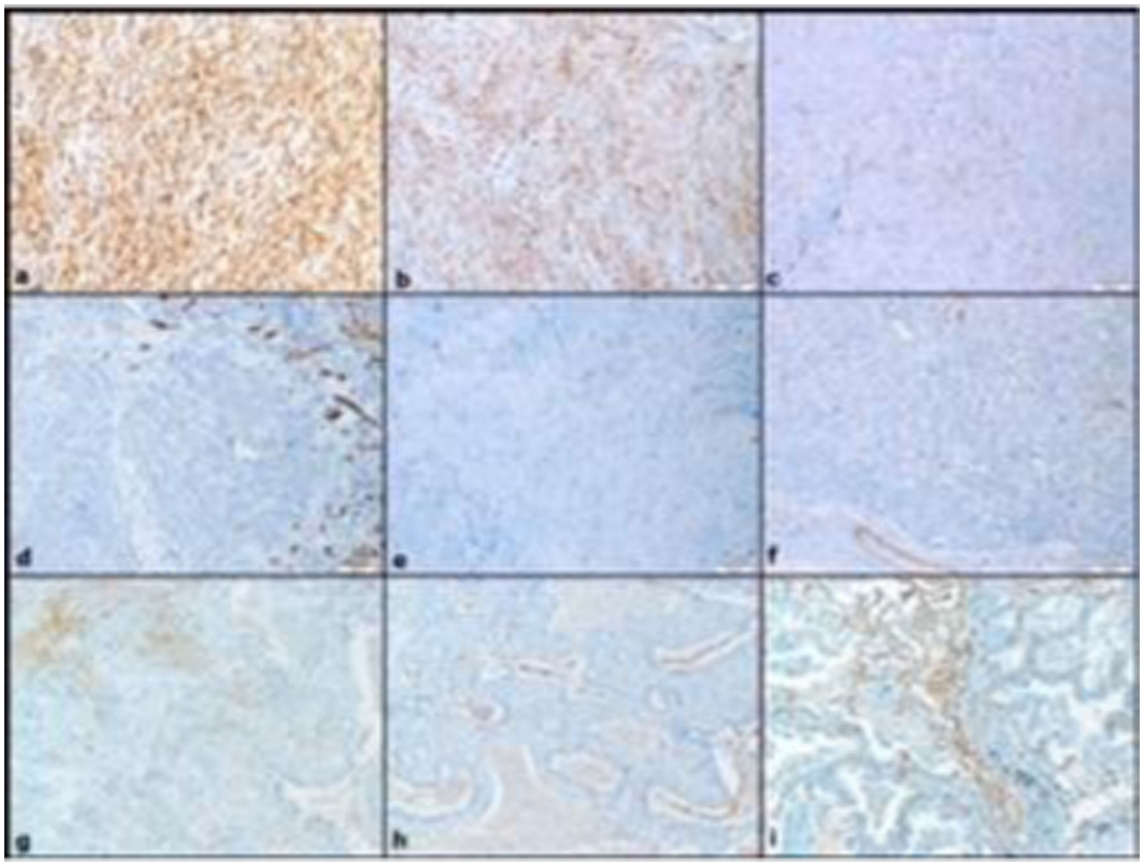

Fig. 1 Cav-1 expression in MPM and PA cases; **a**: strong and score 3 (positivity in >50% of tumor cells) expression in epitheloid mesothelioma (×100), **b**: strong and score 3 expression in biphasic mesothelioma (×100), **c**: weak and score 2 (positivity in 11–50% of tumor cells) expression in sarcomatoid mesothelioma (×100), **d**: negative Cav-1 expression in epitheloid mesothelioma (×100), **e**: negative Cav-1 expression in biphasic mesothelioma (×100), **f**: negative Cav-1 expression in sarcomatoid mesothelioma (×100), **g**: Moderate and score (1 positivity in 1–10% of tumor cells) expression in PA (×100), **h** and **i**: negative Cav-1 expression in PA (×100)

sensitivity and specificity of Cav-1 expression for the differentiation of epithelioid mesothelioma from lung adenocarcinoma were 100% and 92.5%(9). This rate was 94% in Thapa et al.'s study [32], while it was 77% in Righi et al.'s series [33]. We, on the other hand, detected a total of 22(32.35%) positive MPM cases, in our series consisting of 68 cases in total and 15 of 51 were epithelioid. This expression rate was very low compared to other studies.

In different studies based on immunohistochemistry (IHC), different results can be obtained from different series, which changes the sensitivity and specificity rates of the markers used. In studies published by Attanoos et al. containing the results of other studies in the literature, it was demonstrated that there are very different results in different series of biomarkers, including p53, EMA, and P-glycoprotein, used in the differential diagnosis of benign and malign mesothelial proliferation [34].

These differences occurring in IHC studies can have various causes. Among these are the chosen material type (effusion materials, size/type of the biopsy), factors affecting the immunohistochemical staining procedure (fixation, antigen retrieval, antigen dilution rate), the type of selected antibody (clone differences), and differences in staining assessment.

In our study, the paraffin blocks of the resection materials were chosen as Amatya et al. 's study. All cases were containing tumor tissues in large areas. Cases containing a limited amount of tumor tissues were excluded. In the selected cases, attention was paid that there was no fixation problem. The treatment status was also excluded. Only four of MPM cases had a history of neoadjuvant chemotherapy. These patients showed both positive and negative Cav-1 expressions. In antibodies used in appropriate concentrations, strong staining was detected in the alveoli and endothelial cells that were internal controls in all cases. In the assessment, parameters similar to those in the literature were employed. Unlike the other studies in the literature, the antibody we selected in our study was a monoclonal antibody and polyclonal-antibodies were used in the previous three studies. This finding suggests that the choice of poly/monoclonal antibody for Cav-1 presented as a new marker in the differential diagnosis of mesothelioma is important. Advanced studies to be carried out on series in which both clones are used comparatively will reveal

Table 3 The distribution of Caveolin expression score according to their percentage in MPM and PA cases

	Cav-1 positive MPM cases (Subtypes)			Cav-1 positive PA cases (Variant)
Score	Epitheloid	Biphasic	Sarcomatoid	Solid
1+	10	2	0	4
2+	3	2	1	0
3+	2	1	1	0
Total no	15	5	2	4

both the efficacy of Cav-1 and the necessity of polyclonal marker choice used in differential diagnosis in mesothelioma and pulmonary adenocarcinoma, such as pCEA.

Conclusion

Our study shows monoclonal Cav-1 has no significant value in distinguishing MPM and PA.

Compliance with Ethical Standards

Conflict of Interest There is no conflict of interest.

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