Pathol. Oncol. Res. (2017) 23:487–491
DOI 10.1007/s12253-016-0135-9

ORIGINAL ARTICLE

The Role of CD90 in the Differential Diagnosis of Pleural Malignant Mesothelioma, Pulmonary Carcinoma and Comparison with Calretının

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Received: 23 June 2016 / Accepted: 12 October 2016 / Published online: 19 October 2016

Abstract Pleural Malignant Mesothelioma (MM) is a fatal disease that has been associated with asbestos exposure. Differential diagnosis between the pleural infiltration of pulmonary carcinomas and MM is rather difficult particularly for epitheloid type mesothelioma.We aimed to investigate the utility of CD90, a cancer stem cell marker, in the differential diagnosis of MM and lung carcinoma, its prognostic significance and compare its value with that of Calretinin. Ninety pathology specimens including MM (n:30), pulmonary adenocarcinoma (n:30) and pulmonary squamous cell carcinoma (n:30) were used in this study. Immunohistochemical comparision of CD 90 and Calretinin was made in all groups. Calretinin was positive in 20 cases with MM (64.5 %), and was negative in 10 (32.3 %). CD 90 was positive in 25 of these cases (80 %) and negative in 5 (16 %). On the other hand pulmonary adenocarcinomas and squamous cell carcinomas showed positivity with CD90, 63,6 % and 73 %, respectively. We think that CD 90 has no place in the differential diagnosis between mesothelioma and pulmonary carcinoma because of the low specificity in spite of the high sensitivity.

Keywords Mesothelioma · CD 90 · Pulmonary adenocarcinoma · Squamous cell carcinoma

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Introductıon

Pleural Malignant Mesothelioma (MM) is a fatal disease that has been associated with asbestos exposure [1]. Its incidence in industrialized countries is reported to be about 1–6 per 100.000 persons, and known to increase in proportion to asbestos usage [2].

The connection between MM and asbestos exposure was first described by Wagner and colleagues in 1960. These tumors were initially diagnosed according to histologic findings by using histochemical methods in the early 1970s [3, 4]. The Commission of the European Communities recommended the Mesothelioma Histochemistry Panel for MM pathologic diagnosis in 1977. In the 1980s however, with the introduction of immunohistochemistry (IHC) into routine practice, positive and negative immunohistochemical MM markers were incorporated into the diagnostic algorithm of MM [1, 3]. In 2004, the World Health Organization (WHO) suggested the combination of at least two positive and at least two negative markers for the pathologic diagnosis of MM. With this diagnostic criterion, MM was included in the pleural tumors classification [3, 5].

Immunohistochemical markers are mainly used to differentiate MM from benign mesothelial proliferations, and particularly pleural infiltration of pulmonary adenocarcinoma [3, 4]. Diagnosing MM when pleural infiltration of pulmonary adenocarcinoma (PAC) forms part of the differential diagnosis can be challenging particularly for epithelioid type mesothelioma; however, since this differentiation is the primary determinant of treatment approach, an appropriate decision is of vital significance 6). WHO recommends cytokeratin 5/6, Calretinin, WT-1 and D2–40 as positive markers, and CEA(m), CD15, Ber-EP-4, MOC-31 and TTF-1 as negative markers for MM diagnosis [5]. While these markers are recommended for MM diagnosis, use of new markers is required

in the diagnosis of difficult cases. Amatya and colleagues used Caveolin-1 for this differential diagnosis; however, inconsistent results have been obtained with this marker [7, 8].

Over the past century, stem cells, have been reported to be useful in the etiopathogenesis of cancer and their role in the etiopathogenesis of MM have been thoroughly investigated in recent years. CD90, which was tried by Ziegler and colleagues with a surface proteomic analysis in 2012, was studied by Kawamura and colleagues on paraffin-embedded tissues in 2013 [6, 9].

CD90 is a glycoprotein mainly released by leukocytes and is a marker of cancer stem cells [10]. Information related to cancer stem cells come from dates later than 1977 [11]. Since then, studies on stem cell markers in all solid tumors such as glioblastoma and breast, pancreas and liver cancers have been published [10–12].

In this study, we aimed to investigate the utility of CD90, a cancer stem cell marker, in the differential diagnosis of MM and lung carcinoma, its prognostic significance and compare its value with that of Calretinin.

Materıal-Methods

Ninety (90) pathology specimens including MM (n:30), PAC (n:30) and Squamous Cell Carcinoma (SCC) (n:30) were extracted from the pathology archives of Inonu University, Department of Pathology and used for this study. Ethical approval was obtained from the local ethical committee. The diagnosis was based on a combination of clinical presentation, radiological findings, gross observations at surgery, histopathological findings and an immunohistochemical panel including calretinin, CEA, TTF-1, WT-1,p63 and CK5/6 [5]. The H&E stained and immunohistochemistry stained slides of each case were reevaluated under light microscopy by two pathologists. Prognostic parameters such as lymphovascular invasion and lymph node metastasis in SCC and PAC were re-examined as present or absent. Four-micron sections were taken from appropriate formalin-fixed-paraffin-embedded tissue blocks. Immunohistochemical antibodies of anti-CD90 (rabbit polyclonal antibody, Biorbyt, UK; diluted 1:50) and Anti- Calretinin (rabbit polyclonal antibody, Biocare Medical, USA; diluted 1:100) were studied using an automated immunohistochemistry staining device (Ventana BenchMark AutoStainer).

The nuclear and cytoplasmic reactivity for Calretinin, and cytoplasmic staining for CD90 were accepted as positive when more than 10 % of the tumor cells were stained. The intensity of staining was scored as mild (1), moderate (2), or strong (3). Breast carcinoma for CD90 and normal mesothelium for calretinin were included as external positive controls. The omission of the primary antibody was used as negative controls.

The data were summarized as frequencies and percentages. The categorical variables were analyzed using Pearson Chi-Square test with exact method and Fisher's exact test. Sensitivity and specificity were calculated from the related cross tables. *P* values less than 0.05 were accepted as significant. Statistical calculations were made by using IBM SPSS Statistics 23.0 for Windows 7.

Results

Of the thirty cases with MM, there were 20 males (66.6 %) and ten females (33.3 %), with a mean age of 66.3 years. There was a total of 30 PAC cases including, 21 males (70 %) and 9 females (30 %). The mean age of all PAC cases was 63 years. The mean age of the SCC group was 61 years and it consisted of 29 males (96.6 %), and one femaleCalretinin was positive in 20 cases with MM (64.5 %), and negative in 10 (32.3 %). CD 90 was positive in 25 of these cases (80 %) and negative in 5 (16 %) (Table 1) (Fig. 1).

In this study, distribution of staining intensities of Calretinin and CD 90 was also investigated. Calretinin displayed strong staining characteristics in 45 % (n = 14/30) of MM cases. Calretinin showed mild staining in 12 % of cases (n = 4/30), moderate staining in 6.5 % (n = 2/30) and it was negative in 32.3 % of cases (n = 10/30). Distribution of CD 90 in these cases was moderate staining in 29 % (n = 9/30), strong in 25.8 % (n = 8/30) and mild in 25.8 % (n = 8/30), and was negative in 16 % (n = 5/30).

When positivity and negativity of Calretinin and CD90 were evaluated in MM case by case, CD 90 was positive in 8 cases that were negative with Calretinin. In contrast, the number of cases that were positive with Calretinin despite negativity with CD 90 was only 3.

Sensitivity and specificity of Calretinin and CD 90 in MM were also calculated. While the sensitivity of calretinin was 66.6 % and specificity was 100 %, sensitivity of CD 90 was determined as 83 %, and specificity was 36.6 % between MM and PAC.

The entire portion of 30 cases diagnosed with adenocarcinoma was negative with calretinin. CD 90 positivity was observed in 19 of these cases (63.3 %). Positivity was strong in 47 % of the cases.

While calretinin was negative in 30 cases diagnosed with SCC, CD 90 was found positive in 22 cases (73 %), and positivity was strong in half of these cases (50 %).

The correlation between CD 90 positivity and lymphovascular invasion or lymph node metastases was investigated in pulmonary carcinoma cases that are positive for CD 90. Lymphovascular invasion and lymph node metastases were seen in 7 cases (36.8 %) out of 19 PAC cases that were stained positively with CD 90. Staining was strong in 57 % of

Table 1 CD 90 and Calretinin Expression in Mesothelioma and Pulmonary Carcinoma Cases

Tumor Type	CD 90 (n)			Calretinin (n)		
	Positive	Negative	Total	Positive	Negative	Total
Malignant Mesothelioma	25	5	30	20	10	30
Pulmonary adenocarcinoma	19	11	30	0	30	30
Pulmonary squamous cell carcinoma	22	8	30	0	30	30
Total	66	24	90	20	70	90

these cases. Lymphovascular invasion was observed in 17 SCC (77 %) out of 22 that were positive for CD 90 and lymph node metastases were observed in 14 (63.6 %). Of the cases showing lymphovascular invasion, 52.9 % stained strongly.

Statistical analysis of data extracted did not reveal any statistically significant differences between the positivity/negativity of CD 90 in MM, SCC and PAC cases ($p = 0.238$).

The relation between the CD 90 positivity and lymphovascular invasion in SCC and PAC was not statistically significant ($p = 0.078$ and $p = 0.702$, respectively). Also, no statistically significant correlation between the intensity of staining with CD 90 and lymphovascular invasion and metastasis was seen. ($p > 0.05$)

Discussion

MM displays various histologic patterns and cytomorphologic appearances with different variations. Epitheloid, sarcomatoid and biphasic types are the main morphologic types in histology [4, 6]. Differentiation between the epithelioid type MM in particular with PAC is rather difficult [6, 13, 14]. Even for most of the experienced pathologists, a wide immunohistochemical panel is required for differential diagnosis. With this reason, MM has been included in the list of tumors with ever changing differential diagnosis since the day it was defined. Current recommendations by the WHO and International Mesothelial Panel which are not satisfactory, motivated further investigations into possible diagnostic markers.

The role of cancer stem cells in the initiation and progression of breast, pancreas and liver cancers within the previous century have led investigations into their role in the differential diagnosis and prognosis of mesothelioma as well [11]. Ziegler and colleagues studied the cancer stem cell marker 'CD 90' in MM and PAC "cell lines" in 2012 and reported that CD 90 could be accepted as a cell surface marker in differential diagnosis of MM [9]. Following this study, Kawamura et al.evaluated CD 90 in 26 MM, 28 PAC, 33 SCC cases in 2013 and reported that CD 90 displayed a

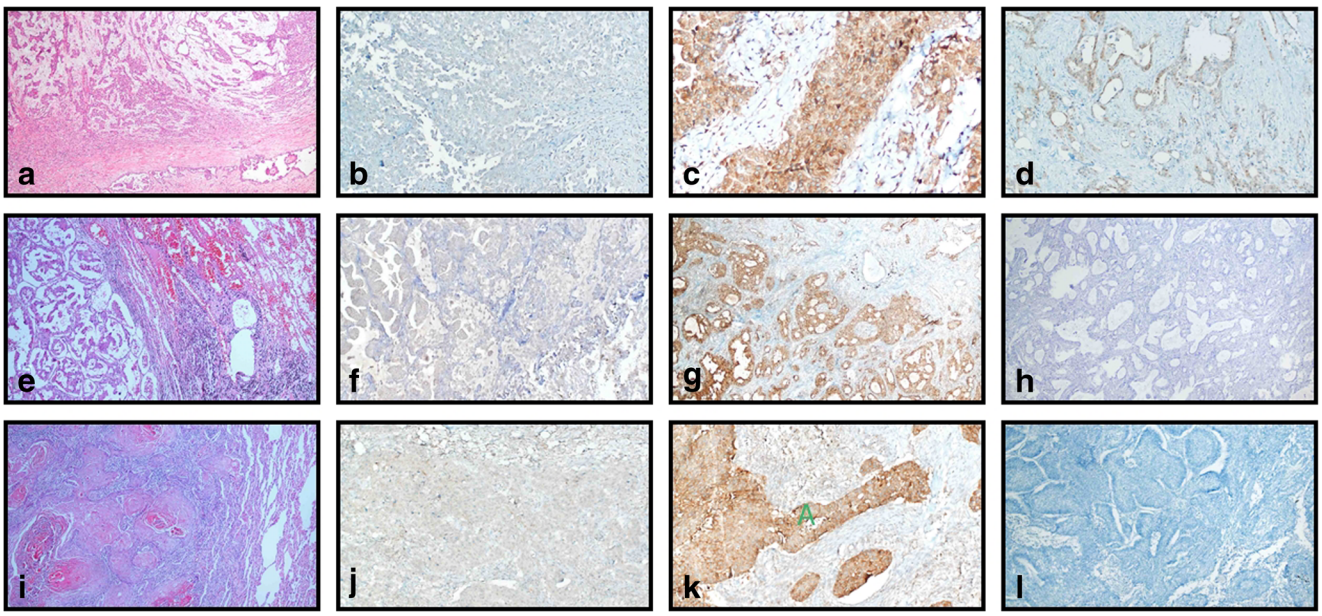

Fig. 1 Epithelioid mesothelioma; **a** with H&E stain (×x40), **b** negativity with CD90 (×x100), **c** positivity with CD90 (×x200), **d** positivity with Calretinin (×x100). Pulmonary Adenocarcinoma; **e** with H&E stain (×x40), **f** negativity with CD90 (×x100), **g** positivity with CD90 (×x40), **h** negativity with Calretinin (×x100)/Pulmonary Squamous Cell Carcinoma; **i** with H&E stain (×x40), **j** negativity with CD90 (×x100), **k** positivity with CD90 (×x100), **l** negativity with Calretinin (×x40)

significantly different pattern of staining in 26 mesothelioma cases [6]. However, there were no comparisons in both studies regarding staining characteristics and intensity with CD 90 or with any of the positive markers recommended by WHO. In our study, we compared the staining distribution and concentration of calretinin [15], which is one of the markers that has been reported with high sensitivity (95 %) and specificity (87 %) for MM. We further examined, the staining distribution and intensity of CD 90 and determined if the differences were statistically significant.

In our study, while calretinin showed positivity in only 64.5 % of 30 cases diagnosed with MM, CD 90 was observed as positive in 80 % of cases. In the study of Kawamura et al., CD 90 positivity was observed in 73 % of cases with MM (6). The sensitivity study we performed on our samples showed, the sensitivity of calretinin as 66.6 % and sensitivity of CD 90 as 83 %. However, calretinin was not positive in any of the PAC and SCC cases, CD 90 displayed positive staining in 19 PAC cases and 22 SCC cases. Therefore, while the calretinin specificity in this study was 100 %, specificity of CD 90 was rather low (36.6 %). To date, there have been no comparative studies in the literature so we hope these data can serve as a guide for future studies that will aim to determine the place of CD 90 in the differential diagnosis of MM.

While it was observed that CD 90 displayed positive staining in 8 MM cases that were calretinin negative, calretinin was positive in only 3 cases that were CD 90 negative. Although these results suggest the question if CD 90 can act as a supportive marker in cases that calretinin is negative, we believe that this staining feature should not be charged with meaning because of the positivity percentages of CD 90 in PAC and SCC.

Immunohistochemical differentiation of MM and SCC is a subject that has not been studied adequately. Ordonez and colleagues have reported that calretinin, mesothelin, and WT-1 can be used as positive markers for this differentiation [16]. In our study, CD 90 positivity was found in 73 % of 30 SCC cases, and strong staining was present in 50 % of the positive cases. However, statistical studies have shown that CD 90 had no differentiating feature among MM, PAC, and SCC ($p = 0.238$).

It has been shown that positivity of cancer stem cells is related to the aggressive course and chemoresistance in many solid tumors [17]. We also investigated the relation between the CD 90 positivity and lymphovascular invasion (LVI) and lymph node metastasis (LNM) in SCC and PAC cases. In PAC cases, LVI and LNM were observed in 7 cases out of 19 (36.8 %) that were positively stained with CD 90; however, this relation was not found to be statistically significant ($p = 0.702$). In SCC cases, however; while LVI was present in 17 cases out of 22 (77 %) with CD 90 positivity, LNM was seen in 14 (63.6 %). LVI positivity in CD 90-positive SCC cases was not found to be statistically significant; however, because of the closeness of the p-value to 0.05 ($p = 0.078$), we think that CD 90 positivity could be significant in pulmonary SCC cases for LVI and the prognostic sense. We believe that this issue should be investigated in larger series.

Conclusıon

In conclusion, in contrast to reports from previous studies that have suggested that CD 90 can be used as a new marker for the differential diagnosis of mesothelioma [6, 9], we found its specificity to be 36.6 % CD 90 sensitivity was calculated as 83 % in this study and these data suggest that CD 90 has no place in the differential diagnosis of mesothelioma and pulmonary carcinoma due to its low specificity. On the other hand, we think that studies on larger series and follow-up are required to determine if CD 90 can be used as an independent prognostic marker for LVI in SCC cases.

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