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From Cell Biology to Tissue Engineering

Expression of plakophilin 3 in diffuse malignant pleural mesothelioma

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Summary. Diffuse malignant pleural mesothelioma (DMPM) is the most common primary malignant pleural neoplasm still posing major diagnostic, prognostic and therapeutic challenges. Plakophilins are structural proteins considered to be important for cell stability and adhesion in both tumor and normal tissues. Plakophilin 3 is a protein present in desmosomes of stratified and simple epithelia of normal tissues with presence in malignant cells of various tumors where it participates in the process of tumorigenesis. The aim of this study was to investigate the expression of plakophilin 3 protein in DMPM, but also to study its prognostic significance and relation to histologically accessible parameters of aggressive growth. Archival samples of tissue with established diagnosis of DMPM and samples of normal pleural tissue were used. Tumor samples were classified into three histological types of DMPM (epithelioid, sarcomatoid and biphasic). Additional subclassification of epithelioid mesotheliomas into nine patterns based on the prevalent histological component of the tumor was then performed. After immunohistochemical staining, cytoplasmic and membrane immunopositivity of tumor cells was assesed by scoring the intensity of the staining from 0 (no staining) to 4 (very strong staining). Prognostic value and expression of plakophilin 3 with consideration to histologically estimated aggression in tumor growth were then statistically analyzed using non-parametric tests. The results demonstrated higher level of plakophilin 3 expression in tumor samples with histologically more aggressive tumor growth, but no significant prognostic value. According to our study, plakophilin 3 appears to be involved in tumor invasion in malignant mesothelioma.

Key words: Plakophilin 3, Diffuse malignant pleural mesothelioma, Invasiveness, Immunohistochemistry

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Introduction

Diffuse malignant pleural mesothelioma (DMPM) is the most common primary malignant pleural neoplasm and represents a diagnostic and clinical challenge due to its histological heterogeneity and clinical outcome (Kadota et al., 2011). Asbestos is the main carcinogen related to this tumor (Robinson and Lake, 2005). DMPM is characterized by poor prognosis and is prone to direct invasion of thoracic structures (Husain, 2010). Histologically, classification includes 3 basic types (epithelioid, sarcomatoid and biphasic) according to the predominant histomorphological growth pattern (Craig, 2005; Kadota et al., 2011). Epithelioid type is the most common type having the most favourable prognosis, while sarcomatoid implies the worst prognosis (Kadota et al., 2011; Tischoff et al., 2011). A great variety of histological patterns is especially present in epithelioid type, some of them seemingly having implications for the biological behaviour of the neoplasm (Kadota et al.,

2011; Tischoff et al., 2011; Brčić et al., 2014). Clinical stage of malignant pleural mesothelioma is based on TNM staging system, and accepted by the UICC (International Union Against Cancer) and the AJCC (American Joint Commission on Cancer) as international staging system for pleural mesothelioma, which represents an important prognostic factor (Geltner et al., 2016; Neumann et al., 2013). Studies on possible therapeutic implications or targets in DMPM aiming to improve treatment and prognosis are also necessary and of great importance and many of them are still in progress (Sekido, 2013; Ortolan et al., 2014; Vinicius Monteiro de Assis and Cesar Isoldi, 2014; Mineo and Ambrogi, 2012). Since malignant mesothelioma incidence is increasing and current treatment modalities including mostly surgical and chemotherapeutic approach do not provide noticeable survival improvement, a need for novel treatment modalities has evolved (Wangpaichitr et al., 2014). Also, new knowledge of molecular malignant mesothelioma characteristics has led to the development of the idea of targeted therapy which would, in contrast to standard approaches, focus only on cancer cells, therefore avoiding destruction of other cells and systemic toxicity (Wangpaichitr et al., 2014; Bononi et al., 2015). Targeted approaches include: molecular targeted approaches such as use of specific antibodies that inhibit angiogenesis (for example, bevacizumab which inhibits vascular endothelial growth factor), use of microRNA mimics which restore deregulated microRNA's and stop tumor growth, gene therapy for restoration of defective components of signaling pathways (for example, restoration of defective tumor suppressor genes). Also, immunotherapy is another targeted approach and includes use of monoclonal antibodies such as tremelimumab which activate immune response in order to destroy tumor cells, then mesothelin- targeted agents such as antibodies or vaccines against mesothelin, which is overexpressed in malignant mesothelioma. Additionally, approach targeting asbestos- induced inflammation using monoclonal antibodies such as infliximab (tumor necrosis factor α antagonist) has also been studied (Bononi et al., 2015).

Plakophilins are structural proteins and therefore part of cytoplasmic plaques of desmosomes. They are considered to be important in connecting other desmosomal proteins, but also for attachment of intermediate filaments to desmosomes, which makes them important for stability and adhesion of cells and tissues (Demirag et al., 2011). They are members of the p120ctn family of armadillo- related proteins important for intercellular interactions and citoskeletal maintenance (Schwarz et al., 2006; Hatzfeld, 2007; Bass-Zubek et al., 2009). Besides a structural role, they are considered to be important in cellular signalization (Bass-Zubek et al., 2009; Hatzfeld, 2007, 2010; Bonne et al., 1999), cell differentiation (Demirag et al., 2011), in the process of translation (Hofmann et al., 2006; Wolf and Hatzfeld, 2010; Roberts et al., 2013), transcription (Munoz et al., 2014), as posttranscriptional regulators of gene expression (Wolf et al., 2010; Fischer- Kešo et al., 2014) and in epithelial- mesenchymal transition (Neuber et al., 2010; Takahashi et al., 2012; Fischer-Kešo et al., 2014). Changes in intercellular adhesion are considered to be related to dedifferentiation of tumor cells and tumor invasion. Still, the biological importance of plakophilins in tumorigenesis and tumor progression is not yet completelly understood (Schwarz et al., 2006; Demirag et al., 2011). Protein plakophilin 3 is part of desmosomes in simple and stratified epithelia (Hofmann et al., 2006; Neuber et al., 2010) present in cytoplasm, membrane and nucleus (Kundu et al., 2008; Schmidt and Jäger, 2005) with different samples of expression and prognostic significance depending on the type of tumor.

In the case of primary oropharyngeal tumors, its weaker immunoreactivity correlated with worse patient's survival (Papagerakis et al., 2003), while in gastric adenocarcinoma connection with higher clinical tumor stage and larger number of metastatically positive lymph nodes was established (Demirag et al., 2011). In bladder cancer, decreased expression was related to higher level of tumor invasiveness (Takahashi et al., 2012). On the other hand, increased plakophilin 3 immunoreactivity in breast adenocarcinoma was related to higher tumor grade and more metastatically involved lymph nodes (Demirag et al., 2012) and to a more aggressive course in prostate and lung adenocarcinoma (Furukawa et al., 2005; Breuninger et al., 2010).

In this study, we decided to examine expression of plakophilin 3 in 3 types and certain subtypes of DMPM and its correlation with histologically estimated aggresiveness of tumor growth. To our knowledge, no studies based on plakophilin 3 expression in DMPM and its correlation to invasiveness have yet been published.

Materials and methods

Patients

Archival material from Institute of Pathology, University of Zagreb, School of Medicine, was used in this retrospective study. Bioptic or operative tissue samples of 100 patients with the histologically and clinically confirmed diagnosis of DMPM established between 1999. and 2009. and also samples of healthy pleural tissue (Institute of Pathology, University of Zagreb, School of Medicine, Zagreb) fixed in neutral buffered formalin and embedded in paraffin were included in the study. Among 100 samples, 3 samples with diagnosis of DMPM were excluded from the study, since they appeared to be technically inadequate. Survival data vere received from Croatian National Cancer Registry (Croatian National Institute of Public Health, Zagreb). No personal data revealing the identity or in any way violating the patient's privacy were used in the study. This paper was performed according to ethical standards.

Histological and immunohistochemical analysis

After collecting the material, hematoxylin-eosin sections of the samples were reexamined by three pathologists in order to confirm the diagnosis and classify tumors in 3 main types of DMPM (epithelioid, sarcomatoid, biphasic) and subtypes of epithelioid type, but also to estimate depth of tumor invasion. Patients with lower degree of tumor invasion were considered those with demonstrable tumor invasion only into connective tissue, and patients with tumor invasion into fatty tissue and/ or skeletal muscle were considered to have higher degree. If enough tumor tissue was present on the slide, immunohistochemical staining with plakophilin 3 followed. Three to four micrometer slices were deparaffinized and predigested in thermo bath (DAKO, Denmark) with "EnVision target retrieval solution" (DAKO, Denmark). The staining was performed by using automatized immunohistochemical system (Dako autostainer, DAKO, Denmark) with Anti-Plakophilin 3 antibody (rabbit monoclonal, IgG1, EPR5560, ab 109441, Abcam, Cambridge, United Kingdom) and dilution 1:100. Paraffin embedded samples of human breast tissue served as positive control. The intensity of cytoplasmic and membranous immunoexpression was estimated on the entire histological section and scored as 0 (no staining), 1 (weak), 2 (moderate), 3 (strong), 4 (very strong staining). The intensity of the staining was considered weak when less than 25% of tumor cells demonstrated positive staining, moderate when 25-50% of tumor cells, strong when 50-75% of cells and very strong in which more than 75% of tumor cells demonstrated positive plakophilin 3 staining.

Statistical analysis

We performed this study in order to analyze the expression of plakophilin 3 in different types and subtypes of diffuse malignant pleural mesothelioma. Statistical analysis of the data was performed using SPSS 20.0 software. Correlation between the intensity of membranous and cytoplasmic staining and depth of invasion in all samples and subtypes of epithelioid mesothelioma was determined by using Mann-Whitney test. Relation of the intensity of membranous and cytoplasmic plakophilin 3 imunoreactivity in all samples of DMPM to survival was determined by using Spearman test. P value <0.05 was considered statistically significant.

Results

The research included 97 patients (83 male, 14 female). The oldest patient at the time of diagnosis was 80 and the youngest was 27 years old. In 87 cases patients had a complete follow- up. The longest survival expressed in days was 3010 and the shortest survival was 11 days.

Our study included 91 patient with epithelioid mesothelioma, 5 with sarcomatoid and 1 with biphasic type of DMPM. Among the subtypes of epithelioid mesothelioma 23 samples were classified as tubulopapillary, 24 as solid, 22 as trabecular,10 as tubular, 2 were microglandular, 2 as microcystic, 2 as micropapillary, 1 as acinar (glandular), 1 pleomorphic. In 4 samples of epithelioid DMPM subtype was not determined. For the analysis of subtypes we used 4 subtypes with adequate number of tumor samples (solid, trabecular, tubular, tubulopapillary), while microglandular, microcystic, micropapillary and acinar were excluded because of small number of samples (Table 1).

In 52 samples, tumor was found to invade only connective tissue. 45 samples revealed tumor cells in surrounding fat and/ or skeletal muscle. As for subtypes, 14 samples with solid subtype, 13 samples with tubulopapillary subtype, 10 of trabecular subtype and 5 of tubular subtype demonstrated lower degree of invasion. Higher degree of invasion was demonstrated by 10, 10, 12 and 5 samples respectivelly.

All samples of normal pleural tissue demonstrated positive cytoplasmic and membranous staining. Immunohistochemical staining for plakophilin 3 revealed positive cytoplasmic reaction in all samples except in one sample of sarcomatoid type (Fig. 1).

Table 1. Clinicopathological characteristics of diffuse malignant pleural mesothelioma patients included in the study.

	Number	%
All patients	97	100
Age range (years)	27 - 80	
Gender		
Female	14	14.4
Male	83	85.6
Overall survival (days)	11- 3010	
Clinical status		
Dead	87	89.7
Alive	10	10.3
Mesothelioma type		
Epithelioid	91	93.8
Sarcomatoid	5	5.2
Biphasic	1	1.0
Epithelioid subtype		
solid	24	26.4
tubulopapillary	23	25.3
trabecular	22	24.2
tubular	10	11.0
microglandular	2	2.2
microcystic	2	2.2
micropapillary	2	2.2
acinar(glandular)	1	1.1
pleomorphic	1	1.1
Depth of invasion (all samples)		
connective tissue	52	54
fatty tissue, skeletal muscle	45	46

Positive membranous staining was present in all samples except for 1 sample of tubular, 2 samples of solid, 1 sample of tubulopapillary, 1 sample of trabecular and 3 samples of sarcomatoid mesothelioma (Table 2, Fig. 2). Statistical analysis showed stronger plakophilin 3 expression both in cytoplasm and membrane of tumor cells with prominent invasion (e.g tumor spread into fatty tissue and/ or skeletal muscle). Correlation between invasiveness and cytoplasmic and membranous immunoreactivity was found in three out of four subtypes (solid, tubulopapillary, trabecular, tubular) of epithelioid malignant mesothelioma.

In samples of trabecular and tubular subtype, correlation between invasiveness and cytoplasmic immunoreactivity showed statistical significance (increased staining, higher degree of invasiveness) (Table 3). In tubulopapillary subtype correlation between membranous immunoreactivity and invasiveness (increased staining, higher degree of invasion) was statistically significant (Table 4), while in samples of solid subtype no statistical significance between membranous and cytoplasmic staining and invasiveness was found.

As for prognostic value, there was no statistical significance (Spearman coefficient of correlation -0.033, p=0.751) in regards of plakophilin 3 cytoplasmic expression and survival in all samples of DMPM. Furthermore, the intensity of membranous staining in all samples correlated to survival also showed a negative result (Spearman coefficient of correlation -0.049, p=0.632).

Discussion

Desmosomes are intercellular junctions located at cell membrane with well- known ultrastructural

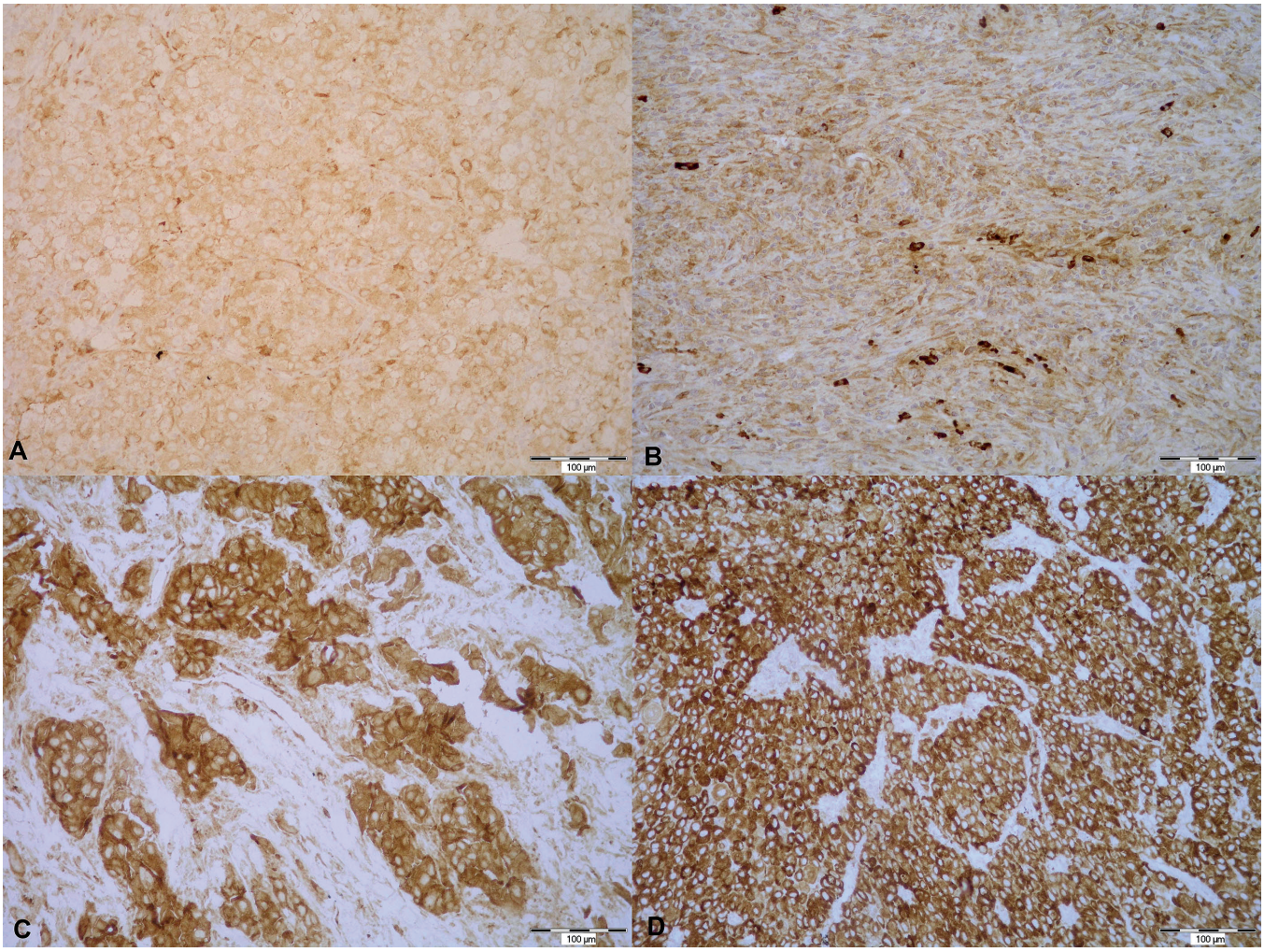

Fig. 1. Immunohistochemical plakophilin 3 cytoplasmic staining: **a)** score 1 (plakophilin 3), **b)** score 2 (plakophilin 3), **c)** score 3 (plakophilin 3), **d)** score 4 (plakophilin 3).

organization (Harrison et al., 2016; Zhou et al., 2017). They are composed of 3 zones: extracellular core region, outer and inner dense plaque. Desmosomal cadherins (desmogleins, desmocollins) extend into extracellular core and outer dense plaque, while their cytoplasmic tails connect to plakoglobin, plakophilins and desmoplakin. Desmoplakin associates to keratin intermediate filaments within inner dense plaque to establish connection between intermediate filaments and plasma membrane (Delva et al., 2009) . However, desmosomal components undergo alterations in their expression and it is considered that most of them are mis- regulated in malignancies, which implies loss of certain proteins, overexpression or reduced expression, and contributes to the process of carcinogenesis (Stahley and Kowalczyk., 2015). They can be identified through immunohistochemical staining for their components or

Table 2. Immunohistochemical plakophilin 3 cytoplasmic and membranous staining in histologic types and subtypes of epithelioid mesothelioma.

	Diagnosis	Histologically estimated invasiveness	Plakophilin 3 cytoplasm	Plakophilin 3 membrane
1.	solid	F/S	4	4
2.	trabecular	C	1	1
3.	microglandular	C	2	1
4.	epithelioid-subtype not determined	F/S	4	3
5.	tubular	F/S	4	3
6.	tubulopapillary	F/S	3	3
7.	microcystic	C	2	2
8.	sarcomatoid	C	2	2
9.	tubular	C	2	0
10.	solid	F/S	4	4
11.	microcystic	C	2	2
12.	tubulopapillary	C	2	2
13.	tubulopapillary	F/S	3	4
14.	tubulopapillary	F/S	2	2
15.	tubulopapillary	F/S	2	3
16.	trabecular	F/S	2	4
17.	solid	F/S	2	2
18.	sarcomatoid	F/S	3	0
19.	solid	F/S	2	3
20.	solid	C	2	0
21.	epithelioid-subtype not determined	F/S	2	4
22.	trabecular	C	2	3
23.	tubulopapillary	C	4	2
24.	pleomorphic	F/S	2	2
25.	trabecular	F/S	4	2
26.	solid	F/S	4	4
27.	tubulopapillary	C	2	0
28.	tubulopapillary	C	2	3
29.	trabecular	C	2	2
30.	micropapillary	F/S	2	2
31.	tubulopapillary	C	1	2
32.	trabecular	C	2	2
33.	tubulopapillary	C	2	1
34.	tubulopapillary	F/S	2	2
35.	trabecular	C	1	0
36.	tubulopapillary	C	2	2
37.	tubulopapillary	F/S	3	4
38.	solid	F/S	2	2
39.	solid	C	1	2
40.	tubular	F/S	3	1
41.	trabecular	F/S	4	4
42.	tubulopapillary	C	2	2
43.	tubular	F/S	2	4
44.	tubular	C	2	4
45.	tubular	C	2	2
46.	solid	F/S	2	4
47.	tubular	C	2	3
48.	solid	F/S	4	1
49.	solid	C	2	2
50.	tubulopapillary	F/S	2	2
51.	tubular	F/S	4	2
52.	sarcomatoid	F/S	2	0
53.	acinar	C	1	2
54.	microglandular	C	2	2
55.	epithelioid-subtype not determined	F/S	2	3
56.	tubular	F/S	4	4
57.	solid	C	3	4
58.	epithelioid-subtype not determined	F/S	3	4
59.	trabecular	F/S	3	2
60.	solid	C	3	4
61.	tubulopapillary	C	4	4
62.	tubulopapillary	F/S	3	4
63.	tubulopapillary	F/S	4	2
64.	trabecular	F/S	2	2
65.	tubulopapillary	C	2	2
66.	solid	C	2	2
67.	sarcomatoid	C	2	2
68.	trabecular	F/S	2	2
69.	trabecular	C	2	2
70.	solid	C	2	3
71.	solid	C	1	1
72.	trabecular	C	1	2
73.	micropapillary	C	1	2
74.	trabecular	F/S	2	2
75.	solid	C	3	3
76.	sarcomatoid	C	0	0
77.	trabecular	F/S	3	2
78.	tubulopapillary	F/S	3	3
79.	tubulopapillary	C	3	3
80.	biphasic	C	1	3
81.	solid	C	2	2
82.	solid	C	1	1
83.	trabecular	C	2	2
84.	tubulopapillary	C	2	2
85.	tubular	C	2	2
86.	solid	C	1	0
87.	tubulopapillary	C	2	2
88.	solid	F/S	2	2
89.	trabecular	F/S	4	4
90.	solid	C	4	4
91.	trabecular	C	2	2
92.	trabecular	F/S	4	4
93.	trabecular	F/S	2	1
94.	solid	C	2	2
95.	trabecular	F/S	3	4
96.	solid	F/S	2	4
97.	trabecular	C	1	2

Table 2. (Continuation).

C- connective tissue, F/S- fatty tissue and/or skeletal muscle.

by ultrastructural analysis. In our study, normal pleural cells have demonstrated positive staining for plakophilin 3 which is an integral part of desmosomes. According to references concerning presence of desmosomes in mesothelioma cells, epithelioid type is characterized by the presence of well-developed desmosomes (Crotty et al., 1994), yet sarcomatoid type contains rare desmosomes (Ascoli et al., 1996). Eimoto et al. described desmosomes in biphasic type of mesothelioma as being rare but more conspicuous in epithelial component of the tumor (Eimoto and Inoue, 1997). In

Table 3. Correlation between invasiveness and cytoplasmic immunoreactivity in main types and certain subtypes of epithelioid mesothelioma.

	Mann- Whitney U	P value
MAIN TYPES	557.500	0.001
TRABECULAR	15.000	0.001
TUBULAR	2.500	0.017

Mann-Whitney U test.

Table 4. Correlation between invasiveness and membranous immunoreactivity in main types and certain subtypes of epithelioid mesothelioma.

	Mann- Whitney U	P value
MAIN TYPES	754.500	0.001
TUBULOPAPILLARY	35.500	0.046

Mann-Whitney U test.

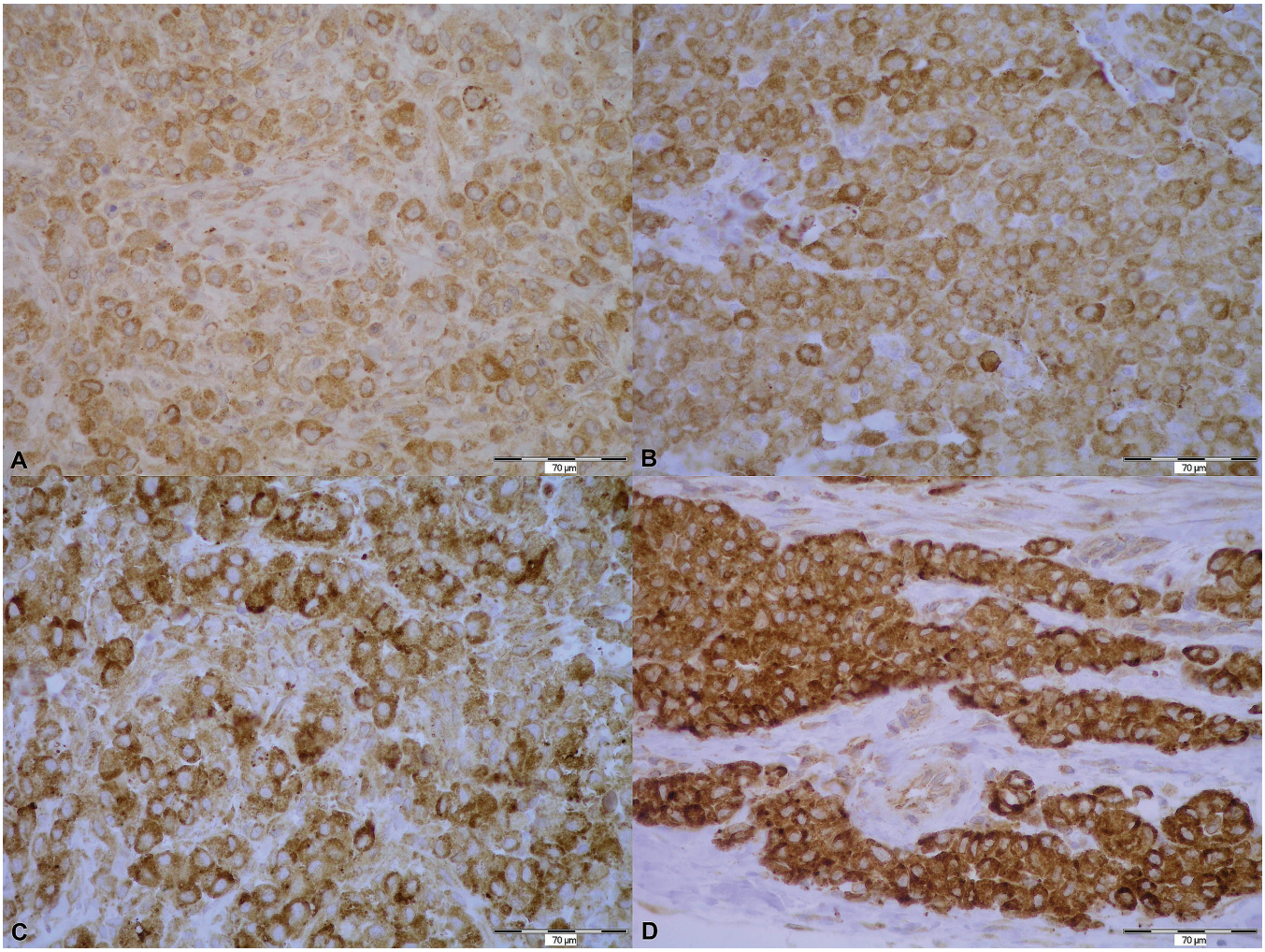

Fig. 2. Immunohistochemical plakophilin 3 membranous staining: **a)** score 1 (plakophilin 3), **b)** score 2 (plakophilin 3), **c)** score 3 (plakophilin 3), **d)** score 4 (plakophilin 3).

our study, epithelioid type has shown cytoplasmic and membranous plakophilin 3 staining intensities from weak to very strong, however, most samples of sarcomatoid type have demonstrated no membranous staining, which can be explained by presence of rare desmosomes in this type. As for biphasic type, more samples are required for valid conclusions.

Unfortunately, due to rarity of biphasic type of malignant mesothelioma, we were not able to collect additional samples of this type. However, collecting more samples of this particular type could be a potential target of a broader (maybe multicentrical) study.

Plakophilins are known as molecules that demonstrate tissue and differentiation specific expression patterns (Delva et al., 2009; Bornslaeger et al., 2010). They were identified as desmosomal plaque proteins and are considered to be nuclear and cytoplasmic proteins that undergo translocation to desmosomes in certain stages of differentiation (Demirag et al., 2011). The importance of plakophilins in desmosomes is much more explored than the ones in nucleus and cytoplasm (Munoz et al., 2014). According to observances about their presence in desmosomes, nucleus and cytoplasm, it was concluded that plakophilins have a role in cell signalization (Hatzfeld, 2010). Still, little is known about the regulation of plakophilin localization, their trafficking among subcellular compartments and their nuclear function (Hatzfeld, 2010).

Plakophilin 3 is a protein present in cytoplasm (stress granules, RNA particles) and membrane (desmosomal plaque in desmosomes), but its presence in nucleus was also observed (Sklyarova et al., 2008). Plakophilin 3 is more widely present in normal tissues (all layers of stratified and simple epithelia, but not in hepatocytes), when compared to other members of the plakophilin family (Kundu et al., 2008; Basu et al., 2015). However, according to the literature, besides in cytoplasm, plakophilin 3 has also been detected in tumor cell nuclei (pharyngeal and oral squamous cell carcinoma) and cell membrane (pancreatic adenocarcinoma, prostatic adenocarcinoma, etc.) (Schwarz et al., 2006). It is considered that plakophilin 3 in cytoplasm might have a different function than in desmosomes, yet the significance of its cytoplasmic presence has so far not been clarified (Schwarz et al., 2006).

As for cytoplasmic and membranous localization, it is considered that this implies a dual role of plakophilin 3 in not only cell adhesion which is suggested by membranous desmosomal localization, but also in the processes of RNA metabolism and translation, which is in accordance with its cytoplasmic presence (Hofmann et al., 2006). Desmosomes are dynamic structures and plakophilins are believed to be translocated from nucleus to membrane (Bonne et al., 1999). According to the points mentioned, and ultrastructural data on desmosomal presence in 3 types of malignant mesothelioma, we discovered that epithelioid type demonstrates both cytoplasmic and membranous plakophilin 3 staining which demonstrates the presence of plakophilin 3 in both localizations. In epithelioid type, desmosomes are well- developed, but it appears that the cytoplasmic plakophilin 3 pool has an important role in tumorigenesis. Most sarcomatoid types showed lack of membranous staining which can be explained by the presence of rare desmosomes, while cytoplasmic staining was weak to strong which implies the importance of cytoplasmic plakophilin 3 localization. Biphasic type showed weak cytoplasmic but strong membranous reaction, however, due to only one tumor sample, no valid conclusions about plakophilin 3 expression in this type can be established.

The role of plakophilins in development and progression of tumors is still not completely cleared. They are considered to function as oncogenes or tumor supressors depending on the type of cells (Neuber et al., 2010). Their role in the process of transcription and translation is also considered to be important in tumorigenesis and tumor progression. Plakophilins are considered to act as tumor suppressors through their structural functions in desmosomes, but as oncogenes through their stimulation of translation (Wolf and Hatzfeld, 2010).

Plakophilin 3 affects carcinogenesis through its involvement in the process of transcription. It modulates transcription by binding to ETV1 (site-specific DNA binding transcription factor important for regulation of matrix metaloproteinases in tumorigenesis) that leads to promotion of transcription (Munoz et al., 2014). Transcription factor ZEB1 demonstrates an important influence on cancer progression. While upregulated, it leads to plakophilin 3 downregulation and consequent loss of intercellular adhesion (loss of plakophilin 3 in membrane) and epithelial- mesenchymal transition, while in the case of ZEB1 knockdown plakophilin 3 partially relocates to plasma membrane, which leads to an increase of intercellular adhesion (Aigner et al., 2007). According to Doi et al. increased expression of MT1- MMP (matrix metaloproteinase) in tumor cells of DMPM is connected to invasive activity of tumor through degradation of extracellular matrix (Doi et al., 2011).

Malignant pleural mesothelioma is characteristic for its local invasiveness, rarely metastasizes, but its spread to adjacent structures is common (Zellos and Sugarbaker, 2002). Our concept of histological mesothelioma invasiveness is based on pleural histological structure and its close relation to adjacent structures, predominantly lungs. Mesothelioma is a malignant neoplasm which originates from mesothelial pleural cells which in normal circumstances form one layer on the pleural surface (Grbac et al., 2002; Vinicius Monteiro de Assis and Cesar Isoldi, 2014). Under the mentioned layer, connective tissue can be found, while fat, muscle or lung represent deeper tissue (Capelozzil et al., 2003). Therefore, mesothelioma infiltrating fatty tissue and/ or skeletal muscle is more locally invasive

and more likely to spread to close structures such as underlying lungs. Here the term " invasive" represents the point of invasion and not biologic potential. However, no papers explaining expression of plakophilin 3 and histological invasiveness in malignant pleural mesothelioma have so far been done.

According to the results of our study, which demonstrate higher degree of plakophilin 3 expression in more invasive samples, we consider the possibility that plakophilin 3 contributes to more aggressive growth pattern of mesothelioma and therefore may be involved in the process of tumor progression.

Based on the knowledge from previous studies, plakophilin 3 is likely to be involved in the process of invasive tumor growth through mediation of cell adhesion. Among other functions, plakophilins represent structural components of desmosomes assembled at the plasma membrane that stabilize and strenghten cell adhesion based on connection of desmosomal cadherins (desmocolin,desmoglein) with intermediary filaments through protein complex consisting of plakoglobin, plakophilins and desmoplakin (Schmidt and Koch, 2007; Bass-Zubek et al., 2009). Plakophilin 3 interacts with desmoglein, desmocolin, plakoglobin,cytokeratin 18 and desmoplakin (Bonne et al., 2003). Loss of plakophilin 3 leads to a decrease of cell adhesion, contributes to increased cell ability to migrate, which enables tumor spread and metastasis (Kundu et al., 2008) in some types of tumors (primary oropharyngeal tumors, gastric adenocarcinoma, bladder cancer) (Papagerakis et al., 2003; Demirag et al., 2011; Takahashi et al., 2012), while its overexpression, as in our case, is likely to be a contributor to aggressive growth of DMPM through its mediation of processes of transcription and translation like in cases of breast, prostate, colorectal adenocarcinoma (Breuninger et al., 2010; Valladares-Ayerbes et al., 2010; Demirag et al., 2012). It has recently been found that plakophilin 3 has a fraction which appears to be stable in desmosomal plaque, while another plakophilin 3 pool undergoes dynamic exchange with the pool in cytoplasm which also affects cell adhesion. Increased exchange of plakophilin 3 in desmosome leads to a decrease in adhesive strength of desmosomes and therefore to higher degree of cell migration (Roberts et al., 2013). This could also be important in the process of tumor metastasis and possibly important in DMPM invasion, however, it remains to be further tested.

In the case of increased cytoplasmic immunoreactivity in trabecular, tubular and also membranous immunoreactivity in tubulopapillary subtype, we believe that studies including more samples are needed in order do determine the role of plakophilin 3 in those subtypes.

Conclusion

This study demonstrated that DMPM exhibits cytoplasmic and membranous expression of plakophilin 3 showing different expression in different histological subtypes, while our results suggest that increased expression of plakophilin 3 may have a significant role as contributor in invasive growth of DMPM, but without correlation to survival, yet further research including larger number of patients in case of DMPM is required in order to confirm our assumption.

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