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Value of PAX8, PAX2, claudin-4, and h-caldesmon immunostaining in distinguishing peritoneal epithelioid mesotheliomas from serous carcinomas

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Distinguishing between peritoneal epithelioid mesotheliomas and papillary serous carcinomas involving the peritoneum can be difficult on routine histological preparations, but this differential diagnosis can be facilitated by the use of immunohistochemistry. Recent investigations have indicated that PAX8, PAX2, claudin-4, and h-caldesmon are immunohistochemical markers that can assist in distinguishing between these two malignancies; however, much of the information published on the value of these markers is either insufficient or contradictory. The purpose of this study is to resolve some of the existing controversies and to fully determine the practical value of these markers for assisting in the differential diagnosis between peritoneal mesotheliomas and serous carcinomas. In order to do so, a total of 40 peritoneal epithelioid mesotheliomas and 45 serous carcinomas (15 primary, 30 metastatic to the peritoneum) were investigated. PAX8 and PAX2 nuclear positivity was demonstrated in 42 (93%) and 25 (56%) of the serous carcinomas, respectively, whereas none of the mesotheliomas expressed either marker. Forty-four (98%) of the serous carcinomas exhibited claudin-4 reactivity along the cell membrane, whereas none of the mesotheliomas were positive for this marker. All of the serous carcinomas and mesotheliomas were negative for h-caldesmon. Based on these results, it is concluded that PAX8 and claudin-4 have a higher sensitivity and specificity for assisting in discriminating between peritoneal epithelioid mesotheliomas and serous carcinomas when compared with all of the other positive carcinoma markers that are, at present, recommended to be included in the immunohistochemical panels used in this differential diagnosis. Even though it is highly specific, PAX2 has little practical value in the diagnosis of peritoneal epithelioid mesotheliomas as its sensitivity is low. The h-caldesmon is not useful.
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The distinction between peritoneal mesotheliomas and serous carcinomas diffusely involving the peritoneum can be challenging because of the overlapping morphological features that exist between these two malignancies. The differential diagnosis, however, can be facilitated by the combined use of markers that are either commonly expressed in mesotheliomas, but not in carcinomas (positive mesothelioma markers) or in carcinomas, but not in mesotheliomas (positive carcinoma markers).[1] A number of comparative studies have been published in which the authors have attempted to determine the best panel of immunohistochemical markers that can assist in discriminating between peritoneal epithelioid mesotheliomas and serous carcinomas.[2–10] The recommended panels, however, have continually been subject to change as a result of the identification of new markers that can be used in the differential diagnosis of these tumors, as well as the publication of new information on the value of the individual markers. In a previous study by this author that was published in 2006 investigating the expression of a large number of positive mesothelioma markers (calretinin, podoplanin, keratin 5/6, and thrombomodulin) and positive carcinoma markers (MOC-31, Ber-EP4, BG-8

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(Lewis^y), TAG-72 (B72.3), CD15 (leu-M1), and CA19-9), it was concluded that thrombomodulin, calretinin, and podoplanin were the best positive mesothelioma markers, and MOC-31 and Ber-EP4 were the best positive carcinoma markers for discriminating between peritoneal epithelioid mesotheliomas and serous carcinomas.[8] Since then, a variety of other markers have become available that some authors have suggested might be useful for assisting in this differential diagnosis;[7,11–14] however, much of the information that has been published on the value of some of these markers is either insufficient or contradictory. The purpose of this study is to determine the utility of some of these markers, specifically PAX8, PAX2, claudin-4, and h-caldesmon, for assisting in distinguishing between peritoneal epithelioid mesotheliomas and serous carcinomas and to compare these markers with those that have previously been recommended.

Materials and methods

The material used in this study was obtained from the files of the Department of Pathology at the University of Texas MD Anderson Cancer Center. It consisted of 40 cases of peritoneal malignant epithelioid mesotheliomas and 45 serous carcinomas of the ovary (15 primary, 30 metastatic to the peritoneum). In all, 25 of the mesotheliomas occurred in women and 15 in men. The diagnosis of mesothelioma was made using WHO criteria on hematoxylin-and-eosin-stained sections combined with immunohistochemical findings and clinical information. Immunohistochemical studies were performed on 5-μm thick, formalin-fixed, paraffin-embedded tissue sections using the polymeric biotin-free horseradish peroxidase method on a Leica Microsystems Bond Max Stainer (Bannockburn, IL). The primary antibodies are listed in Table 1. In brief, slides were deparaffinized and hydrated, followed by heat-induced antigen retrieval in which a citrate buffer solution, pH 6.0, was used. Incubation with the primary antibody was followed by development of the immunostaining with 3,3′-diaminobenzidine. The secondary antibody and detection was applied as per instructions from the manufacturer. To evaluate the specificity of the immunoreaction, known positive and negative tissues were used as controls. The immunostaining was graded on a sliding scale of 1+ to 4+ according to the percentage of reactive cells (1+, 1–25%; 2+, 26–50%; 3+, 51–75%; 4+, >75%).

Results

The immunohistochemical results are summarized in Table 2.

PAX8

Of the 45 papillary serous carcinomas, 42 (93%) demonstrated PAX8 nuclear positivity (Figure 1a). In the large majority of the cases, the reaction was strong and diffuse (in 40 of the cases, the reaction was graded as 4+ or 3+), and it was focal (2+) in

Table 1 Antibodies used in this study

Marker	*Source*	*Type*	*Dilution*	*Antigen retrieval*
Claudin-4	Invitrogen (Camarillo, CA)	3E2C1 MAb	1:250	Yes (citrate)
h-caldesmon	Dako Corporation (Carpinteria, CA)	h-CD MAb	1:50	Yes (citrate)
PAX 2	Invitrogen	Z-RX2 PAb	1:25	Yes (citrate)
PAX 8	ProteinTech Group (Chicago, IL)	PAb (rabbit)	1:100	Yes (citrate)

Abbreviation: MAb, monoclonal antibody; PAb, polyclonal antibody.

Table 2 Immunohistochemical results

	Papillary serous carcinomas						*Peritoneal epithelioid mesotheliomas*					
	n = *45*		*Grade of reactivity*				n = *40*		*Grade of reactivity*			
	Positive cases	*%*	*1+*	*2+*	*3+*	*4+*	*Positive cases*	*%*	*1+*	*2+*	*3+*	*4+*
PAX8	42	93	0	2	12	28	0	0	0	0	0	0
PAX2	25	56	4	7	8	6	0	0	0	0	0	0
Claudin-4	44	98	1	3	6	34	0	0	0	0	0	0
h-caldesmon	0	0	0	0	0	0	0	0	0	0	0	0

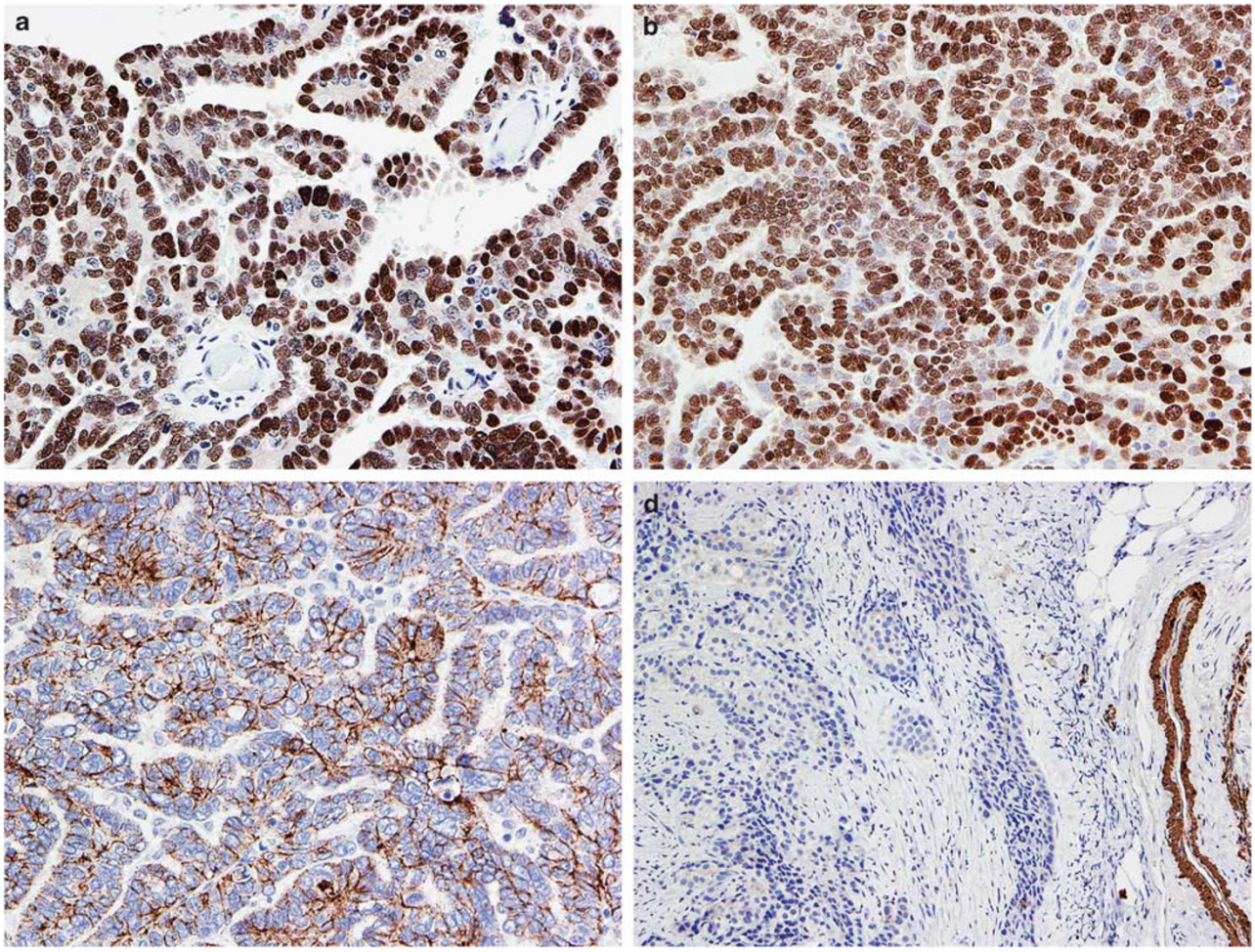

Figure 1 (**a**) Serous carcinoma exhibiting strong nuclear positivity for PAX8. (**b**) Serous carcinoma displaying nuclear positivity for PAX2. (**c**) Serous carcinoma showing claudin-4 positivity along the cell membrane. (**d**) Epithelioid peritoneal mesothelioma demonstrating a lack of h-caldesmon expression in the neoplastic cells (left). Strong positivity for this marker is seen in the smooth muscle cells of the vascular walls of blood vessels, which serve as an internal positive control.

the remaining 2 cases. All of the mesotheliomas were PAX8 negative.

PAX2

Twenty-five (56%) of the 45 papillary serous carcinomas exhibited nuclear positivity for PAX2 (Figure 1b). The staining in these cases was strong (3+ or 4+) in 14 cases, whereas in the remaining cases, it was focal (1+ or 2+). None of the mesotheliomas expressed PAX2.

Claudin-4

Forty-four (98%) of the 45 papillary serous carcinomas exhibited claudin-4 expression. The staining in these cases occurred along the cell membrane in a continuous or punctated pattern, and it was strong and diffuse in 40 of the cases (3+ or 4+) (Figure 1c). None of the mesotheliomas showed claudin-4 positivity.

h-caldesmon

All of the mesotheliomas and serous carcinomas were h-caldesmon negative (Figure 1d).

Discussion

Primary serous carcinomas of the peritoneum and serous carcinomas of the ovary are two malignancies of Müllerian lineage that exhibit similar histological features and have a tendency to involve the peritoneum, mimicking both clinically and morphologically, diffuse peritoneal mesothelioma. Traditionally, the differential diagnosis between peritoneal mesotheliomas and serous carcinomas has been based on the use of immunohistochemical panels composed of positive mesothelioma markers and broad-spectrum positive carcinoma markers.[1,8] Although no absolutely specific marker for Müllerian lineage has yet been identified, several Müllerian-associated markers that are commonly

expressed in serous carcinomas, such as PAX8 and PAX2, have recently become available.[15,16]

PAX8 is one of the nine members of the paired-box (PAX) family of transcription factors that regulates organogenesis during fetal development and has an important role in maintaining the normal function of certain cells after birth.[17–20] During embryogenesis, PAX8 is thought to be a crucial transcription factor for the development of several organs, including the kidney, thyroid, certain areas of the nervous system, and organs derived from the mesonephric (wolffian) duct, and those related to the Müllerian system, the precursor of the female genital tract.[21,22] Deletion of the PAX8 gene in mice results in the lack of a functional uterus with absence of the endometrium and poor development of endometrial tissue, as well as the absence of a vaginal opening.[22] The fallopian tubes, cervix, and upper parts of the vagina, however, are not affected, thus suggesting that other transcription factors, such as PAX2, may have a compensatory role in Müllerian organogenesis. Among ovarian tumors, PAX8 is expressed in the vast majority of non-mucinous carcinomas, including serous carcinomas (79–100%),[13,23–28] endometrioid carcinomas (38–92%),[13,23–25,27,28] clear cell carcinomas (76–100%),[13,23–25,27] and transitional cell carcinomas (67–100%).[13,27] In a combined review of 713 primary and 118 metastatic ovarian serous carcinomas from 10 published studies, 650 (91%) and 107 (91%), respectively, were reported to be PAX8 positive.[13,23–26,28–32] These results are comparable to those in the present investigation in which 14 (93%) of the 15 primary and 28 (93%) of the 30 metastatic ovarian serous carcinomas were found to express PAX8. To my knowledge, only one study on the expression of PAX8 in peritoneal epithelioid mesotheliomas has been published.[13] In that study, only 2 (9%) of the 23 cases investigated exhibited focal, weak reactivity for this marker. In the present study, none of the 40 cases of peritoneal mesothelioma that were stained for PAX8 were positive. These findings indicate that, because PAX8 is usually absent in peritoneal epithelioid mesotheliomas, immunostaining for this marker could be useful in distinguishing these tumors from serous carcinomas involving the peritoneum.

PAX2 is a transcription factor that has an essential role in the organogenesis and development of the central nervous system, eye, ear, mammary gland, and urogenital tract.[33,34] PAX2 null mice of both sexes completely lack the entire genital tract.[33] Among ovarian carcinomas, PAX2 expression has been reported to be often expressed in serous (27–100%),[14,35–38] endometrioid (40%),[38] and clear cell (43%) carcinomas.[39] In a combined review of 132 cases of primary and 53 metastatic ovarian serous carcinomas from six published studies, 63 (48%) and 23 (43%), respectively, were reported to be PAX2 positive.[14,31,35–38] In addition, 2 (29%) of the 7 primary peritoneal serous carcinomas that have been investigated for PAX2 expression were positive for this marker.[31] That only 8 (53%) of the 15 primary and 17 (57%) of the 30 metastatic ovarian serous carcinomas in the present investigation were PAX2 positive indicates that the sensitivity of this marker for serous carcinomas is lower than that of PAX8. Because the same anti-PAX2 antibody that was obtained from the same commercial source was used in all of the published studies, as well as in the current investigation, it is unclear as to whether the low sensitivity reported for this marker in serous carcinomas is due to a true low expression or if it is actually the result of a low sensitivity of this particular antibody.[16] To my knowledge, only two studies have been published on PAX2 expression in mesotheliomas.[14,35] The first of these was by Tong *et al*,[35] in 2007, who reported PAX2 positivity in 2 (12%) of 17 peritoneal mesotheliomas in women, whereas all 37 peritoneal mesotheliomas in men were negative. In a more recent study by Gao *et al*,[14] in 2012, none of the 25 cases of peritoneal mesotheliomas investigated were PAX2 positive. That all 40 of the peritoneal epithelioid mesotheliomas in the present study were PAX2 negative is an indication that this marker is usually absent in these tumors.

Similar to PAX8 and PAX2, the expression of estrogen receptor (ER) and progesterone receptor (PR) is rather restricted to some types of carcinomas. ER and PR have been reported to be frequently expressed in carcinomas of the breast, ovary, and endometrium.[11,40–42] The percentage of ER and PR positivity reported in serous carcinomas of the ovary and peritoneum has ranged from 80 to 95% (Table 3)[7,9–11,35] and 29 to 65% (Table 4),[7,9,11,35] respectively. Several studies have been published on the expression of ER and PR in epithelioid mesotheliomas.[7,9–11,35,43] The results of these investigations indicate that ER expression is usually absent in peritoneal epithelioid mesotheliomas. Only two studies have reported ER positivity in 2 (8%) of 24[43] and 2 (4%) of 54[35] peritoneal mesotheliomas, respectively. All of the positive cases occurred in women, whereas all of the

Table 3 Estrogen receptor expression reported in serous carcinomas and peritoneal epithelioid mesotheliomas

	Serous carcinomas			*Mesotheliomas*		
	n	*Positive cases*	%	n	*Positive cases*	%
Trupiano *et al*[43]		Not studied		24	2	8
Ordóñez[11]	47	41	87	30	0	0
Barnetson *et al*[7]	28	26	93	20	0	0
Comin *et al*[9]	40	38	95	15	0	0
Tong *et al*[35]	36	33	92	54	2	4
Takeshima *et al*[10]	20	16	80	15	0	0

Table 4 Progesterone receptor expression reported in serous carcinomas and peritoneal epithelioid mesotheliomas

	Serous carcinomas			*Mesotheliomas*		
	n	*Positive cases*	%	n	*Positive cases*	%
Trupiano *et al*[43]		Not studied		24	0	0
Ordóñez[11]	47	28	(60	30	0	0
Barnetson *et al*[7]	28	8	29	20	0	0
Comin *et al*[9]	40	26	65	15	0	0
Tong *et al*[35]	36	15	42	54	0	0

Table 5 MOC-31 reactivity reported in serous carcinomas and peritoneal epithelioid mesotheliomas

	Serous carcinomas			*Mesotheliomas*		
	n	*Positive cases*	%	n	*Positive cases*	%
Ordóñez[5]	30	29	97	40	2[a]	5
Barnetson *et al*[7]	28	28	100	20	3[b]	15
Ordóñez[8]	45	44	98	40	2[a]	5
Takeshima *et al*[10]	20	18	90	19	1[b]	5

[a]Few positive cells.
[b]Weakly positive (1%).

Table 6 Ber-EP4 reactivity reported in serous carcinomas and peritoneal epithelioid mesotheliomas

	Serous carcinomas			*Mesotheliomas*		
	n	*Positive cases*	%	n	*Positive cases*	%
Ordóñez[5]	45	45	100	35	4[a]	11
Attanoos *et al*[6]	23	20	87	32	3[b]	9
Barnetson *et al*[7]	28	27	96	20	2[b]	10
Ordóñez[8]	45	45	100	40	5[a]	13
Comin *et al*[9]	40	38	95	15	1[b]	7
Takeshima *et al*[10]	20	20	100	21	0	0

[a]Positive in a limited number of cells.
[b]Focal or weakly positive.

Table 7 TAG-72 expression reported in serous carcinomas and peritoneal epithelioid mesotheliomas

	Serous carcinomas			*Mesotheliomas*		
	n	*Positive cases*	%	n	*Positive cases*	%
Bollinger *et al*[2]	46	33	72	28	0	0
Khoury *et al*[3]	20	13	65	10	3[a]	30
Ordóñez[5]	45	39	87	35	0	0
Ordóñez[8]	45	33	73	40	0	0
Comin *et al*[9]	40	29	73	15	0	0

[a]<1% of the cells were positive.

cases investigated in men were negative for this marker. It should be noted that the two ER-positive mesothelioma cases in one of these studies[35] were also reported to be PAX2 positive, a marker that is frequently expressed in serous carcinomas,[16] but not in mesotheliomas.[14,35] In addition to the two previously mentioned studies, other investigations on ER expression in peritoneal mesotheliomas, including one by this author, have been published (Table 3).[7,9–11] No ER expression was demonstrated in any of the cases, including those in women, investigated in these studies.[7,9,11] PR has been reported to be consistently negative in all cases of peritoneal epithelioid mesotheliomas investigated.[7,9,11] Based on these results, it has been concluded that both ER and PR immunostaining can be useful for assisting in distinguishing between epithelioid mesotheliomas and serous carcinomas involving the peritoneum. ER, however, has the advantage of being more sensitive for serous carcinomas than PR, thus making it the more useful of the two.

In 1979, Wang *et al*[44] introduced carcinoembryonic antigen as a positive carcinoma marker that could be useful in discriminating between epithelioid mesotheliomas and lung adenocarcinomas. Since then, a relatively large number of other broad-spectrum positive carcinoma markers that could be helpful in the differential diagnosis of mesotheliomas have been recognized.[1,45,46] Among these, MOC-31, Ber-EP4, TAG-72 (B72.3), CD15 (leu-M1), and CA19-9 are the ones that, based on their sensitivity and specificity, have been suggested as being the most useful in distinguishing between peritoneal mesotheliomas and serous carcinomas.[5,8,10] In serous carcinomas, the percentage of positivity has ranged from 90 to 100% for MOC-31 (Table 5),[5,7,8,10] 87 to 100% for Ber-EP4 (Table 6),[5–10] 65 to 87% for TAG-72 (Table 7),[2,3,5,8,9] 30 to 80% for CD15 (Table 8),[2–10] and 60 to 80% for CA19-9 (Table 9).[5,8–10] The percentage of positivity reported for these markers in peritoneal epithelioid mesotheliomas has been much lower, ranging 0–15% for MOC-31,[5,7,8,10] 0–13% for Ber-EP4,[5–10] and 0–15% for CD15[2,4,6] (Tables 5, 6 and 8). With the exception of one study each, all other investigations into the expression of TAG-72 or CA19-9 in peritoneal epithelioid mesotheliomas have been unable to demonstrate any reactivity for either of these markers in these tumors (Tables 7 and 9). In all of the mesothelioma cases positive for these markers, the staining was described as weak and focal or limited to a few cells, which is in contrast to serous carcinomas in which the reaction was often reported to be strong and diffuse.

Claudin-4 is one of the 24 members of the claudin family of transmembrane proteins that are essential in the formation and maintenance of tight junctions.[47] The tight junction is part of the apical junctional complex and is involved in both paracellular permeability and cell polarity.[48,49]

Table 8 CD15 reactivity reported in serous carcinomas and peritoneal epithelioid mesotheliomas

	Serous carcinomas			*Mesotheliomas*		
	n	*Positive cases*	*%*	n	*Positive cases*	*%*
Bollinger *et al*[2]	46	34	74	28	3	11
Khoury *et al*[3]	20	6	30	4	0	0
Wick *et al*[4]	10	8	80	20	3	15
Ordóñez[5]	45	28	62	35	0	0
Attanoos *et al*[6]	23	7	30	32	2	6
Barnetson *et al*[7]	28	14	50	20	0	0
Ordóñez[8]	45	26	58	40	0	0
Comin *et al*[9]	40	18	45	15	0	0
Takeshima *et al*[10]	20	12	60	12	0	0

Table 9 CA19-9 expression reported in serous carcinomas and peritoneal epithelioid mesotheliomas

	Serous carcinomas			*Mesotheliomas*		
	n	*Positive cases*	*%*	n	*Positive cases*	*%*
Ordóñez[5]	45	31	69	35	0	0
Ordóñez[8]	45	30	67	40	0	0
Comin *et al*[9]	40	24	60	15	0	0
Takeshima *et al*[10]	20	16	80	23	3[a]	13

[a]Focally and weakly positive.

Claudin-4, also known as *Clostridium perfringens* enterotoxin receptor, is expressed in most epithelial cells, including those of the lung, kidney, breast, thyroid, thymus, and bladder, and in follicular dendritic cells, but it is not expressed in hepatocytes or mesothelial cells.[12,50,51] Because claudin-4 is commonly demonstrated in a wide variety of carcinomas, including adenocarcinomas of the lung, breast, thyroid and pancreas, renal cell carcinomas, and various subtypes of ovarian carcinomas, including papillary serous carcinomas, as well as in most squamous cell carcinomas and transitional cell carcinomas, but it is often absent in mesotheliomas,[12,51] this marker can be regarded as a broad-spectrum positive carcinoma marker.[1] The first investigation on the potential utility of claudin-4 immunostaining for assisting in the differential diagnosis of mesotheliomas was by Soini *et al*[51] in 2006. In all, 7 (29%) of 24 epithelioid, 1 (25%) of 4 sarcomatoid, and none of the 7 biphasic mesotheliomas included in the study were reported to exhibit claudin-4 expression, whereas all (100%) of the 23 metastatic adenocarcinomas were positive for this marker.[51] Because the degree of claudin-4 positivity in the epithelioid mesotheliomas was lower than that seen in the adenocarcinomas, the authors concluded that claudin-4 could have some utility as an additional marker for assisting in distinguishing between epithelioid mesotheliomas and metastatic adenocarcinomas to the serosal membranes. This observation, however, is in contrast to that of Facchetti *et al*,[12] who, in a subsequent study, were unable to demonstrate claudin-4 expression in any of their 60 epithelioid or 11 sarcomatoid mesotheliomas. Because 245 (88%) of 278 primary carcinomas of various sites of origin and 57 (98%) of 58 serosal metastases included in the study were claudin-4 positive, they concluded that this marker should be considered as a primary immunohistochemical marker for assisting in the differential diagnosis between epithelioid mesotheliomas and metastatic carcinomas. Only a few studies have been published on the expression of claudin-4 in serous carcinomas of the ovary and peritoneum, and the percentage of positivity reported in these tumors has ranged from 64 to 100%.[12,52–54] That expression for this marker was demonstrated in 98% of the serous carcinomas, but in none of the peritoneal epithelioid mesotheliomas, in the present investigation indicates that immunostaining for this marker could be very useful for assisting in the differential diagnosis between the latter tumors and serous carcinomas involving the peritoneum. When compared with other broad-spectrum positive carcinoma markers, such as MOC-31, Ber-EP4, TAG-72, CD15, and CA19-9, which are at present considered to be useful for assisting in this differential diagnosis, the results of this study indicate that, in general, claudin-4 has a higher sensitivity and specificity than the previously mentioned markers (Tables 5–9).

Since the identification of thrombomodulin in 1992 by Collins *et al,*[55] as a positive mesothelioma marker that could be useful in distinguishing epithelioid mesotheliomas from metastatic carcinomas to the serosal membranes, a relatively large number of other markers that can assist in this differential diagnosis have also become available.[1,45,46] Because of their sensitivity and specificity, thrombomodulin, calretinin, and podoplanin have been reported to be the most useful for assisting in the differential diagnosis between peritoneal epithelioid mesotheliomas and serous carcinomas involving the peritoneum.[8] In peritoneal epithelioid mesotheliomas, the percentage of expression has ranged from 45 to 95% for thrombomodulin (Table 10),[5–10] from 85 to 100% for calretinin (Table 11),[5–10,14,56] and from 93 to 96% for podoplanin (Table 12).[8–10,57] The percentage of positivity reported for these markers in serous carcinomas is much lower, ranging from 2 to 26% for thrombomodulin (Table 10),[5–10] 0 to 40% for calretinin (Table 11),[5–10,14,56] and 13 to 65% for podoplanin (Table 12).[8–10,42,57]

Caldesmon, an actin-interacting and calmodulin-binding protein found in smooth muscle cells and other cell types, has an important role in the regulation of smooth muscle and non-smooth

Table 10 Thrombomodulin expression reported in peritoneal epithelioid mesotheliomas and serous carcinomas

	Mesotheliomas			*Serous carcinomas*		
	n	*Positive* cases	%	n	*Positive* cases	%
Ordóñez[5]	35	26	74	45	1[a]	2
Attanoos *et al*[6]	32	18	56	23	6[a]	26
Barnetson *et al*[7]	20	9	45	28	1[a]	4
Ordóñez[8]	40	29	73	45	2[a]	4
Comin *et al*[9]	15	9	60	40	2[a]	5
Takeshima *et al*[10]	22	21	95	20	1[a]	5

[a]Positive either focally or in a few tumor cells.

Table 11 Calretinin expression reported in peritoneal epithelioid mesotheliomas and serous carcinomas

	Mesotheliomas			*Serous carcinomas*		
	n	*Positive cases*	%	n	*Positive cases*	%
Doglioni *et al*[56]	3	3	100	16	1	6
Ordóñez[5]	35	35	100	45	4[a]	9
Attanoos *et al*[6]	32	28	88	23	0	0
Barnetson *et al*[7]	20	17	85	28	0	0
Ordóñez[8]	40	40	100	45	14[b]	31
Comin *et al*[9]	15	15	100	40	5[c]	13
Takeshima *et al*[10]	29	29	100	20	8[d]	40
Gao *et al*[14]	25	22	88	34	0	0

[a]Three cases, 1–25% + cells; one case, <1% + cells.
[b]Three cases, 50–75% + cells; three cases, 25–50% + cells; seven cases, 1–25% + cells; one case, <1% + cells.
[c]Two cases, 50–75% + cells; three cases, 25–50% + cells.
[d]Three cases, 25–50% + cells; five cases, <25% + cells.

Table 12 Podoplanin expression reported in epithelioid mesotheliomas and serous carcinomas

	Mesotheliomas[a]			*Serous carcinomas*		
	n	*Positive cases*	%	n	*Positive cases*	%
Chu *et al*[57]	53	51	96	26	17[b]	65
Ordóñez[8]	40	37	93	45	6[c]	13
Comin *et al*[9]	15	14	93	40	8[d]	20
Nofech-Mozes *et al*[42]	Not studied			56	13[e]	23
Takeshima *et al*[10]	23	22	96	20	9[f]	45

[a]All of the cases investigated by Chu *et al* were pleural, whereas all of those in the remaining publications were peritoneal.
[b]33% mean positive cells.
[c]Two cases, 25–50% + cells; four cases, 1–25% + cells.
[d]Three cases, 25–50% + cells; five cases, 1–25% + cells.
[e]Six cases, > 50% + cells; seven cases, 1–25% + cells.
[f]Two cases, 25–50% + cells, seven cases, 1–25% + cells.

muscle contraction.[58–62] Two human caldesmon isoforms, which are generated from a single gene by alternative splicing and which differ in their molecular weight, electrophoretic mobility, and cellular distribution, have been identified.[58] The h-caldesmon, also referred to as high-molecular weight, variant (120–150 kDa) is predominantly expressed in smooth muscle cells, and a low-molecular weight variant known as l-caldesmon (70–80 kDa) is found in non-smooth muscle cells. Neither of these variants is expressed in skeletal muscle cells. Only a few studies, which reported conflicting results, have investigated the potential utility of h-caldesmon immunostaining for assisting in the differential diagnosis of mesotheliomas. The first of these studies was by Comin *et al*,[63] in 2006, who reported h-caldesmon expression in 68 (97%) of 70 epithelioid mesotheliomas, but none was seen in any of the 70 lung adenocarcinomas investigated, and they concluded that this marker could be useful in distinguishing between these two malignancies. In a subsequent study published the following year, the same group of investigators reported h-caldesmon positivity in all 15 (100%) peritoneal epithelioid mesotheliomas, whereas only 2 (5%) of 40 ovarian serous carcinomas were found to express this marker.[9] These results differ from those obtained in a more recent investigation by Laury *et al*,[13] in which only 1 (4%) of the 24 epithelioid pleural mesotheliomas and none of the 26 mesothelial tumors of the peritoneum (23 malignant mesotheliomas, 2 well-differentiated papillary mesotheliomas, and 1 multicystic mesothelioma) investigated were reported to be h-caldesmon positive. In addition, none of the 254 ovarian serous tumors included in the study exhibited h-caldesmon expression. Based on these results, the authors concluded that h-caldesmon immunostaining had no utility in discriminating between peritoneal epithelioid mesotheliomas and serous carcinomas involving the peritoneum. That none of the peritoneal epithelioid mesotheliomas or serous carcinomas in the present study exhibited h-caldesmon expression indicates that this marker is not expressed in these types of tumors. The cause of the discrepancy between the results reported by Comin *et al,* and those obtained by Laury *et al*, as well as those in the present investigation, is unclear as the only difference between the two studies was the dilution of the antibody, which was obtained from the same commercial source. In the present study, the antibody dilution was 1:50, whereas, in the study by Comin *et al*,[9] it was 1:100, and in that by Laury *et al*,[13] it was 1:300.

The results of this investigation indicate that, because of their sensitivity and specificity, claudin-4 and PAX8 should be considered to be the best positive carcinoma markers. In serous carcinomas, positivity for claudin-4 was observed in nearly all (98%) of the cases, and PAX8 was demonstrated in 93%, whereas all mesotheliomas were negative for both markers. Because of its low sensitivity for serous carcinomas, PAX2 has little or no practical value for assisting in the differential diagnosis

between these tumors and peritoneal epithelioid mesotheliomas. Because h-caldesmon is not expressed in either epithelioid mesotheliomas or serous carcinomas, it has no value in discriminating between these malignancies. Therefore, it is concluded that both claudin-4 and PAX8 should be considered as two primary immunohistochemical markers for assisting in the differential diagnosis between peritoneal epithelioid mesotheliomas and papillary serous carcinomas involving the peritoneum. Finally, because the International Mesothelioma Interest Group[64] recommends an immunohistochemical panel composed of two positive carcinoma markers and two positive mesothelioma markers for distinguishing between epithelioid mesotheliomas and metastatic carcinomas to the serosal membranes, based on the findings of this study, as well as the results obtained in previous investigations by this author, claudin-4 and PAX8 (or ER) combined with thrombomodulin and calretinin will usually make it possible to distinguish between peritoneal epithelioid mesotheliomas and serous carcinomas involving the peritoneum.

Disclosure/conflict of interest

The author declares no conflict of interest.

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