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Claudin 3, 4, and 15 expression in solid tumors of lung adenocarcinoma versus malignant pleural mesothelioma

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ABSTRACT

Epithelioid malignant pleural mesothelioma (MPM) can easily be confused with lung adenocarcinomas (ACAs). In serous effusion, claudin (cldn) 3 is shown to be useful in the diagnosis of mesothelioma vs ACAs. Cldn15 is reported to be overexpressed in epithelioid mesothelioma and absent in human airway epithelium. The aim was to assess the value of cldn3 and cldn4 compared to that of BerEp4 and thyroid transcription factor–1 (TTF1) in differentiating lung ACA from epithelioid MPM and to examine the expression of cldn15 in these tumors. The expression of cldn3, cldn4, cldn15, BerEp4, and TTF1 was examined by immunohistochemistry in a total of 62 human specimen including 28 epithelioid MPMs and 34 ACAs of the lung. In lung ACA, cldn4 was strongly expressed in all 34 (100%) specimens followed by cldn3 in 33 (97%) of 34. BerEp4 was expressed in 32 (94.1%) of 34. TTF1 reacted for only 20 (58.82%) of 34 cases of lung ACA. In MPM specimens, the expression of cldn3 and4 as well as that of TTF1 was completely absent. In contrast, BerEp4 was focally expressed in 5 (17.85%) of 28 cases of epithelioid MPM. Cldn15 was strongly expressed in 53% pf epithelioid MPMs but also in 50% of lung ACAs. Its expression was moderate in normal pleura and limited in normal lung. Cldn3 and cldn4 appear to be the best performing carcinoma markers in discriminating lung ACA from mesothelioma compared with BerEp4 and TTF1. There is no differential expression of cldn15 between the 2 pathologies. However, the limited cldn15 expression in normal tissues and high expression in tumors make it an attractive candidate for cancer therapy.

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1. Introduction

Malignant pleural mesothelioma (MPM) is a highly aggressive tumor with difficult diagnosis. Differentiating between the most common, epithelioid form of mesothelioma and lung adenocarcinoma (ACA) is often problematic. Immunohistochemistry has greatly improved the diagnosis of mesothelioma. However, as there is no specific biomarker, the diagnosis is based in part on the negativity of markers that characterize other pathological pleural entities [1,2].

Emerging studies suggest the interest of claudin expression in cancer diagnosis and therapy [3]. Claudins are a family of 27 proteins that constitute the major components of tight junctions [4–7]. In the last decade, the role of some claudins in distinguishing mesothelioma from carcinomas has been reported. In 2002, Gordon et al [8] showed a highly differential gene expression of *claudin 7* between mesothelioma and lung ACA. Some years later, Holloway et al [9] listed *claudin 3* and *claudin 7* among the highly differentially expressed genes between the 2 pathologies. Simultaneously, Soini et al [10] showed that cldn1, 3, 4, 5, and 7 protein expression could be used as an adjunct in the differential diagnosis between these tumors. Kleinberg et al [11] reported the usefulness of claudin1, 3, and 7 in serous effusion; and Facchetti et al [12,13] reported the usefulness of cldn4 in pleural and peritoneal biopsies and effusions. We previously found, in a limited number of specimens, the absence of cldn3 and 4 in MPM and their expression in 100% of lung ACAs [14]. Recently, Ordóñez [15] and Ohta et al [16] confirmed the diagnostic utility of cldn4 in distinguishing mesothelioma from ACAs.

Taken together, these data highlight the interest of cldn3 and 4 in mesothelioma diagnosis. However, the number of published reports is limited; and the use of these proteins in routine practice still requires validation by further studies especially for claudin 3. In the present work, we compare the value of cldn3 and 4 with that of the 2 markers, BerEp4 and thyroid transcription factor–1 (TTF1), in 62 human

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Table 1
Antibodies used

Antibody	Clone	Host and clonality	Source	Dilution	Incubation (min)	Pretreatment
Calretinin	5A5	Mouse monoclonal	Leica	Prediluted	32	Citrate buffer pH 6.0 60 min
CK5/6	D5/16B4	Mouse monoclonal	Dako	1:50	32	EDTA, pH 8.4 60 mn
WT1	6F-H2	Mouse monoclonal	Roche	Prediluted	44	EDTA, pH 8.4 60 min
BerEp4	Ber-Ep4	Mouse monoclonal	Dako	1:25	32	None
TTF1	8G7G3/1	Rabbit polyclonal	Dako	1:50	32	Citrate buffer, pH 6.0 60 min
Cldn3	Ab15102	Rabbit polyclonal	Abcam	1:100	20	Citrate buffer, pH 6.0 60 min
Cldn4	Ab15104	Rabbit polyclonal	Abcam	1:200	20	EDTA, pH 8.4 36 min
Cldn15	NBP2-13842	Rabbit polyclonal	Novus Bio	1:200	32	Citrate buffer, pH 6.0 90 min

specimens of lung ACA and epithelioid MPM, currently used in the panel for differentiating between the 2 pathologies.

Moreover, *claudin 15* has been listed among the highly expressed genes in epithelioid MPM [17] and in diffuse peritoneal malignant mesothelioma [18]. We previously found high levels of cldn15 protein in MPM cell lines [14]. The expression of cldn15 was detected in mesothelial cells of the pleura and peritoneum in mice [19] but was absent in bronchial human airways [20]. In this study, we examined the expression of cldn15 in human specimens of epithelioid mesothelioma and lung ACA to see whether it is differentially expressed between these tumors.

2. Material and methods

Sixty-two (62) human specimens including epithelioid MPMs (n= 28) and lung ACAs (n=34), in addition to normal control tissues (colon, pleura, bronchi, and bronchioles), were used to examine the expression of cldn3, cldn4, cldn15, BerEp4, and TTF1 in addition to calretinin, cytokeratin 5/6, and WT1 to confirm the diagnosis of mesothelioma. Surgical resection specimens were provided by the CHU of Mustapha Pacha (Algiers, Algeria) and the Public Hospital of Blida (Algeria). No biopsy material was used because there was insufficient material for immunohistochemical analysis. The diagnosis of all specimens was validated by pathologists using both morphologic criteria and immunohistochemistry with a broad antibody panel that recognizes carcinoma or mesothelioma. In case of TTF1-negative cases, the diagnosis of primary lung carcinoma was based on clinicopathological correlation. Nevertheless, the primary vs secondary origin of ACA was less important for this study because it was aimed at differentiating between ACAs and epithelioid form of mesotheliomas.

2.1. Immunohistochemistry

Formalin-fixed, paraffin-embedded samples were cut (4 μm–thick sections) and placed on silane-covered slides. Morphological assessment of the samples was obtained by examining sections stained with hematoxylin-eosin-saffron. Slides were stained with anti-cldn3, anti-cldn4, anti-cldn15, anti-BerEp4, anti-TTF1, anti-calretinin, anti-keratin 5/6, and anti-WT1 using a Ventana Ultraview DAB detection kit in a Ventana BenchMark XT processor (Ventana, Tucson, AZ). For the antibody dilutions, pretreatment, and sources, see Table 1. An external positive control section was included in each immunohistochemical analysis (1 section for each slide). The signal was interpreted as present (positive) or absent (negative) irrespective of the intensity of the signal. When present, there were at least several cells stained; and the intensity was at least moderate in all cases.

3. Results

In lung ACAs, cldn4 (Fig. 1A) was strongly expressed along the cell membrane in all 34 specimens (100%). Cldn3 (Fig. 1B) was expressed along the cell membrane in 33 (97%) of 34 cases with less intensity of immunostaining than cld4. The immunostaining of BerEp4 (Fig. 1C) was as intense as that of cldn4 along the cell membrane in 32 (94.1%)

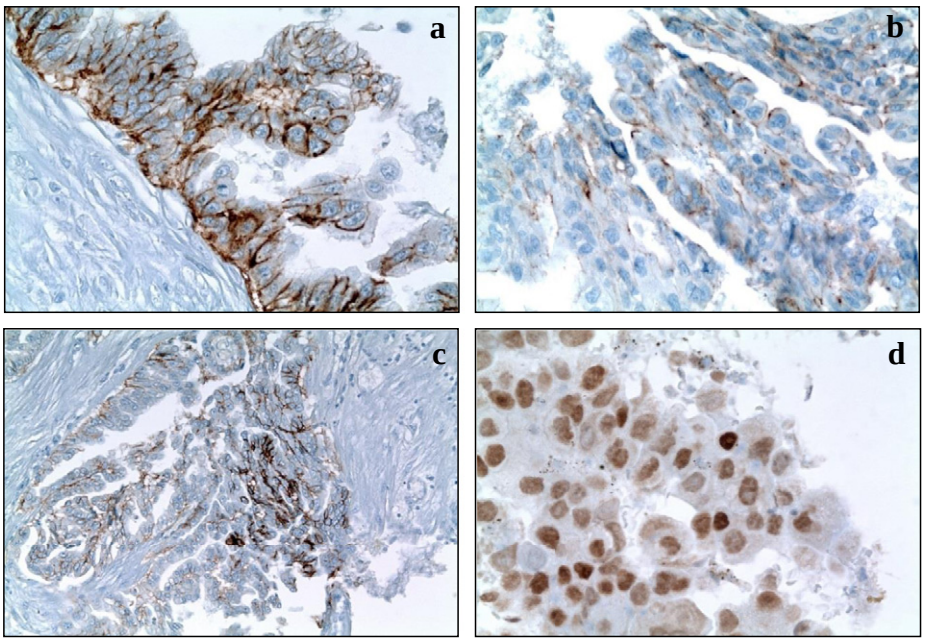

Fig. 1. Lung ACA immunostaining. (A) cldn-4 immunostaining (intense membrane positivity) ×400. (B) cldn-3 immunostaining (membrane positivity) ×400. (C) BerEp4 immunostaining (intense membrane positivity) ×200. (D) TTF1 immunostaining (nuclear positivity) ×400.

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