TIMELY REVIEWS

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CD147 Immunohistochemistry Discriminates Between Reactive Mesothelial Cells and Malignant Mesothelioma

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Malignant mesothelioma (MM) is a rare form of cancer. Its histopathological diagnosis is very difficult, as it exhibits a number of different appearances that can be misinterpreted as metastatic invasion or atypical hyperplasia. Thus, there is an urgent need to identify adequate markers to distinguish between benign and malignant cells, allowing the implementation of appropriate therapies and, possibly, specific directed therapies. MM, like other tumors, show an increase in glucose uptake, due to high rates of glycolysis, inducing an intracellular overload of acids. In this context, monocarboxylate transporters (MCTs) emerge as important players, by mediating the transmembranar co-transport of lactate with a proton, thereby, regulating pH and allowing continuous glycolysis. Importantly, proper MCT expression and activity depend on its co-expression with a chaperone, CD147, which is associated with poor prognosis in cancer. Twenty-two samples including reactive

mesothelial cells, MM, and atypical mesothelial hyperplasias were evaluated for immunoeexpression of MCT1, MCT4, and CD147. Expression of these proteins was compared with GLUT1 as a new promising marker for MM. Although MCT isoforms were not differentially expressed in the two types of cytological specimens, CD147, as GLUT1, was almost exclusively expressed in MM. Both MCT1 and MCT4 are not able to discriminate between mesothelial reactive cells and mesothelial malignant cells, while CD147 was able to distinguish these two proliferations. If confirmed, besides being a good marker for identification of MM, CD147 may also be a target for therapeutical strategies in this rare type of tumor. Diagn. Cytopathol. 2011;00:000–000. © 2011 Wiley Periodicals, Inc.

Key Words: monocarboxylate transporters; CD147/EMMPRIN; malignant mesothelioma; lactate

Malignant mesothelioma (MM), a neoplasm of mesothelial cells from the serosal membranes occurs predominantly in the pleura, less often in the peritoneum, seldom in pericardium, and rarely in the tunica vaginalis testis. Asbestos exposure is the most common cause of MM and its connection with this rare form of cancer has been well established for the last half a century.¹ The long-latency period from initial asbestos exposure to development of MM is responsible for an incidence of nearly 3,000 new cases a year in the U.S., a number that may remain at that level for the foreseeable future.² The number of MM diagnosed worldwide may also grow larger due to pathologists' increasing ability in recognizing MM and to the emergence of newer immunohistochemical markers with restrictive specificity for this neoplasm.

Current MM treatment is complex and the results are far from ideal. Surgery cannot remove all the residual mi-

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oscopic cancer deposits and as a result, surgical treatment for operable MM even in able hands cannot be considered a definite form of therapy.³ Even with new forms of systemic therapy, survival beyond 12–18 months is extremely rare.⁴ Attempts at combined chemotherapy,⁵ gene therapy,⁶ and immunotherapy with vaccines⁷ have not fared any better. The attention, therefore, has shifted to targeted therapy directed at molecular events involved in the survival and growth of malignant transformed mesothelial cells. Specific-targeted therapy has been a success story in the treatment of GIST and other tumors and constitutes a promising option for the treatment of MM.⁸

A precise pathological diagnosis of MM remains crucial for instituting appropriate therapeutic intervention. Early diagnosis offers the best opportunity for successful outcomes in any treatment strategy and cytological examination of effusion specimens offers the greatest chance for achieving it. However, in its early stage, the distinction between MM and atypical mesothelial hyperplasia still represents a very difficult challenge.⁹ Cytological results have vastly improved with the use of newer antibody panels but no infallible marker has been developed to distinguish reactive mesothelial hyperplasia from MM.^{10,11} More recently, the combination of positive epithelial membrane antigen (EMA) and negative desmin was interpreted to strongly favor MM, whereas a combination of negative EMA and positive desmin favors a reactive process.¹²

Some new promising markers have emerged recently, including some like GLUT1,¹³ which play a role in cellular glycolytic metabolism. The expression of GLUT1 may even discriminate between reactive mesothelium and MM,¹³ although with a lower specificity and sensitivity than originally claimed.¹⁴ Following this line of evidence, some studies suggest that positron emission tomography with ¹⁸F-fluorodeoxyglucose (FDG-PET) imaging could be an effective tool for differentiating between benign and malignant pleural diseases,^{15–17} indicating a higher glycolytic metabolism in the malignant context. It is widely known that, under hypoxic conditions, cancer cells obtain energy by increasing their rates of glycolysis leading to an overload of intracellular lactic acid, which must be transported out of the cell, a process that results in extracellular acidification. Low interstitial pH is associated with the upregulation of various angiogenic molecules such as VEGF, IL-8, bFGF, and Heparanase, all of which may provide autocrine stimulation and contribute to tumor aggressiveness.¹⁸ Cancer lactate accumulation has also been correlated with poor clinical outcomes,^{19–21} a property that remains yet to be explored as part of tumoral treatment strategies. One of the most important players in this context is monocarboxylate transporters (MCTs), which are also responsible for transmembrane co-transport of lactate with a proton, thus regulating

pH and enabling continuous glycolysis. The authors have observed that some MCT isoforms (MCT1 and MCT4) and their chaperone CD147 are overexpressed in a variety of solid tumors, including cervical carcinoma,^{22,23} colorectal carcinoma,²⁴ and basal-like carcinoma of the breast²⁵ but not in gastric carcinoma.²⁶ Since MCT activity supports cancer cell survival, their capacity to transport lactate can be explored as a new therapeutic target. In addition, CD147, the MCT1/MCT4 chaperone, was shown to contribute to tumoral progression and metastasis. Therefore, the metabolic modification of the tumor's microenvironment mediated by MCT1/CD147 holds promise as a target for future therapeutic options.²⁷ Despite their potential as molecular therapeutic targets for neoplasms relying on glycolytic metabolism, MCTs have not been investigated in MM. The aim of this study was to characterize the expression of monocarboxylate transporters 1 and 4 and their chaperone CD147 in benign and malignant mesothelial cells, and compare their expression with GLUT1, a new promising MM marker.

Material and Methods

Samples were obtained from 20 patients including 11 mesothelial reactive cells and 9 MM. Samples were retrieved from the consultation files of one of the authors (CWB). Relevant data available included patient's age and gender, source of the effusion as well as the final histopathological diagnosis in pleural biopsies or decortication specimens.

Immunohistochemistry

MCT, CD147, and GLUT1 detection. MCT immunohistochemistry was performed according to the avidin–biotin–peroxidase complex method (R.T.U. VECTASTAIN Elite ABC Kit (Universal), Vector Laboratories, Burlingame, CA), with primary antibodies for MCT1 (AB3538P, Chemicon International, Temecula, CA), and MCT4 (AB3316P, Chemicon International, Temecula, CA), diluted 1:200, as previously described.²⁴ Immunohistochemistry for CD147 and GLUT1 was performed according to the streptavidin–biotin–peroxidase complex principle Ultravision Detection System Anti-polyvalent, HRP, Lab Vision Corporation, Fremont, CA), using primary antibodies raised against CD147 (18-7344, ZYMED Laboratories Inc., South San Francisco, CA, diluted 1:750, as previously described²³) and GLUT1 (ab15309, AbCam, Cambridge, UK, diluted 1:500, same protocol as for CD147). Negative controls were performed using appropriate serum controls for the primary antibodies (N1699, Dako, Carpinteria, CA), colon carcinoma tissue was used as positive control for both MCT1 and MCT4, cervical squamous carcinoma for CD147 and skin for GLUT1. Tissue sections were counterstained with hematoxylin and permanently mounted.

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Table I. Immunohistochemical Characterization of MCT1, MCT4, CD147, and GLUT1 in 20 Patients with Mesothelioma and Benign Mesothelial Reaction

Case	Gender	Age	Histopathological diagnosis	MCT1 score	MCT1 membrane	MCT4 score	CD147 score	CD147 membrane	GLUT1 membrane
1	Male	82	Mesothelioma	+	+	+	+	+	+
2	Male	70	Benign	+	+	+	—	—	—
3	Male	80	Mesothelioma	+	+	—	+	+	+
4	Male	47	Benign	—	—	+	—	—	—
5	Male	69	Mesothelioma	+	+	+	+	+	+
6	Male	78	Mesothelioma	+	+	+	+	+	+
7	Female	78	Benign	+	+	+	—	—	—
8	Male	82	Benign	+	+	—	n.a.	n.a.	—
9	Male	81	Mesothelioma	+	+	—	+	+	+
10	Male	81	Benign	+	+	+	+	+	—
11	Male	58	Benign	—	—	+	—	—	—
12	Female	72	Mesothelioma	+	+	+	+	+	+
13	Male	69	Benign	+	+	—	—	—	—
14	Male	76	Benign	+	+	—	—	—	—
15	Female	52	Benign	+	—	—	+	—	—
16	Male	62	Benign	+	+	+	—	—	—
17	Male	89	Benign	+	+	+	—	—	+
18	Male	55	Mesothelioma	+	+	+	+	+	+
19	Female	73	Mesothelioma	+	+	+	—	—	+
20	Male	55	Mesothelioma	+	+	—	+	+	—

Benign means hyperplastic reactive mesothelia.n.a., not available.

Immunohistochemical evaluation. Sections were scored semi-quantitatively for immunoreaction as follows: 0: 0% of immunoreactive cells; 1: <5% of immunoreactive cells; 2: 5–50% of immunoreactive cells; and 3: >50% of immunoreactive cells. Also, intensity of staining was scored semi-qualitatively as follows: 0: negative; 1: weak; 2: intermediate; and 3: strong. The final score was defined as the sum of both parameters (extent and intensity), and grouped as negative (score 0 and 2) and positive (score 3–6), as previously described.²⁴ Since plasma membrane location of these proteins is essential for activity, when present, the significance of plasma membrane positivity for MCTs, CD147, and GLUT1 was evaluated separately. Immunohistochemical evaluation was performed blindly by two independent observers and discordant cases were discussed using a double-head microscope to determine the final score.

Statistical Analysis

Data were stored and analyzed using the SPSS statistical software (version 16.0, SPSS, Chicago, IL). All comparisons were examined for statistical significance using Pearson's chi-square (χ^2) test and Fisher's exact test (when $n < 5$), being threshold for significance P values <0.05.

Results

Twenty samples from 16 male and 4 female patients were assessed for MCT1, MCT4, CD147, and GLUT1 immunohistochemical expression. The results obtained for each case are summarized in Table I. Positive MCT1 expression was observed in both plasma membrane and cytoplasm (Figs. 1A and 1E), while MCT4 was only found in the cytoplasm (Figs. 1B and 1F). Regarding CD147,

except for one case, expression was always present in the plasma membrane (Fig. 1G), with some cytoplasmic staining. GLUT1 expression was present in the plasma membrane of all positive cases (Fig. 1H), showing also a strong plasma membrane expression in red blood cells (Fig. 1D).

Comparison between the expression frequencies of all markers in MM cells and mesothelial reactive cells is depicted in Table II. No statistically significant difference was observed between the benign and malignant samples with regards to their MCT1 and MCT4 expression. In contrast, the expression of CD147 was significantly increased in the MM samples, being expressed in about 90% (8/9) of MM vs. 9% (1/11) for mesothelial reactive cells ($P = 0.001$). The power of CD147 to distinguish benign from malignant mesothelial cells was the same as that of GLUT1, with about 90% of positivity for MM vs. 9% for mesothelial reactive cells ($P = 0.001$), corresponding to a sensitivity of 88.8% and specificity of 90.9%. No associations were found between either of the two MCTs, CD147, or GLUT1 and age or gender (data not shown). In addition, MCTs were not significantly co-expressed with CD147 (data not shown).

Discussion

Appropriate therapeutic intervention in MM can only be achieved if an accurate pathological diagnosis is made. However, the distinction between MM and atypical mesothelial hyperplasia at early stages remains very difficult.⁹ Therefore, there is an urgent need to establish efficient markers to distinguish between these two cytological entities.

Fig. 1. Immunohistochemical expression of monocarboxylate transporter 1 (MCT1), MCT4, CD147, and glucose transporter 1 (GLUT1) in mesothelial samples. (A) MCT1 in benign mesothelial cells; (B) MCT4 in benign mesothelial cells; (C) CD147 in benign mesothelial cells; (D) GLUT1 in benign mesothelial cells; (E) MCT1 in malignant mesothelial cells; (F) MCT4 in malignant mesothelial cells; (G) CD147 in malignant mesothelial cells; (H) GLUT1 in malignant mesothelial cells. Note the strong expression of GLUT1 in the plasma membrane of red blood cells. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

To the best of our knowledge, the results in this study reported described for the first time the expression of monocarboxylate transporters 1 and 4 in MM. However, neither MCT1 nor MCT4 showed any significant discriminating value in the recognition of malignancy. Actually, the values observed show a very similar pattern between mesothelial-derived malignancy and benign cells. Of note, MCT4 positive reaction was predominantly found in the cytoplasm which can explain, in part, the lack of significance of MCT4 in MM. Conversely, despite detection of MCT1 within the plasma membrane, MCT1 reaction did not show significant differences either. The overexpression of monocarboxylate transporters in both reactive mesothelium and MM supports the intriguing notion that benign mesothelial proliferations may be a precursor lesion of MM. In fact, the evolution of atypical mesothelial hyperplasia to invasive peritoneal MM has been documented, 8 years from the original non-MM diagnosis.²⁸ Unfortunately, no laparoscopy or biopsy was available in the intervening years, to document or dispel the presence of an in situ stage.

CD147, also known as extracellular matrix metalloproteinase inducer (EMMPRIN), is recognized to promote tumor angiogenesis mostly through its protease-inducing function. Remarkably, the plasma membrane expression of CD147 was associated with MM, since the majority of MM cases were positive for CD147 and only 1 case of mesothelial hyperplasia was decorated by CD147 immunoreaction. This fact may be important on two different bases: first, membranous positive reaction for CD147 revealed to be a reliable marker of MM; second, it could be considered as a promising candidate for specific-targeted therapy. A more recent study revealed that CD147

Table II. Frequency of MCT1, MCT4, CD147, and GLUT1 Expressions in Mesothelioma Compared to Mesothelial Reactive Cells

	<i>n</i>	Positive (%)	<i>P</i>
MCT1 (membrane)			0.218
Mesothelial reactive cells	11	8 (72.7)	
Mesothelioma	9	9 (100.0)	
MCT4 (cytoplasm)			1.000
Mesothelial reactive cells	11	7 (63.6)	
Mesothelioma	9	7 (66.7)	
CD147 (membrane)			0.001
Mesothelial reactive cells	11	1 (9.1)	
Mesothelioma	9	8 (88.9)	
GLUT1 (membrane)			0.001
Mesothelial reactive cells	11	1 (9.1)	
Mesothelioma	9	8 (88.9)	

may directly contribute to the angiogenic process regulation by association with upregulation of HIF-2 α , VEGFR-2, and the soluble forms of VEGF in endothelial cells.²⁹ This finding corroborated, in part, our previous report, where we found that VEGFR-3, a lymphatic vessel receptor which may be highly expressed in tumor cells and blood vessels of certain malignancies, was overexpressed in MM.³⁰ Importantly, these results also suggest that, in addition to increasing protease production, CD147 may contribute to the upregulation of soluble forms of VEGF in endothelial cells, thus directly regulating the angiogenic process. However, CD147 was not found to be accurate in predicting MM aggressiveness.³¹

MM responds unpredictably to therapy and treatment results seem to be related to biological properties, rather than to cytological grading or clinical staging. Molecular markers provide evergrowing insight into the biology of MM, but fall short of being prognostic factors or as gages

of treatment response. Currently, GLUT1 appears promising for guiding therapeutic strategies and evaluating their effectiveness. The strong GLUT1 expression the authors observed in MM confirms the experience of other investigators and suggests that MM has a preference for glycolysis.¹³ This property has been utilized in positron emission tomography (PET) in the evaluation of response to therapy.³² Based on these prior observations one would expect that either MCT1 or MCT4 would be upregulated in MM, but this was not the case in our study. In fact, both MCT1 and MCT4 were equally positive in MM and mesothelial hyperplasia suggesting that these molecules behave as other proliferative markers that are positive in these two settings but cannot discriminate between them. On the other hand, the immunopositivity for CD147 paralleled the results of GLUT1, supporting the notion that CD147 is a valuable discriminator between benign and malignant mesothelial proliferations. In addition, we found that CD147 positive reactions were easier to interpret, since this marker did not stain red blood cells, as is the case with GLUT1 in the study of bloody effusions. Just as hyperthermic intraperitoneal chemotherapy enhances the results of cytoreductive surgery,³³ it seems plausible to assume that altering the pH in the microenvironment of MM may enhance other modalities of therapy. This possibility has not been investigated.

In this study, although MCT expression was not increased in MM, when compared with reactive mesothelial cells, CD147 was found to discriminate between these two cytological entities, in a similar method to that described for GLUT1. However, our current findings deserve further study to substantiate their validity and to explore the potential use of CD147 as a factor in novel treatment strategies.

Ethics

The present study has been approved by the local Ethic Committees.

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