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The prognostic value of CD147/EMMPRIN is associated with monocarboxylate transporter 1 co-expression in gastric cancer

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ARTICLE INFO

Article history:
Received 21 February 2009
Received in revised form 7 June 2009
Accepted 16 June 2009
Available online 21 July 2009

Keywords:
Monocarboxylate transporters
CD147/EMMPRIN
Gastric cancer
Immunohistochemistry

ABSTRACT

The aim of the present work was to assess the role of monocarboxylate transporters (MCTs), namely MCT1 and MCT4 as well as MCT/CD147 co-expression in gastric tissues and evaluate their clinico-pathological significance in gastric carcinoma. For that, we analysed the immunohistochemical expression of MCT1, MCT4 and CD147, in a large series of gastric samples, including non-neoplastic, tumour and metastatic tissues. A significant decrease in MCT4 plasma membrane expression was observed from non-neoplastic to gastric primary malignant tissues and to lymph-node metastasis and both MCT1 and MCT4 correlated with CD147. Importantly, both MCT4 and CD147 were more frequently expressed in Lauren's intestinal-type tumours and MCT1/CD147 co-expression was associated with advanced gastric carcinoma, Lauren's intestinal type, TNM staging and lymph-node metastasis. Our results showed that the prognostic value of CD147 was associated with MCT1 co-expression in gastric cancer cells, supporting the view that CD147 plasma membrane activity is dependent on MCT co-expression.

1. Introduction

Despite the worldwide decline in incidence and mortality of gastric carcinoma along the second half of the 20th century, it is still the 4th most common cancer and the 2nd leading cause of cancer-related deaths in the world.[1] Prognosis of gastric carcinoma patients depends on several pathological variables. Among them, histological typing seems to have some prognostic relevance. The most prestigious histological classifications include: Lauren's and World Health Organization (WHO). Lauren's histological classification identifies two major patterns: intestinal-type (which principally includes papillary, well-differentiated adenocarcinomas, moderately differentiated or mucinous adenocarcinomas without signet

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2009.06.018

ring cell carcinoma (SRC) cells of WHO classification) and diffuse-type carcinomas (usually corresponding to poorly differentiated adenocarcinomas, SRC and undifferentiated adenocarcinomas of WHO classification). This classification distinguishes, by microscopic morphology, two main cancer entities with different clinical and epidemiological features (for review see [2]). The prognosis of gastric carcinoma depends also largely on tumour classification in early gastric cancer, which presents a better prognosis, or advanced gastric carcinoma, with an unfavourable prognosis.[3] Although this nomenclature may suggest a progression from one entity to the other, it is still not clear whether these are different stages of the same tumour or independent entities.[4]

Early epithelial carcinogenesis occurs under hypoxic conditions, since altered cells are separated from the vascularised stroma that supplies oxygen and nutrients. To maintain ATP levels, cancer cells increase their rates of glycolysis, developing a significant proliferative advantage. However, this phenotype leads to an overload of lactic acid, which must be expelled out of the cell, causing a decrease in the extracellular pH. Constitutive up-regulation of glycolysis requires additional adaptations, namely, resistance to apoptosis and up-regulation of membrane transporters to maintain normal intracellular pH.[5] Besides being an adaptation to high glycolytic phenotype, acidic environment represents *per se* a significant advantage for tumour cells since it is associated with increased migration, invasion and metastases, among others.[5–7] One of the most important groups of proteins involved in intracellular pH regulation is monocarboxylate transporters (MCTs), which are also responsible for transmembrane transport of lactate.[8] Indeed, there are evidences for the up-regulation of MCTs in tumours, such as high grade glial neoplasms,[9,10] colorectal,[11,12] cervical[13] and lung carcinomas,[14] but only our studies[12,13] evaluated the clinico-pathological significance of MCT altered expression. Besides an increased MCT expression in colorectal[12] and cervical carcinomas,[13] we found an association between MCT1 positivity and vascular invasion in colorectal carcinomas[12] and both MCT1 and MCT4 expressions with high risk HPV infection in uterine cervix carcinomas.[13]

MCT expression appears to be influenced by altered physiologic conditions, however, the underlying molecular events involved in MCT regulation are poorly understood. Recently, it was demonstrated that proper expression and activity of MCT1 and MCT4 require co-expression of CD147, also known as EMMPRIN or Basigin.[15–19] On the other hand, silencing studies showed that maturation and cell surface expression of CD147 depend on MCT1 and MCT4 expressions.[18,19] Recently, our group described a close association of both MCT1 and MCT4 with CD147, in cervical cancer.[20] CD147 alone has already been described as a key element in oncogenesis by stimulating the synthesis of several matrix metalloproteinases, leading to enhanced tumour cell invasion.[21,22] This protein is described to be up-regulated in human cancers,[21–23] including gastric carcinomas,[24] however if its role in cancer is associated with MCTs is not known.

One of the aims of the present study is to assess the role of MCTs in gastric cancer, by analysing the immunohistochemical expression of the MCT isoforms 1 and 4 in a large series of gastric samples, including non-neoplastic, tumour and metastatic tissues, and evaluate its clinico-pathological value. We also intend to infer about the significance of MCT and CD147 co-expression.

2. Materials and methods

2.1. Case selection

Gastric tissues were obtained from 190 patients with gastric carcinoma (including 71 non-neoplastic tissues, 190 primary tumours and 42 lymph-node metastases). Samples were retrieved from the files of the Department of Pathology, Hospital das Clinicas, University of São Paulo, School of Medicine, São Paulo, Brazil, and organised in 10 tissue microarrays (TMAs). To achieve representative sampling and minimise sample loss, sample duplicates were included in the TMAs. Even so, some cases were lost during the immunohistochemical procedure. Relevant data available included patient's age and gender as well as tumour size and location, macroscopic classification, Lauren's classification, TNM staging, depth of invasion, lymph-node metastases and both lymphatic and vascular invasions.

2.2. Immunohistochemistry

2.2.1. MCT and CD147 detections

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.[12] Immunohistochemistry for CD147 was performed according to the streptavidin–biotin-peroxidase complex principle (Ultravision Detection System Anti-polyvalent, HRP, Lab Vision Corporation, Fremont, CA), using a primary antibody raised against CD147 (18-7344, ZYMED Laboratories Inc., South San Francisco, CA) diluted 1:750, as previously described.[20] Negative controls were performed by 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 and cervical squamous carcinoma for CD147. Tissue sections were counterstained with haematoxylin and permanently mounted.

2.2.2. Immunohistochemical evaluation

Since plasma membrane location is essential for protein activity, immunoreactions for MCTs and CD147 were only considered positive when plasma membrane staining was present. Sections were scored semi-quantitatively for plasma membrane 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 (scores 0 and 2) and positive (scores 3–6), as previously described.[12] Immunohistochemical evaluation was performed blindly

by two independent observers and discordant cases were discussed in a double-head microscope in order to determine the final score.

2.3. Statistical analysis

Data were stored and analysed using the SPSS statistical software (version 16.0, SPSS Inc., 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.

3. Results

A total of 303 gastric samples organised into TMAs (Tissue Microarrays), including 71 non-neoplastic mucosas, 190 primary tumours and 42 metastatic tissues, were assessed for MCT1 and MCT4 immunohistochemical expressions. We also evaluated CD147 as putative regulator of MCT expression in gastric cells.

In general, positive MCT1 expression was observed in both plasma membrane and cytoplasm (Fig. 1A), while MCT4 expression was mainly observed in the cytoplasm, with few cases presenting plasma membrane staining (Fig. 1B). Regarding CD147, expression was mainly found in the plasma membrane (Fig. 1C). Table 1 summarises MCT and CD147 expressions in non-neoplastic and primary tumour tissues, as well as in lymph-node metastasis. As can be seen, no significant differences were observed for MCT1 and CD147 expressions. On the other hand, a significant decrease was observed for MCT4 plasma membrane expression in the transition from non-neoplastic to gastric primary malignant tissues and to lymph-node metastasis ($p < 0.001$).

In order to assess the role of CD147 in MCT regulation, as described in the literature,[15–19] we searched for associations between the expression of this regulator and the expressions of MCT1 and MCT4 in gastric carcinoma (Table 2). This analysis showed that both MCT1 and MCT4 correlated with CD147 ($p = 0.021$ and $p = 0.001$, respectively). Fig. 1A and C shows sequential tumour sections stained for MCT1 and CD147, in which positive cells for both proteins can be seen.

The clinico-pathological data available allowed assessment of correlations with MCTs (Table 3) and CD147 expressions (Table 4). Importantly, both MCT4 and CD147 were more frequently expressed in Lauren's intestinal-type tumours ($p < 0.001$ and $p = 0.010$, respectively). Additionally, although not significant ($p = 0.065$), MCT4 was more frequently expressed in early gastric cancer. CD147 expression was also associated with male gender ($p = 0.031$), advanced gastric carcinoma ($p = 0.001$), TNM stages III/IV ($p = 0.006$), depth of invasion ($p = 0.002$) and presence of lymph-node metastasis ($p = 0.003$). As proper membrane expression and activity of both MCT1 and MCT4 require association with CD147,[15–19] we also analysed the clinico-pathological significance of MCT/CD147 co-expression (Table 4). Simultaneous expression of MCT1 and CD147 was associated with advanced gastric carcinoma ($p = 0.030$), Lauren's intestinal-type ($p = 0.020$), TNM stages III/IV ($p = 0.004$) and presence of lymph-node metastasis ($p = 0.018$), and, although not significant, a tendency for invading tumours to co-express MCT1 and CD147 was also observed ($p = 0.073$). Co-expression of MCT4 and CD147 was only associated with Lauren's intestinal type ($p = 0.023$). Since MCT4 and CD147 were more frequently expressed in Lauren's intestinal type, we also evaluated the clinico-pathological significance of the co-expression of these molecules, in the tumours included in this group. Similar associations were found when analysing all primary tumours, with the addition of an association between MCT4 and younger patients ($p = 0.044$) and between CD147 and bigger tumours ($p = 0.024$) (data not shown).

4. Discussion

Up-regulation of glycolysis and adaptation to acidosis are key events in the transition from *in situ* to invasive cancer.[5] Owing to their essential function in exporting lactate, the end-product of glycolysis, MCTs are likely to be key elements in the regulation of tumour intracellular pH and induction of extracellular acidosis. Thus, the role of these transporters in tumours must be clarified in order to understand their contribution to the glycolytic and acidic phenotypes of tumours. Few reports lay on this matter and none tackled

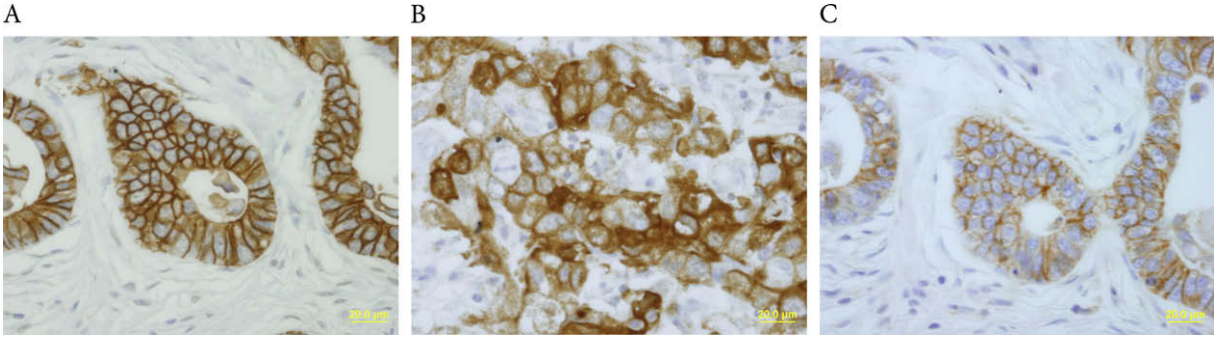

Fig. 1 – Immunohistochemical expression of monocarboxylate transporter 1 (MCT1), monocarboxylate transporter 4 (MCT4) and CD147 in gastric cancer samples. MCT1 expression was observed in both plasma membrane and cytoplasm (A), MCT4 expression was mainly observed in the cytoplasm, although few cases presented plasma membrane staining (B), while CD147 expression was clearly found in the plasma membrane (C). Tumour cells positive for both MCT1 and CD147 can be seen in the sequential sections A and C.

Table 1 – Frequency of monocarboxylate transporters (MCTs) and CD147 expressions in gastric samples.

	MCT1			MCT4			CD147		
	n	Positive (%)	*p*	*n*	Positive (%)	*p*	*n*	Positive (%)	*p*
			0.654			<0.001			0.624
Non-neoplastic	71	84.5		61	50.8		66	39.4	
Primary tumour	177	79.7		175	18.3		160	41.2	
Lymph-node metastasis	35	82.9		35	8.6		39	48.7	

Table 2 – Association between monocarboxylate transporters (MCTs) and CD147 expressions in gastric primary tumours.

	MCT1			MCT4		
	n	Positive (%)	*p*	*n*	Positive (%)	*p*
CD147			0.021			0.001
Negative	89	73.0		84	10.7	
Positive	61	88.5		61	32.8	

Table 3 – Correlations between monocarboxylate transporter 1 (MCT1) and 4 (MCT4) membrane expressions in primary tumours and the clinico-pathological data.

Clinico-pathological data	MCT1			MCT4		
	n	Positive (%)	*p*	*n*	Positive (%)	*p*
Age			0.788			0.380
>61	86	81.4		85	15.3	
⩽61	84	79.8		83	20.5	
Gender			0.333			0.208
Female	56	83.9		56	12.5	
Male	116	77.6		113	20.4	
Tumour size (cm)			0.664			0.223
<4	69	81.2		67	22.4	
⩾4	102	78.4		100	15.0	
Tumour localisation			0.402			0.503
Body	42	76.2		40	17.5	
Antrum	114	83.3		112	18.8	
Others	6	66.7		6	0.0	
Macroscopic classification			0.397			0.065
Early gastric cancer	54	75.9		50	26.0	
Advanced gastric cancer	119	81.5		120	14.2	
Lauren's classification			0.269			<0.001
Intestinal-type	105	82.9		107	26.2	
Diffuse-type	62	75.8		59	3.4	
TNM			0.604			0.789
IB + II	145	78.6		142	18.3	
III + IV	27	85.2		27	14.8	
pT			0.405			0.120
Mucosa	54	75.9		50	26.0	
Muscular propria/subserosa	111	82.9		111	13.5	
Adjacent structures	6	66.7		7	28.6	
Lymph-node metastasis			0.189			0.440
Absent	76	75.0		76	19.7	
Present	95	83.2		92	15.2	
Lymphatic invasion			0.557			0.288
Absent	97	81.4		94	20.2	
Present	72	77.8		72	13.9	
Vascular invasion			0.950			0.194
Absent	143	79.7		140	19.3	
Present	24	79.2		24	8.3	

Median was used for age and tumour size cut-off.

Table 4 – Correlations between CD147 and MCT (monocarboxylate transporter)/CD147 expressions in primary tumours and the clinico-pathological data.

Clinico-pathological data	Plasma membrane								
	CD147			MCT1/CD147			MCT4/CD147		
	n	Positive (%)	*p*	*n*	Positive (%)	*p*	*n*	Positive (%)	*p*
Age			0.894			0.634			0.355
>61	83	41.0		80	35.0		78	11.5	
⩽61	69	42.0		67	38.8		65	16.9	
Gender			0.031			0.109			0.209
Female	51	29.4		48	27.1		48	8.3	
Male	103	47.6		101	40.6		96	16.7	
Tumour size (cm)			0.055			0.456			0.517
<4	60	31.7		59	32.2		55	16.4	
⩾4	93	47.3		89	38.2		88	12.5	
Tumour localisation			0.891			0.983			0.649
Body	36	38.9		35	37.1		33	15.2	
Antrum	103	41.7		100	37.0		97	13.4	
Others	6	33.3		6	33.3		5	0.0	
Macroscopic classification			0.001			0.030			0.626
Early gastric cancer	52	23.1		50	24.0		44	15.9	
Advanced gastric cancer	103	50.5		100	42.0		101	12.9	
Lauren's classification			0.010			0.020			0.023
Intestinal-type	101	49.5		97	43.3		96	18.8	
Diffuse-type	48	27.1		47	23.4		45	4.4	
TNM			0.006			0.004			0.304
IB + II	134	37.3		129	31.8		125	12.8	
III + IV	14	70.0		20	65.0		19	21.1	
pT			0.002			0.073			0.307
Mucosa	52	23.1		50	24.0		44	15.9	
Muscular propria/subserosa	95	50.5		92	42.4		93	11.8	
Adjacent structures	6	66.7		6	50.0		6	33.3	
Lymph-node metastasis			0.003			0.018			0.815
Absent	74	29.7		71	26.8		68	14.7	
Present	79	53.2		77	45.5		75	13.3	
Lymphatic invasion			0.474			0.864			0.913
Absent	89	39.3		84	35.7		80	13.8	
Present	62	45.2		62	37.1		61	13.1	
Vascular invasion			0.692			0.649			0.738
Absent	127	40.9		122	36.9		117	14.5	
Present	22	45.5		22	31.8		22	9.1	

Median was used for age and tumour size cut-off.

gastric tumours. Therefore, the present study is an attempt to shed light on the involvement of MCTs in tumours arising from the gastric mucosa. With this purpose, we analysed MCT immunohistochemical expression in gastric samples organised into TMAs. The TMA technology allows simultaneous screening of large series of samples without losing tumour representativity.[25]

Although MCTs have been little explored in cancer, there are evidences for the up-regulation of these proteins in a variety of tumours[9–14] but also reports of down-regulation.[26,27] Interestingly, in the present study, no alteration in expression frequency was identified for MCT1, whereas a significant decrease in MCT4 expression was observed. It is important to highlight that the gastric milieu is not comparable to the majority of body tissues, since gastric mucosa is permanently under acidic conditions. This particular condition demands a very high metabolic conversion which may involve high MCT4 activity in homeostasis. Considering the acidic environment of gastric cells, the role of MCTs in cellular pH regulation,[8] as well as the capacity of MCT4 to export lactate through a proton symport, it is, perhaps, not surprising to find important levels of MCT4 in normal gastric cells. Similarly to what we observed for MCT4, carbonic anhydrase IX, also involved in the maintenance of intracellular pH and commonly up-regulated in cancer, is less frequently expressed in gastric carcinoma than in normal gastric epithelium.[28] To note that both MCT4 and carbonic anhydrase IX are HIF-1α (hypoxia-inducible factor 1) target proteins,[28,29] which may give some clues about the metabolic and molecular alterations occurring in the transition from normal gastric epithelium to gastric carcinoma. However, the reason why these two molecules are down-regulated in gastric cancer cells, as well as the mechanism by which these events take place, remains to be unveiled.

Despite the fact that MCT4 was down-regulated in our series of gastric tumour samples, a significant association with Lauren's intestinal-type tumours was found. This group of tumours includes well-differentiated carcinomas in opposition to the diffuse-type group, which are poorly-differentiated carcinomas with worse prognosis.[2,3] Therefore, intestinal-type

tumours are more alike normal gastric epithelium and the increased expression of MCT4 in this type of tumours is in agreement with our observation that the non-neoplastic epithelium expresses more MCT4.

CD147 is described as an important key element in oncogenesis[21,22] and there are some published data regarding its significance in gastric carcinoma.[24,30] In the present work, we found a higher CD147 expression in intestinal-type carcinomas, which is in agreement with the results reported by others.[30] Additionally, CD147 has been pointed as a good marker for local invasion and prognosis, being associated with tumour size, depth of invasion and both lymphatic and blood vessel invasions.[24] We confirmed some of these associations, namely tumour size (only for intestinal tumour type) and depth of invasion, and also found positive associations with higher TNM staging and lymph-node metastasis, supporting the role of CD147 as a good indicator of prognosis. Although the last associations were not described by Zheng and collaborators,[24] our results are in agreement with the enhanced metastatic capacity of CD147 expressing tumour cells, due to its role as a matrix metalloproteinase-inducing factor as well as inducer of VEGF and hyaluronan production.[21,22] Importantly, we assessed the correlation between MCTs and CD147, as this molecule is described as an important regulator of MCT activity and expression.[15–19] Here, we observed a close association between the expressions of CD147 and both MCT1 and MCT4, which provides further evidence for the regulation of MCT1/MCT4 by CD147, specifically in gastric tumour cells. On the other hand, there are a significant number of MCT positive cases, negative for CD147, which led us to speculate that MCT plasma membrane expression may depend on a yet non-identified regulation mechanism. Nevertheless, most clinico-pathological associations found for CD147 were maintained for MCT1/CD147 co-expression, which supports previous data showing that CD147 activity is dependent on MCT1 co-expression.[19] Although CD147 expression was also associated with MCT4, the co-expression of MCT4/CD147 gave no additional information than the expression of MCT4 alone, showing that, in opposition to MCT1, MCT4 associated to CD147, is not contributing to the aggressive phenotype of gastric cancer.

In the present study, we analysed the expression of MCT1 and MCT4 in non-neoplastic gastric mucosa, primary gastric tumours and lymph-node metastases, as well as CD147 as MCT regulator, in gastric tissues. Importantly, we evaluated for the first time the correlation between MCT expression and clinico-pathological data in gastric cancer and found important associations, especially with intestinal-type carcinomas. Notably, our data confirm the prognostic value of CD147 in cancer and show for the first time that this value is associated with MCT1 co-expression in gastric cancer cells.

Ethics

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

Conflict of interest statement

None declared.

Acknowledgement

We thank the Portuguese Science and Technology Foundation for C.P.'s PhD fellowship (SFRH/BD/27465/2006).

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