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GLUT1 and CAIX expression profiles in breast cancer correlate with adverse prognostic factors and MCT1 overexpression

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Summary. The goal of the present work was to evaluate the correlation of glucose transporter 1 (GLUT1) and carbonic anhydrase IX (CAIX) with the monocarboxylate transporters 1 (MCT1) and 4 (MCT4) and their chaperone, CD147, in breast cancer. The clinico-pathological value of GLUT1 and CAIX was also evaluated. For that, we analysed the immunohistochemical expression of GLUT1 and CAIX, in a large series of invasive breast carcinoma samples (n=124), previously characterized for MCT1, MCT4 and CD147 expression. GLUT1 expression was found in 46% of the cases (57/124), while CAIX was found in 18% of the cases (22/122). Importantly, both MCT1 and CD147, but not MCT4, were associated with GLUT1 and CAIX expression. Also, GLUT1 and CAIX correlated with each other. Concerning the clinico-pathological values, GLUT1 was associated with high grade tumours, basal-like subtype, absence of progesterone receptor, presence of vimentin and high proliferative index as measured by Ki-67. Additionally, CAIX was associated with large tumour size, high histological grade, basal-like subtype, absence of estrogen and progesterone receptors and presence of basal cytokeratins and vimentin expression. Finally, patients with CAIX positive tumours had a significantly shorter disease-free survival.

The association between MCT1 and both GLUT1 and CAIX may result from hypoxia-mediated metabolic adaptations, which confer a glycolytic, acid-resistant and more aggressive phenotype to cancer cells.

Key words: GLUT1, CAIX, Monocarboxylate transporters (MCTs), CD147/EMMPRIN, Breast carcinoma, Immunohistochemistry

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Introduction

Early epithelial carcinogenesis occurs under hypoxic conditions, since altered cells are separated from the vascularised stroma, source of oxygen and nutrients. To maintain the needed ATP levels, cancer cells increase their rates of glycolysis, acquiring a significant proliferative advantage. However, this phenotype leads to an overload of lactic acid, which must be exported from the cell, causing a decrease in the extracellular pH.

Constitutive upregulation of glycolysis requires additional adaptations, namely, resistance to apoptosis and upregulation of membrane transporters to maintain normal intracellular pH (Gatenby and Gilles, 2004). The need to increase glucose uptake, to allow high glucose consumption rates, is achieved by upregulation of glucose transporters (GLUT) in the plasma membrane of cancer cells, especially the hypoxia-responsive GLUT1 (Ganapathy et al., 2009). However, the role of GLUT1 in breast cancer remains poorly elucidated (Younes et al., 1995; Kang et al., 2002).

Besides being an adaptation to high glycolytic phenotype, the acidic environment represents, *per se,* a

Abbreviations: CA (carbonic anhydrase); ER (estrogen receptor); GLUT (glucose transporter); HIF-1α (hypoxia inducible-factor 1 alpha); MCT (monocarboxylate transporter); PR (progesterone receptor)

significant advantage for tumour cells since it is associated with increased migration, invasion and metastases, among others (Gatenby and Gilles, 2004; Gatenby et al., 2006). Although lactate produced by glycolysis under hypoxic conditions is a significant contributor to acidic extracellular pH, there is also a substantial contribution from carbonic acid (Helmlinger et al., 2002). In this context, the hypoxia-responsive carbonic anhydrase isoforms, CAIX and CAXII, emerge as important contributors to the regulation of cancer cell intracellular pH (Swietach et al., 2007; Chiche et al., 2009), with CAIX, in particular, being associated with poor prognosis in breast cancer (Chia et al., 2001; Trastour et al., 2007; Hussain et al., 2007; Tan et al., 2009; Chen et al., 2010).

Another important group of proteins involved in intracellular pH regulation are monocarboxylate transporters (MCTs), which are also responsible for transmembrane transport of lactate (Izumi et al., 2003). By performing these two inter-dependent activities (lactate transport coupled with a proton), MCTs appear as strong potential targets for cancer therapy. Indeed, there is evidence for the up-regulation of MCTs in tumours, such as high grade glial neoplasms (Mathupala et al., 2004), colorectal (Koukourakis et al., 2006; Pinheiro et al., 2008a), cervical (Pinheiro et al., 2008b), and breast carcinomas (Pinheiro et al., 2010). Besides analysing MCT expression in tumours, our group also assessed for the first time the clinico-pathological value of their overexpression (Pinheiro et al., 2008a,b, 2009a, 2010). MCT expression appears to be influenced by altered physiological 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 requires co-expression of CD147, also known as EMMPRIN or Basigin (Kirk et al., 2000; Wilson et al., 2005). Based on this, we described the association between CD147 and both MCT1 and MCT4 in human cervical (Pinheiro et al., 2009b), gastric (Pinheiro et al., 2009a) and breast cancer (Pinheiro et al., 2010). Furthermore, the hypoxia inducible-factor 1 alpha (HIF-1α), which regulates many genes codifying proteins involved in the glycolytic pathway (like GLUT1) and pH regulation (like the Na^+/H^+ exchanger NHE1 and both CAIX and CAXII) (Vaupel and Harrison, 2004), also regulates MCT1 (Perez et al., 2010) and MCT4 (Ullah et al., 2006; Perez et al., 2010). However, there is some controversy around MCT1 regulation by hypoxia, with some studies reporting MCT1 repression by hypoxia (Ullah et al., 2006; Sonveaux et al., 2008).

One of the goals of the present study was to evaluate the association between the HIF-1α downstream targets GLUT1 and CAIX and both MCT1 and MCT4, as well as their chaperone, CD147, in invasive breast carcinomas. We also intended to strengthen the clinico-pathological value of GLUT1 and CAIX in breast cancer.

Materials and methods

Case selection

Case selection was based on availability of follow up information and amount of material, ensuring adequate numbers for statistical analysis. Thus, a series of 124 formalin-fixed paraffin embedded breast carcinoma tissues was retrieved from the files of the Department of Pathology, Hospital do Divino Espírito Santo, Azores, Portugal, and from the Federal University of Santa Catarina, Florianopolis-SC, Brazil. Haematoxylin/eosin stained sections of all cases were reviewed by three pathologists (R.D., D.V. and F.S.). Tumour samples were organized into 14 tissue microarrays (TMAs), with 20 tumour cores (2 mm diameter) each, also including several samples of normal breast tissue. Each case was represented in the TMA by at least two cores. Relevant clinico-pathological data included tumour size (TNM), molecular subtype, histological grade, estrogen receptor (ER) and progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor 1 (EGFR), basal cytokeratins (CK5 and CK14), vimentin and Ki67 expression status. Information on lymph-node metastasis, disease-free survival and overall survival was also available. These tumour samples were previously analysed by our group for MCT1, MCT4 and CD147 expressions (Pinheiro et al., 2010).

The molecular classification was done by translating the immunohistochemistry results for ER, PR, HER2, EGFR, CK5, CK14, vimentin and Ki-67. Tumours positive for ER and/or PR were classified as luminal. Cases positive for ER/PR and for HER2 and/or high Ki-67 index were subclassified as luminal B. Cases classified as HER2 overexpressing were characterized by HER2 overexpression and negativity for ER/PR, and cases defined as "basal-like" were negative for ER/PR and HER2 and positive for at least one of the "basal markers" tested.

Immunohistochemistry

GLUT1 and CAIX detection

Immunohistochemistry was performed based on the streptavidin–biotin–peroxidase complex principle (Ultravision Detection System Anti-polyvalent, HRP, Lab Vision Corporation, Fremont, CA), using rabbit polyclonal primary antibodies raised against GLUT1 (ab15309, AbCam, Cambridge, UK, diluted 1:500) and CAIX (ab15086, AbCam, Cambridge, UK, diluted 1:2000). Briefly, deparaffinized and rehydrated sections were immersed in citrate buffer (0.01M, pH 6.0) heated up to 98ºC, in a water bath, for 10 minutes (GLUT1) or 20 minutes (CAIX) and washed in PBS. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 10 minutes, followed by

washing with PBS. Tissue sections were incubated with blocking solution for 10 minutes and incubated at room temperature with the primary antibody for 2 hours. Sections were then sequentially washed in PBS and incubated with biotinylated goat anti-polyvalent antibody for 10 minutes, streptavidin peroxidase for 10 minutes, and developed with 3,3'-diamino-benzidine (DAB+ Substrate System, Dako, Carpinteria, CA) for 10 minutes. Negative controls were performed by using adequate serum controls for the primary antibodies (N1699, Dako, Carpinteria, CA) and skin and gastric mucosa were used as positive controls for GLUT1 and CAIX, respectively. Tissue sections were counterstained with haematoxylin and permanently mounted.

Immunohistochemical evaluation

As described for MCTs and CD147 (Pinheiro et al., 2010), GLUT1 and CAIX immunohistochemical reactions were scored semi-quantitatively for plasma membrane staining 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 (extension and intensity), and grouped as negative (score 0 and 2) and positive (score 3-6). Evaluation of GLUT1 and CAIX immunohistochemical reactions were performed blindly by two independent observers (F.S., J.P.). Discordant results between observers were discussed in a double-head microscope and a final score was agreed, while discordant results between different cores of the same tumour were handled by choosing the highest staining intensity and finding the mean value for immunoreactive cells.

Statistical analysis

Data were stored and analysed using the Statview statistical software (SAS Institute Inc., Cary, NC). All comparisons were examined for statistical significance using Pearson's chi-square (χ^2) test or Fisher's exact

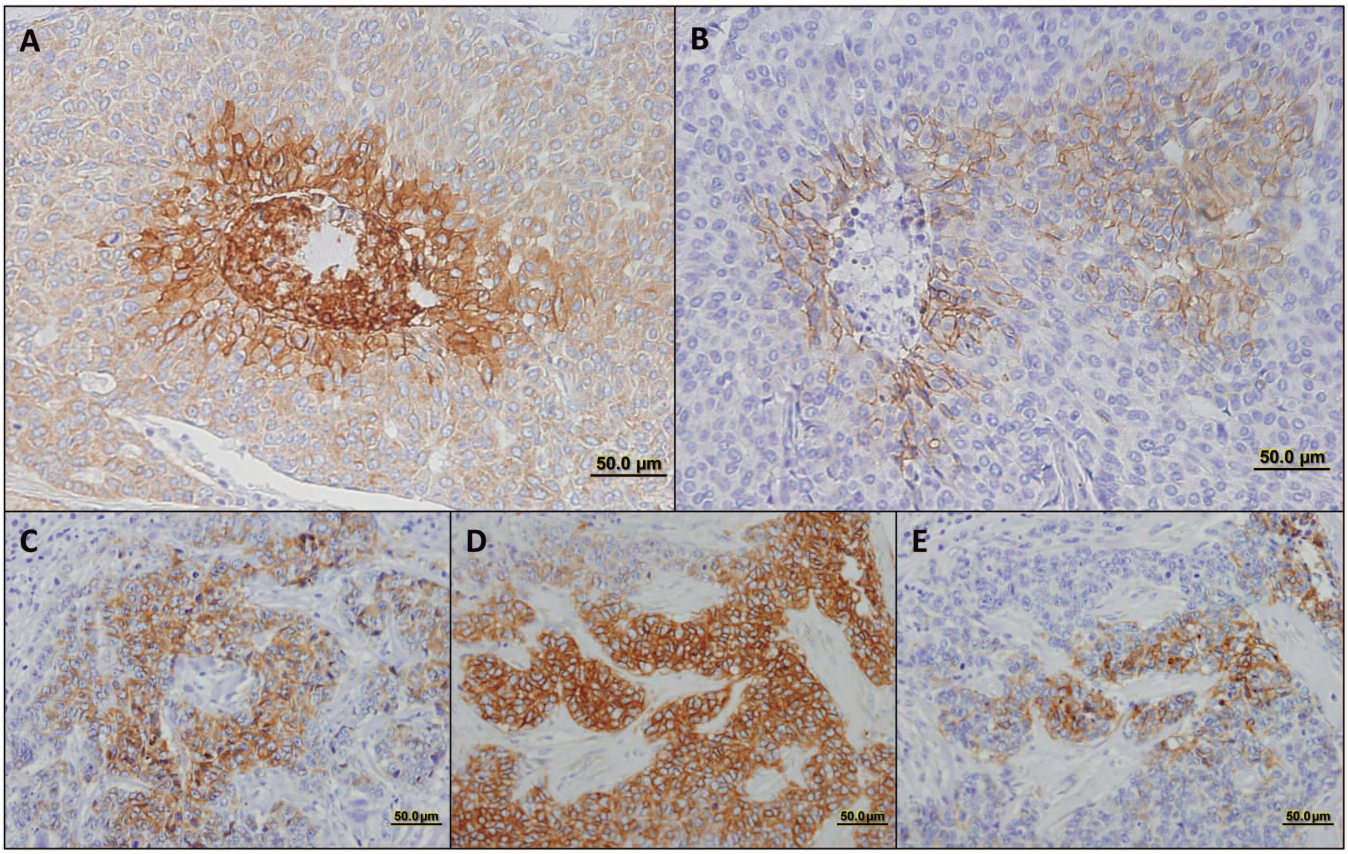

Fig. 1. Immunohistochemcial expression of glucose transporter 1 (GLUT1), carbonic anhydrase IX (CAIX) and monocarboxylate transporter 1 (MCT1), in breast carcinoma samples. GLUT1 and CAIX expression was frequently observed in perinecrotic regions (**A** and **B**, respectively). CAIX expression was usually focal and mainly restricted to tumour cells. Lower panel shows a breast ancer case simultaneously positive for MCT1 (**C),** GLUT1 (**D)** and CAIX **(E),** with staining in the same tumour region.

test, as appropriate, the threshold for significance p values being <0.05. Disease-free and overall survival curves were plotted using the method of Kaplan-Meier and data were compared using the log-rank test. The first 5 years following primary therapy was considered because recurrence rates are expected to be highest in

Table 1. Association of CAIX and GLUT1 with MCT1, MCT4 and CD147 expression in breast carcinoma samples.

	n	MCT1 Positive (%)	*p*	MCT4 Positive (%)	*p*	CD147 Positive (%)	*p*
GLUT1	106		**<0.0001**		0.3473		**0.0032**
Negative	55	1 (1.8)		4 (7.3)		2 (3.6)	
Positive	51	15 (29.4)		7 (13.7)		12 (23.5)	
CAIX	105		**<0.0001**		0.6897		**0.0005**
Negative	84	6 (7.1)		8 (9.5)		7 (8.3)	
Positive	21	10 (47.6)		3 (14.3)		8 (38.0)	

Table 2. Associations of CAIX and GLUT1 expression with clinico-pathological data from breast cancer cases.

Clinico-pathological data	GLUT1			CAIX		
	n	Positive (%)	*p*	n	Positive (%)	*p*
T size (TNM)	121		0.5218	119		0.0034
T1	46	23 (50.0)		45	6 (13.3)	
T2	63	25 (39.7)		63	9 (14.3)	
T3	12	6 (50.0)		11	6 (54.5)	
Histological grade	124		**0.0014**	122		0.0263
I	23	5 (21.7)		24	2 (8.3)	
II	55	22 (40.0)		51	6 (11.8)	
III	46	30 (65.2)		47	14 (29.8)	
Subtype	114		**0.0008**	113		0.0050
Luminal	78	31 (39.7)		79	8 (10.1)	
Basal-like	25	20 (80.0)		24	9 (37.5)	
HER2 overexpressing	11	3 (27.3)		10	3 (30.0)	
Estrogen receptor	124		0.1059	122		0.0014
Negative	45	25 (55.6)		42	14 (33.3)	
Positive	79	32 (40.5)		80	8 (10.0)	
Progesterone receptor	124		**0.0162**	122		0.0292
Negative	75	41 (54.7)		73	18 (24.6)	
Positive	49	16 (32.6)		49	4 (8.2)	
HER2 overexpression	123		**0.5885**	121		**0.4556**
Negative	110	51 (46.4)		109	19 (17.4)	
Positive	13	5 (38.5)		12	3 (25.0)	
EGFR	124		**>0.9999**	122		**0.6643**
Negative	115	53 (46.1)		113	20 (17.7)	
Positive	9	4 (44.4)		9	2 (22.2)	
CK5	124		**0.1188**	122		0.0002
Negative	98	42 (42.8)		97	11 (11.3)	
Positive	26	15 (57.7)		25	11 (44.0)	
CK14	121		**0.0508**	121		0.0102
Negative	114	51 (44.7)		115	18 (15.6)	
Positive	7	6 (85.7)		6	4 (66.7)	
Vimentin	106		0.0033	106		0.0004
Negative	89	38 (42.7)		88	12 (13.6)	
Positive	17	14 (82.4)		18	9 (50.0)	
Ki67	124		0.0339	122		**0.5214**
< 20%	65	24 (36.9)		63	10 (15.9)	
> 20%	59	33 (55.9)		59	12 (20.3)	
Lymph-node metastasis	117		**0.6326**	115		**0.5840**
Absent	58	24 (41.4)		55	8 (14.5)	
Present	59	27 (45.8)		60	11 (18.3)	

this period of time, especially in series with high number of ER negative cases like the one herein studied (Emens and Davidson, 2003). Cases lacking one or more of the clinico-pathological variables were not included in the specific statistical analysis.

Results

A total of 124 breast carcinoma samples, organised into TMAs (Tissue Microarrays), were assessed for GLUT1 and CAIX immunohistochemical expressions.

In general, positive GLUT1 expression was observed in both plasma membrane and cytoplasm (Fig. 1A), while CAIX expression was mainly observed in the plasma membrane, with some cases also presenting cytoplasm staining (Fig. 1B). GLUT1 expression was observed only in the epithelial cells of 1 out of 20 normal samples evaluated (5%), with a significantly higher frequency (46%) in tumour samples (57/124, p=0.0005), while CAIX expression was absent in normal tissue but was found in 18% (22/122) of breast cancer cases (p=0.0388). Importantly, the expression of both molecules was frequently observed in perinecrotic regions, as can be observed in Figure 1A,B, especially CAIX, which was usually focal and mainly restricted to tumour cells adjacent to areas of necrosis.

Importantly, when comparing the expression of the previously analysed MCT1, MCT4 and CD147 (Pinheiro et al., 2010) with GLUT1 and CAIX, we found that both MCT1 and CD147, but not MCT4, were more frequently expressed in GLUT1 and CAIX positive tumour samples (Table 1). Also, as expected, GLUT1 and CAIX were significantly co-expressed, with 81.8% (18/22) of CAIX positive cases also positive for GLUT1, versus 37.5% (36/96) GLUT1 positive in the CAIX negative group, p=0.0002. Figure 1C-E shows a breast cancer case simultaneously positive for MCT1, GLUT1 and CAIX, in the same tumour area.

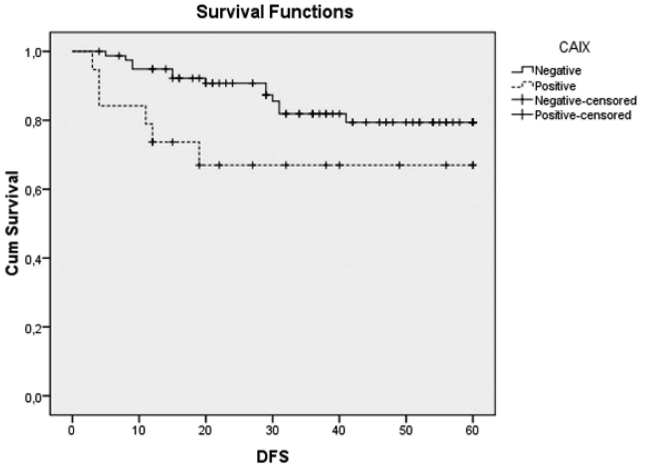

Fig. 2. Disease-free survival (DFS) curve regarding CAIX immunoreaction in breast cancer patients. Patients with positive tumours for CAIX expression show shorter disease-free survival (interrupted line) than patients with CAIX negative tumours (continuous line) (p=0.045).

Assessment of the clinico-pathological value of GLUT1 and CAIX also retrieved important results (Table 2). We found significant associations between GLUT1 expression and high grade tumours (p=0.0014), basal-like subtype (p=0.0008), absence of PR (p=0.0162), presence of vimentin (p=0.0033) and high proliferative index as measured by Ki67 expression (p=0.0339). Additionally, CAIX was associated with the majority of the clinico-pathological parameters analysed, including tumour size (p=0.0034), histological grade (p=0.0263), molecular subtype (p=0.0050), ER and PR negativity (p=0.0014 and p=0.0292, respectively), expression of CK5 (p=0.0002), CK14 (p=0.0102) and vimentin (p=0.0004).

Analysis of GLUT1 expression and patients' survival (disease-free survival and overall survival) showed no significant differences between negative and positive groups (data not shown), but, importantly, patients with CAIX positive tumours had a lower disease-free survival than patients with CAIX negative tumours (43.2 *versus* 52.4 months, respectively, p=0.045) (Fig. 2). No significant differences in overall survival were observed between the CAIX negative group and the CAIX positive group.

Discussion

Upregulation of glucose conversion into lactate, even in the presence of oxygen (Warburg effect), has been described as a possible adaptive mechanism to overcome intermittent hypoxia in pre-malignant lesions. This metabolic switch leads to an increase in acid production by cancer cells and, therefore, the need for further adaptation by means of intracellular pH regulation and resistance to extracellular acidity (Gatenby and Gilles, 2004). In this perspective, MCTs emerge as important contributors to cancer cell adaptation due to their function, on one hand, of lactate export, allowing continuous glycolysis, and, on the other hand, of tumour intracellular pH regulation and induction of extracellular acidosis, by co-transporting lactate and a proton. Although the contribution of MCTs to the glycolytic and acidic phenotype of tumours is suggested (Mathupala et al., 2007), the significance of tumour MCT expression in this context is still not clear. Thus, the main aim of the present work was to determine whether glycolytic and acid-resistant tumours, with upregulation of GLUT1 and CAIX, present a higher expression of MCTs, supporting the involvement of these transporters in the metabolic adaptations of cancer cells.

GLUT1, involved in glucose uptake, is upregulated in a variety of tumours (for a review see Macheda et al., 2005), being the hypoxia transcription factor HIF-1α the major regulator of its expression in cancer cells

(Baumann et al., 2007). HIF-1α, the master transcriptional regulator of tumour cell adaptation to hypoxic stress, activates a number of genes, many of which code for proteins involved in O_2 delivery, angiogenesis, energy preservation (including glucose transporters and glycolytic enzymes), and other processes essential to tumour cell survival, proliferation, and spread (Vaupel and Harrison, 2004). Moreover, CAIX and MCT4 are also downstream targets of HIF-1α (Wykoff et al., 2000; Ullah et al., 2006; Perez et al., 2010) and both GLUT1 and CAIX are recognised as tumour hypoxia markers (Vordermark and Brown, 2003). However, in our study, MCT4 expression was not increased in GLUT1 or CAIX positive tumours. In contrast, MCT1 was more frequently expressed in both GLUT1 and CAIX positive tumours, pointing to a hypoxia dependent upregulation of this MCT isoform, which is accompanied by co-expression of its chaperone CD147, essential for plasma membrane localization and transporter activity (Kirk et al., 2000; Wilson et al., 2005). This finding is of major importance since it supports the induction of MCT1 expression by hypoxia (Perez et al., 2010), which is contested by some groups (Ullah et al., 2006; Sonveaux et al., 2008). With these associations, one can support the role of functional MCT1 as the lactate transporter responsible for lactate efflux in highly glycolytic breast cancer cells, especially in basal-like tumours. The transport activity of MCT1 is considered one of the most important mechanisms of intracellular pH regulation (Izumi et al., 2003). Besides MCTs, carbonic anhydrases, especially CAIX, play a major role in maintenance of intracellular (and extracellular) pH levels, by contributing to the extrusion of the protons generated by the high metabolic rates of glycolytic cancer cells. At the tumour cell surface, CAIX catalyses the extracellular trapping of acid, by hydrating the cell-generated CO_2 into HCO_3^- and H^+ (Swietach et al., 2007). Thus, it is not surprising to see an association between MCT1 and both GLUT1 and CAIX, which is likely a result of the overall HIF-1α-mediated metabolic adaptations, conferring a glycolytic, acid-resistant phenotype to cancer cells.

As described previously (Chia et al., 2001; Vleugel et al., 2005), GLUT1 and CAIX were mainly observed in the vicinity of necrotic areas (a consequence of tumour hypoxia) in our tumour series, which supports the hypoxia-mediated regulation of the expression of these proteins. Both hypoxia markers were absent in normal breast tissue, but upregulated in breast tumour tissues, with expression frequencies concordant with previous reports (Kang et al., 2002; Younes et al., 1995; Hussain et al., 2007; Trastour et al., 2007; Tan et al., 2009; Chen et al., 2010). Although the clinico-pathological value of GLUT1 and CAIX in breast cancer has already been studied by others (Chia et al., 2001; Younes et al., 1995; Hussain et al., 2007; Trastour et al., 2007; Tan et al., 2009), our data strengthens the importance of these proteins as prognostic markers, especially GLUT1, which has been little explored in breast cancer (Kang et al., 2002; Younes et al., 1995; Chen et al., 2010). So far, GLUT1 has been associated with lower disease-free survival, loss of ER and PR (Kang et al., 2002) and both higher grade (Kang et al., 2002; Younes et al., 2005; Chen et al., 2010) and proliferative activity (through Ki67 expression) (Younes et al., 1995). In the present work, although we did not find associations of GLUT1 with disease-free or overall survival, GLUT1 was more frequently expressed in high grade tumours, negative for PR and with high proliferative index (Ki67). Importantly, we found GLUT1 to be more frequently expressed in basal-like tumours, as well as in vimentin positive tumours. With respect to CAIX, more data has been published in breast carcinomas, which identifies CAIX as a marker of aggressive tumour behaviour. This protein was positively correlated with larger tumour size, basal-like (Tan et al., 2009) and high grade tumours (Chia et al., 2001; Span et al., 2003; Trastour et al., 2007; Tan et al., 2009, Chen et al., 2010), loss of ER (Chia et al., 2001; Span et al., 2003; Trastour et al., 2007; Tan et al., 2009) and PR (Span et al., 2003) as well as with shorter disease-free survival (Chia et al., 2001; Hussain et al., 2007; Tan et al., 2009). Here, we support all the previous findings described by other groups, by associating CAIX expression with high histological grade, loss of ER and PR and, importantly, basal-like subtype and disease-free survival. These results suggest that the basal-like subtype tumours may be more representative of the glycolytic, acid-resistant phenotype proposed for cancer cells and this hypoxia mediated phenotype may explain, at least partly, the more aggressive phenotype of this breast carcinoma subtype.

In the present study, we investigated the expression of the key hypoxia regulated proteins GLUT1 and CAIX. Importantly, they were positively associated with the major lactate transporter, MCT1, especially in a subset of aggressive breast carcinomas (basal-like), where these proteins are more frequently expressed. Since this subtype of tumours does not have a specific molecular therapy (Matos et al., 2005), the development of therapeutic approaches targeting these particular metabolic features could be a promising strategy to be explored in the treatment of basal-like breast tumours.

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Conflict. The authors declare no conflict of interest.

Ethics. The present study has been approved by the local Ethics Committee.

References

Baumann M.U., Zamudio S. and Illsley N.P. (2007). Hypoxic upregulation of glucose transporters in BeWo choriocarcinoma cells is mediated by hypoxia-inducible factor-1. Am. J. Physiol. Cell Physiol. 293, C477-485.

Chen C.L., Chu J.S., Su W.C., Huang S.C. and Lee W.Y. (2010). Hypoxia and metabolic phenotypes during breast carcinogenesis: expression of HIF-1α, GLUT1, and CAIX. Virchows Arch. 457, 53-61.

Chia S.K., Wykoff C.C., Watson P.H., Han C., Leek R.D., Pastorek J., Gatter K.C., Ratcliffe P. and Harris A.L. (2001). Prognostic significance of a novel hypoxia-regulated marker, carbonic anhydrase IX, in invasive breast carcinoma. J. Clin. Oncol. 19, 3660-3668.

Chiche J., Ilc K., Laferriere J., Trottier E., Dayan F., Mazure N.M., Brahimi-Horn M.C. and Pouyssegur J. (2009). Hypoxia-inducible carbonic anhydrase IX and XII promote tumor cell growth by counteracting acidosis through the regulation of the intracellular pH. Cancer Res. 69, 358-368.

Emens L.A. and Davidson N.E. (2003). The follow-up of breast cancer. Semin. Oncol. 30, 338-348.

Ganapathy V., Thangaraju M. and Prasad P.D. (2009). Nutrient transporters in cancer: relevance to Warburg hypothesis and beyond. Pharmacol. Ther. 121, 29-40.

Gatenby R.A. and Gillies R.J. (2004). Why do cancers have high aerobic glycolysis? Nat. Rev. Cancer 4, 891-899-899.

Gatenby R.A., Gawlinski E.T., Gmitro A.F., Kaylor B., Gillies R.J. (2006). Acid-mediated tumor invasion: a multidisciplinary study. Cancer Res. 66, 5216-5223.

Helmlinger G., Sckell A., Dellian M., Forbes N.S. and Jain R.K. (2002). Acid production in glycolysis-impaired tumors provides new insights into tumor metabolism. Clin. Cancer Res. 8, 1284-1291.

Hussain S.A., Ganesan R., Reynolds G., Gross L., Stevens A., Pastorek J., Murray P.G., Perunovic B., Anwar M.S., Billingham L., James N.D., Spooner D., Poole C.J., Rea D.W. and Palmer D.H. (2007). Hypoxia-regulated carbonic anhydrase IX expression is associated with poor survival in patients with invasive breast cancer. Br. J. Cancer 96, 104-109.

Izumi H., Torigoe T., Ishiguchi H., Uramoto H., Yoshida Y., Tanabe M., Ise T., Murakami T., Yoshida T., Nomoto M. and Kohno K. (2003). Cellular pH regulators: potentially promising molecular targets for cancer chemotherapy. Cancer Treat. Rev. 29, 541-549.

Kang S.S., Chun Y.K., Hur M.H., Lee H.K., Kim Y.J., Hong S.R., Lee J.H., Lee S.G. and Park Y.K. (2002). Clinical significance of glucose transporter 1 (GLUT1) expression in human breast carcinoma. Jpn. J. Cancer Res. 93, 1123-1128.

Kirk P., Wilson M.C., Heddle C., Brown M.H., Barclay A.N. and Halestrap A.P. (2000). CD147 is tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface expression. EMBO J. 19, 3896-904.

Koukourakis M.I., Giatromanolaki A., Harris A.L. and Sivridis E. (2006). Comparison of metabolic pathways between cancer cells and stromal cells in colorectal carcinomas: a metabolic survival role for tumor-associated stroma. Cancer Res. 66, 632-637.

Macheda M.L., Rogers S. and Best J.D. (2005). Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. J. Cell. Physiol. 202, 654-662.

Mathupala S.P., Parajuli P. and Sloan A.E. (2004). Silencing of monocarboxylate transporters via small interfering ribonucleic acid inhibits glycolysis and induces cell death in malignant glioma: an in vitro study. Neurosurgery 55, 1410-1419.

Mathupala S.P., Colen C.B., Parajuli P. and Sloan A.E. (2007). Lactate and malignant tumors: a therapeutic target at the end stage of glycolysis. J. Bioenerg. Biomembr. 39, 73-77.

Matos I., Dufloth R., Alvarenga M., Zeferino L.C. and Schmitt F. (2005). p63, cytokeratin 5, and P-cadherin: three molecular markers to distinguish basal phenotype in breast carcinomas. Virchows Arch. 447, 688-94.

Perez de H.F., Wood I.S. and Trayhurn P. (2010). Hypoxia stimulates lactate release and modulates monocarboxylate transporter (MCT1, MCT2, and MCT4) expression in human adipocytes. Pflugers Arch. 459, 509-518.

Pinheiro C., Longatto-Filho A., Scapulatempo C., Ferreira L., Martins S., Pellerin L., Rodrigues M., Alves V.A., Schmitt F. and Baltazar F. (2008a). Increased expression of monocarboxylate transporters 1, 2, and 4 in colorectal carcinomas. Virchows Arch. 452, 139-146.

Pinheiro C., Longatto-Filho A., Ferreira L., Pereira S.M., Etlinger D., Moreira M.A., Jube L.F., Queiroz G.S., Schmitt F. and Baltazar F. (2008b). Increasing expression of monocarboxylate transporters 1 and 4 along progression to invasive cervical carcinoma. Int. J. Gynecol. Pathol. 27, 568-574.

Pinheiro C., Longatto-Filho A., Simoes K., Jacob C.E., Bresciani C.J., Zilberstein B., Cecconello I., Alves V.A., Schmitt F. and Baltazar F. (2009a). The prognostic value of CD147/EMMPRIN is associated with monocarboxylate transporter 1 co-expression in gastric cancer. Eur. J. Cancer 45, 2418-24.

Pinheiro C., Longatto A., Pereira S.M.M., Etlinger D., Moreira M.A.R., Jube L.F., Queiroz G.S., Schmitt F. and Baltazar F. (2009b). Monocarboxylate transporters 1 and 4 are associated with CD147 in cervical carcinoma. Dis. Markers 26, 97-103.

Pinheiro C., Albergaria A., Paredes J., Sousa B., Dufloth R., Vieira D., Schmitt F. and Baltazar F. (2010). Monocarboxylate transporter 1 is up-regulated in basal-like breast carcinoma. Histopathology 56, 860-867.

Sonveaux P., Vegran F., Schroeder T., Wergin M.C., Verrax J., Rabbani Z.N., De Saedeleer C.J., Kennedy K.M., Diepart C., Jordan B.F., Kelley M.J., Gallez B., Wahl M.L., Feron O. and Dewhirst M.W. (2008). Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. J. Clin. Invest. 118, 3930-42.

Span P.N., Bussink J., Manders P., Beex L.V. and Sweep C.G. (2003). Carbonic anhydrase-9 expression levels and prognosis in human breast cancer: association with treatment outcome. Br. J. Cancer 89, 271-6.

Swietach P., Vaughan-Jones R.D. and Harris A.L. (2007). Regulation of tumor pH and the role of carbonic anhydrase 9. Cancer Metastasis Rev. 26, 299-310.

Tan E.Y., Yan M., Campo L., Han C., Takano E., Turley H., Candiloro I., Pezzella F., Gatter K.C., Millar E.K., O'Toole S.A., McNeil C.M., Crea P., Segara D., Sutherland R.L., Harris A.L. and Fox S.B. (2009). The key hypoxia regulated gene CAIX is upregulated in basal-like breast tumours and is associated with resistance to chemotherapy. Br. J. Cancer 100, 405-411.

Trastour C., Benizri E., Ettore F., Ramaioli A., Chamorey E., Pouyssegur J. and Berra E. (2007). HIF-1alpha and CA IX staining in invasive breast carcinomas: prognosis and treatment outcome. Int. J. Cancer 120, 1451-1458.

Ullah M.S., Davies A.J. and Halestrap A.P. (2006). The plasma

membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism. J. Biol. Chem. 281, 9030-9037.

Vaupel P., Harrison L. (2004). Tumor hypoxia: causative factors, compensatory mechanisms, and cellular response. Oncologist 9 (Suppl. 5), 4-9.

Vleugel M.M., Greijer A.E., Shvarts A., van der G.P., van B.M., Aarbodem Y., van T.H., Harris A.L, van Diest P.J. and van der Wall E. (2005). Differential prognostic impact of hypoxia induced and diffuse HIF-1alpha expression in invasive breast cancer. J. Clin. Pathol. 58, 172-717.

Vordermark D. and Brown J.M. (2003). Endogenous markers of tumor hypoxia predictors of clinical radiation resistance? Strahlenther Onkol. 179, 801-811.

Wilson M.C., Meredith D., Fox J.E., Manoharan C., Davies A.J. and Halestrap A.P. (2005). Basigin (CD147) is the target for organomercurial inhibition of monocarboxylate transporter isoforms 1 and 4: the ancillary protein for the insensitive MCT2 is EMBIGIN (gp70). J. Biol. Chem. 280, 27213-27221.

Wykoff C.C., Beasley N.J., Watson P.H., Turner K.J., Pastorek J., Sibtain A. Wilson G.D., Turley H., Talks K.L., Maxwell P.H., Pugh C.W., Ratcliffe P.J. and Harris A.L. (2000). Hypoxia-inducible expression of tumor-associated carbonic anhydrases. Cancer Res. 60, 7075-7083.

Younes M., Brown R.W., Mody D.R., Fernandez L. and Laucirica R. (1995). GLUT1 expression in human breast carcinoma: correlation with known prognostic markers. Anticancer Res. 15, 2895-2898.

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