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Original Article

Expression of monocarboxylate transporters in gallbladder cancer and their prognostic clinical significance

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Abstract: Objective: Gallbladder cancer (GBC), one of the most common and aggressive malignancies of the biliary duct, is associated with a dismal prognosis. Monocarboxylate transporters (MCTs) have been reported to play critical roles in the development and progression of cancer. The present study investigated the expression of the MCT isoforms 1, 2 and 4 and explored their roles in GBC. Methods: We evaluated the protein expression levels of MCT1, 2 and 4 in 80 samples of histologically confirmed GBC tissues and analysed the relationships between their expression levels and clinicopathological parameters and their prognostic significance. Results: The results from IHC analysis showed that expression of MCT1, MCT2 and MCT4 was significantly higher in GBC tissues than in adjacent normal tissues. Interestingly, overexpression of MCT1 and MCT4 was associated with lymph node metastasis, distant metastasis and poor survival. Moreover, high levels of MCT1 may be an independent predictor of unfavorable survival in patients with GBC. Conclusion: Overexpressed MCTs may play critical roles in GBC. MCT1 and MCT4 expression may be associated with GBC regional and distant metastases. MCT1 and MCT4 may be potential targets for improving treatments for GBC.

Keywords: Monocarboxylate transporter, gallbladder cancer, poor prognosis

Introduction

Gallbladder cancer (GBC), the most common malignancy of the bile duct, is characterized by its high lethality [1]. Despite the progress that has been made in the prognosis and treatment of GBC in recent years, a prognosis of GBC is still dismal. The 5-year survival rate for GBC is only 5%, and the overall mean survival time is 6 months [2, 3]. Thus, identifying novel molecular mechanisms that underlie the development and progression of GBC may lead to more effective treatment strategies.

Metabolic reprogramming is a known hallmark of cancer [4]. Rapidly proliferative cancer cells rely on the glycolysis pathway to rapidly generate ATP, which they require to meet the demands of tumour progression. Acid by-products, especially lactate, are produced by tumour cells during this process. To prevent the induction of apoptosis by the accumulation of cellular acids, cancer cells accelerate the export of lactate into the extracellular milieu by increasing the expression of pH regulators, such as proton pumps, bicarbonate transporters, sodium-proton exchangers and monocarboxylate transporters (MCTs) [5].

The monocarboxylate transporters (MCT) family members, which are encoded by the SLC16 gene, contain fourteen members [6, 7]. However, only MCT 1-MCT4 has been demonstrated to facilitate the transport of proton-linked monocarboxylate, and the varying properties of each isoforms are associated with their substrate specificity, tissue distribution and intracellular localization [7]. MCT1 has a broader expression pattern wherein it is expressed in almost all tissues, and its major function is to facilitate the shuttling of L-lactic acid from the cell to the extracellular milieu. MCT2 has a high

affinity for pyruvate and lactate and is reported to be primarily expressed in the liver and in the proximal convoluted tubules of the kidneys. MCT3 is exclusively distributed in choroid plexus epithelium, while MCT4 has a ubiquitous expression pattern, especially in tissues that rely on glycolysis, and is a low-affinity but important transporter.

Recently, MCTs have been demonstrated to be aberrantly expressed in several cancer types. Céline et al. [8] demonstrated that the expression levels of MCT1, 2 and 4 were significantly higher in colorectal carcinomas (CRC) than in adjacent normal epithelium. Mathupala et al. [9] reported that MCT1 and MCT2 were frequently present in GBM tumours, whereas MCT4 was absent in all tumour tissues. In prostate cancer, MCT1 and MCT4 were found to be overexpressed compared to normal prostate tissues, and their expression levels were closely associated with several clinicopathological parameters [10]. Overexpression of MCT4 was reported to be associated with poor prognosis in pancreatic cancer patients [11]. However, research describing the expression of MCTs in GBC is relatively rare.

Cancer metabolism is an important link between the tumour microenvironment and tumour progression [12]. The increased acidity of the extracellular milieu that is caused by enhanced tumour glycolysis has been verified to be associated with tumour progression. The acidic tumour microenvironment provides a favourable atmosphere for the activation of some critical proteases, such as MMPs [13] and urokinase-type plasminogen activator [14]. The activation of these proteases induces the degradation of the extracellular matrix and facilitates the invasion and metastasis of cancer cells. Moreover, the acidity of the extracellular milieu has been reported to be associated with many angiogenic molecules, including vascular endothelial growth factor (VEGF) [15]. Thus, targeting MCTs to inhibit lactic acid efflux from cells may cause tumour cells to become apoptotic in addition to inhibiting tumour invasion, migration and angiogenesis.

In the present study, to explore the role of MCTs in GBC, we examined the expression profiles of MCT1, 2 and 4 in clinical GBC samples using immunohistochemistry and analysed their clinical and prognostic significance.

Materials and methods

Patients and tissue specimens

For this study of GBC in a Chinese cohort, a total of 80 specimens were obtained at the time of surgery in the Department of Hepatobiliary Surgery, Xijing Hospital, Fourth Military Medical University between 2007 and 2012. Related clinicopathological information (age, gender, tumour size, histological grade, tumour depth, lymph node metastasis and distant metastasis) was collected from medical records. The pathologic diagnoses were confirmed for all slides by two pathologists. Written informed consent was provided by each patient or their legal guardians. The protocol of this study was approved by the ethics committee of Xijing Hospital.

Immunohistochemical staining

Paraffin-embedded GBC samples and their surrounding non-tumour tissues were used to construct tissue microarrays (in collaboration with Shanghai Outdo Biochip Co., LTD. Shanghai, China). The TMAs were stained to detect MCT1, MCT2 and MCT4 expression using IHC. Briefly, the sections were routinely deparaffinized and rehydrated in xylene and alcohol solutions, then heated in boiling sodium citrate buffer (10 Mm, pH 6.0) and maintained at a sub-boiling temperature for 15 minutes for antigen retrieval. The slides were then incubated in 3% hydrogen peroxide for 10 minutes for endogenous peroxidase inactivation. After blocking the sections in 5% normal goat serum for 1 hour at room temperature, the tissue slides were incubated with rabbit anti-MCT1 (Santa Cruz Biotechnology), -MCT2 (Abcam) or -MCT4 (Santa Cruz Biotechnology) polyclonal antibody all at 1:500 dilution overnight at 4°C. After rinsing the slides with PBS, the sections were incubated with horseradish peroxidase (HRP)-conjugated goat anti rabbit antibodies (Zhongshan Biotechnology, China) according to manufacturer's instructions. Then, the sections were stained with fresh 0.05% 3,3'-diaminobenzidine (DAB) for several minutes and counterstained with haematoxylin. As a negative control, some sections were incubated with PBS instead of primary antibodies. The results of immunohistochemical staining were independently examined by two pathologists, and immunohistochemical scoring was performed

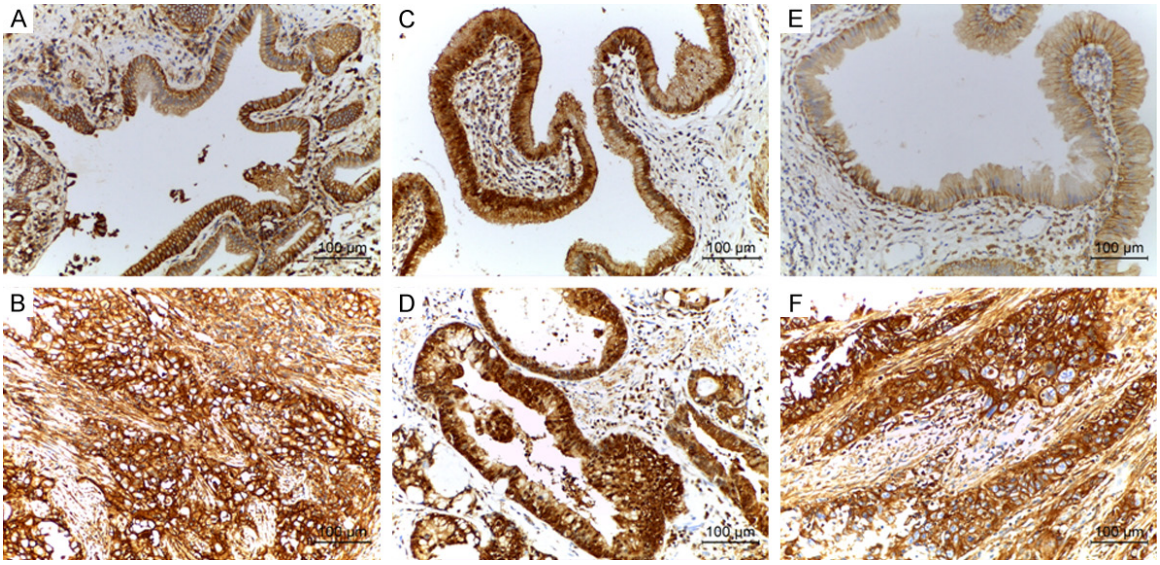

Figure 1. Expression analysis of MCTs in GBC using immunohistochemistry (A-F). (A) Staining for MCT1 in adjacent non-tumorous tissue. (B) Staining for MCT1 in GBC tissue. (C) Staining for MCT2 in adjacent non-tumorous tissue. (D) Staining for MCT2 in GBC tissue. (E) Staining for MCT4 in adjacent non-tumorous tissue. (F) Staining for MCT4 in GBC tissue. SP ×200.

Table 1. Expression of MCTs in GBCs using IHC

Isoform	GBC tissues (n=80)	Adjacent normal tissues (n=80)	*P*
MCT1			0.026
Positive	62	48	
Negative	18	32	
MCT2			0.015
Positive	68	54	
Negative	12	26	
MCT4			<0.001
Positive	67	32	
Negative	13	48	

using a semiquantitative grading system, as previous reported [16]. The percentage of positive cells (staining area) was scored as follows: 0, <5%; 1, 5-30%; 2, 31-70%; and 3, >71%. Staining intensity was scored as follows: 0, no staining; 1, weakly positive, 2, moderate staining; and 3, strongly positive. The immunostaining score of each specimen was calculated by adding the staining percentage score and the staining intensity score. The product of the quantity scores was calculated, and a final score of 0-2 was determined to indicate negative while a score of 3-6 indicated positive.

Statistical analysis

The expression of MCTs in GBC samples and their relationship with clinicopathological characteristics were analysed using x^2 tests. Survival curves were calculated using the Kaplan-Meier method. Multivariate analyses were applied using Cox proportional hazard regression analysis to evaluate correlations between survival times and multiple variables. Statistical analyses were conducted using SPSS 17.0 statistical software (Chicago, IL). $P<0.05$ was considered to indicate a significant result.

Results

Expression of MCT1, MCT2 and MCT4 in GBC

To explore the expression of MCT1, MCT2 and MCT4 in GBC, 80 samples were acquired from GBC patients enrolled in this study. Immunohistochemical assays were performed to assess the protein expression of MCTs in 80 paired GBC and adjacent non-cancerous tissues using a tissue microarray (TMA). The samples that were positive for MCT isoforms 1, 2 and 4 in tumour tissues and adjacent non-tumorous tissues are shown in **Figure 1**. The results of the analysis of immunohistochemical expression are summarized in **Table 1**.

MCT1 and MCT4 were expressed mainly in plasma membranes as well as in the cytoplasm. MCT2 was mainly expressed in the cytoplasm. Interestingly, in some samples, MCT2 was localized in the nucleus (**Figure 1D**) and plasma

Table 2. Correlation between MCT expression levels and clinicopathological features of patients

Clinical variables	MCT1			MCT2			MCT4		
	Positive	Negative	*P*	Positive	Negative	*P*	Positive	Negative	*P*
Age			0.396			0.742			1.000
<61	19	8		24	3		23	4	
≥61	43	10		44	9		44	9	
Gender			0.774			0.331			1.000
Male	18	6		22	2		20	4	
Female	44	12		46	10		47	9	
Tumour Size			0.104			0.529			1.000
<4 cm	34	14		42	6		40	8	
≥4 cm	28	4		26	6		27	5	
Histological grade			0.593			0.760			0.366
Well	28	10		33	5		30	8	
Moderately/Poorly	34	8		35	7		37	5	
Tumour depth			0.157			0.679			0.164
T1	11	5		14	2		12	4	
T2	19	4		20	3		21	2	
T3	21	9		26	4		23	7	
T4	11	0		8	3		11	0	
Lymph node metastasis			0.001			0.056			0.002
Negative	27	16		40	3		31	12	
Positive	35	2		28	9		36	1	
Distant metastasis			<0.001			0.755			0.005
Negative	28	17		39	6		33	12	
Positive	34	1		29	6		34	1	

membrane. The expression of MCT1 was significantly increased in GBC tissues compared to adjacent non-tumorous tissues (77.5% vs. 60%, $P<0.05$). The positive staining rate for MCT2 was the highest among the three MCT isoforms. Positive staining for MCT2 was observed in 85% (68/80) of the GBC samples, while only 67.5% (54/80) of the adjacent non-cancerous samples demonstrated positive MCT2 staining. Positive expression of MCT4 in tumour samples was also more frequent than positive expression in adjacent normal samples (83.8% vs 40%, $P<0.05$).

Correlation between MCTs expression and the clinicopathological features

We next compared the expression of the MCT isoforms to the clinical features of the patients to evaluate the clinical relevance of up-regulated expression of MCTs in GBC specimens. As shown in **Table 2**, positive staining for either MCT1 or MCT4 was associated with lymph node metastasis and distant metastasis ($P<0.05$). No correlation was found between MCT2 expression and the analysed clinicopathological parameters ($P>0.05$).

Relationship between MCTs expression and overall survival

To investigate the relationship between MCT expression levels and clinical outcomes, we compared the survival rates in GBC patients according to the level of MCT1, MCT2 and MCT4 expressed in their tumour tissues. According to the results of a Kaplan-Meier survival analysis, we found that patients in the high MCT1 and high MCT4 expression groups had reduced overall survival rates compared to patients in the corresponding low expression groups ($P<0.05$, **Figure 2**). According to univariate Cox regression analyses, histological grade, distant metastasis, MCT1 expression and MCT4 expression were correlated with overall survival rates in GBC patients ($P<0.05$, **Table 3**). Multivariate Cox regression further confirmed the prognostic value of MCT1 as an independent predictor of OS in GBC ($P<0.05$, **Table 3**).

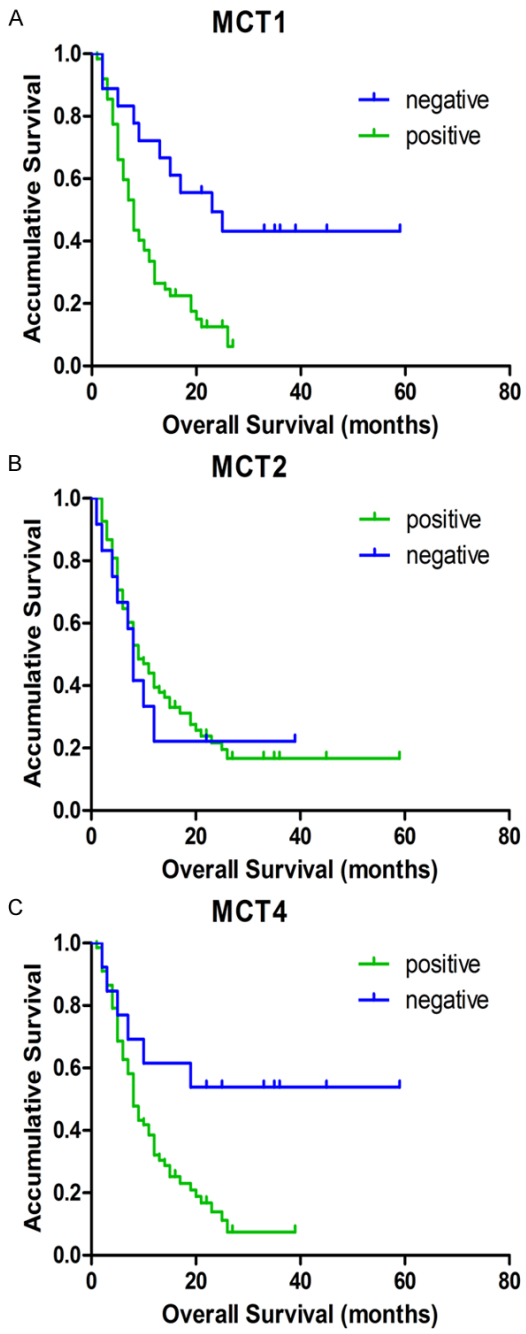

Figure 2. Overall survival curves for patients with GBC, subdivided according to MCT1 expression levels (A), MCT2 expression levels (B) and MCT4 expression levels (C).

Discussion

The human monocarboxylate transporter (MCT) family comprises 14 members that are encoded by the human SLC16 genes. Currently, only a few members of this family have well-characterized functions. MCT10 is a TH transport with a preference for T3 that is ubiquitously expressed in the intestines, kidneys, liver, muscle, and placenta [17]. MCT8 is also a high-affinity thyroid hormone transporter [18]. MCT6 has been reported to transport a number of drugs, such as bumetanide, probenecid and nateglinide; however, neither L-lactic acid nor L-tryptophan is transported by MCT6 [19]. MCT1-MCT4 are the only four family members that have been demonstrated to transport monocarboxylates [6]. MCT3 expression was confined and was usually located in the basal membrane of the retinal pigment epithelium and the choroid plexus epithelia [20, 21]. The physiological function of MCT3 is to facilitate the transport of glycolytically derived lactic acid out of the retina, a process that also involves MCT1 [7]. MCT1 has a ubiquitous expression pattern in most human tissues. MCT1 transports L-lactic acid and ketone bodies by facilitating their influx into or efflux out of cells across the plasma membrane under different metabolic states [6, 7]. In skeletal and heart muscle tissues, MCT1 facilitates the transport of lactate and ketone bodies into myocytes when they accumulate in the extracellular milieu. Under hypoxic conditions (which is common near solid tumour tissues), cells mostly rely on the glycolysis pathway to generate ATP and produce the end-product lactate, which is then exported to the extracellular matrix by MCT1 to maintain intracellular pH homeostasis. MCT4 is another widely expressed MCT isoform; however, it is mainly expressed in tissues that rely on glycolysis, such as astrocytes, skeletal muscle and tumour tissues. In hyper-glycolytic tissues, the export of lactate by MCT4 allows the continuous conversion of pyruvate to lactate, which sustains aerobic glycolysis [22]. The major function of MCT2 is different from the functions of other isoforms. MCT2 is more frequently expressed in the cytoplasm of tissues that use lactic acid as a respiratory fuel, suggesting its role in transporting lactate across the mitochondrial membrane [7, 8, 23].

The aberrant expression of MCT isoforms 1, 2 and 4, especially MCT1 and MCT4, in tumours has been noted in recent studies. In prostate cancer, the expression levels of MCT1 and MCT4 were significantly up-regulated compared to their levels in normal prostate tissues, and their expression was associated with pretreatment PSA levels, Gleason score, pathological

Table 3. High expression of MCT1 is an independent predictor of a poor prognosis in patients with GBC

Factors	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Age	1.255	0.738-2.136	0.402			
Gender	1.041	0.606-1.789	0.883			
Tumour size	1.597	0.961-2.653	0.071			
Histological grade	1.733	1.043-2.881	0.034*	1.539	0.923-2.566	0.098
Tumour depth	1.244	0.961-1.612	0.097			
Lymph node metastasis	1.573	0.944-2.623	0.082			
Distant metastasis	1.750	1.041-2.944	0.035*	1.142	0.649-2.009	0.645
MCT1	2.930	1.451-5.917	0.003*	2.316	1.094-4.904	0.028*
MCT2	0.864	0.426-1.756	0.687			
MCT4	2.991	1.266-7.065	0.012*	2.225	0.916-5.401	0.077

*P<0.05.

stage and nodal involvement [10]. Céline et al. [8] showed that the expression levels of MCT1, 2 and 4 were all significantly increased in colorectal carcinomas (CRC) and evaluated their clinicopathological significance. Their resulting study of breast, colon, lung and ovary neoplasms showed that high and heterogeneous expression of MCT1, 2 and 4 was present in different cancer types [24]. Moreover, the heterogeneous expression of MCTs has been found to be associated with poor prognosis in various cancers, such as pancreatic cancer [11] and soft tissue sarcomas [25]. In the present study, we found that the protein level of MCT1 and MCT4 was significantly increased in GBC tissues. Positive staining for MCT1 and MCT4 was observed in the plasma membrane and the cytoplasm, and these finding indicate that MCT1 and MCT4 may play important roles in lactate efflux, which allows continuous aerobic glycolysis in GBC. We also observed that MCT2 was mainly expressed in the cytoplasm, a finding that is consistent with those of Céline's [8] study in CRC. This implies that MCT2 is localized in mitochondrial membranes and that it plays a physiological role in the transport of pyruvate into mitochondria. Interestingly, we found that in some GBC samples and adjacent normal samples, there was clear nuclear staining for MCT2, which has never been previously reported. The same expression pattern has been reported for MCT1 in soft tissue sarcomas [25]. The detailed mechanisms and the biological effects of nuclear-translocated MCT2, therefore, deserve investigation. Furthermore, our analysis of clinicopathological factors showed that MCT1 and MCT4 expression levels were significantly correlated with lymph node metastasis and distant metastasis. Moreover, a worse prognosis was observed in patients with positive MCT1 and MCT4 expression than in those with negative expression. Univariate and multivariate analyses also showed that positive expression of MCT1 was an independent predictor of overall survival in GBC patients. Thus, MCTs, especially MCT1 and MCT4, may play critical roles in GBC development and progression.

The switch from oxidative metabolism to glycolysis and the production of lactate, even under normal oxygen conditions, is a key characteristic of cancer cells that is referred as to the Warburg effect [26]. An important consequence of this switch is to increase the production of the end-product lactate. The accumulation of lactate leads to cell apoptosis via the caspase-mediated activation of the p53-dependent apoptotic pathway [27]. However, cancer cells express sufficient MCT1, MCT4 and Na+-H+ exchanger, which are activated by growth factors, hypoxia and low intracellular pH [28], to avoid apoptosis, which results in the acidification of the microenvironment. The acidic tumour microenvironment further activates critical proteases, such as MMPs [13], and urokinase-type plasminogen activator [14], which results in the degradation of the extracellular matrix and facilitates the invasion and metastasis of cancer cells. Moreover, hypoxia-inducible factor (HIF) has been demonstrated to be activated by lactate [29]. The activation of HIF-1α leads to the down-regulation of E-cadherin, whereas the up-regulation of twist and the met proto-oncogene have been demonstrated to be induced during EMT [30-32]. Inhibition of MCT1 was reported

to decrease intracellular pH and inhibit tumour growth in mice [33]. Silencing both MCT1 and MCT2 inhibited glycolysis and induced cell death in malignant gliomas in vitro [9]. In addition, down-regulating MCT4 inhibited the migration of breast cancer cells [34]. Therefore, relying on glycolysis for energy may be the Achilles' heel of tumours [12], and targeting MCTs may provide a new strategy for cancer therapies.

Collectively, our study is the first to explore the expression of MCTs in GBC samples and assess their associations with clinicopathological features and prognostic significance in GBC patients. We demonstrated that the levels of the MCT1, MCT2 and MCT4 proteins are increased in GBC tissues compared to adjacent normal tissues. High expression of either MCT1 or MCT4 in GBC was significantly associated with lymph node metastasis and distant metastasis. We also found that MCT1 is an independent prognostic indicator that predicts a poor prognosis in GBC. Therefore, MCTs might be potential targets for future GBC treatments.

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Disclosure of conflict of interest

None.

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References

[1] Siegel R, Ma J, Zou Z and Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014; 64: 9-29.

[2] Stinton LM and Shaffer EA. Epidemiology of gallbladder disease: cholelithiasis and cancer. Gut Liver 2012; 6: 172-187.

[3] Hundal R and Shaffer EA. Gallbladder cancer: epidemiology and outcome. Clin Epidemiol 2014; 6: 99-109.

[4] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.

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

[6] Halestrap AP. The SLC16 gene family-structure, role and regulation in health and disease. Mol Aspects Med 2013; 34: 337-349.

[7] Halestrap AP and Wilson MC. The monocarboxylate transporter family--role and regulation. IUBMB Life 2012; 64: 109-119.

[8] Pinheiro C, Longatto-Filho A, Scapulatempo C, Ferreira L, Martins S, Pellerin L, Rodrigues M, Alves VA, Schmitt F and Baltazar F. Increased expression of monocarboxylate transporters 1, 2, and 4 in colorectal carcinomas. Virchows Arch 2008; 452: 139-146.

[9] Mathupala SP, Parajuli P and Sloan AE. Silencing of monocarboxylate transporters via small interfering ribonucleic acid inhibits glycolysis and induces cell death in malignant glioma: an in vitro study. Neurosurgery 2004; 55: 1410-1419, 1419.

[10] Hao J, Chen H, Madigan MC, Cozzi PJ, Beretov J, Xiao W, Delprado WJ, Russell PJ and Li Y. Co-expression of CD147 (EMMPRIN), CD44v3-10, MDR1 and monocarboxylate transporters is associated with prostate cancer drug resistance and progression. Br J Cancer 2010; 103: 1008-1018.

[11] Baek G, Tse YF, Hu Z, Cox D, Buboltz N, McCue P, Yeo CJ, White MA, DeBerardinis RJ, Knudsen ES and Witkiewicz AK. MCT4 defines a glycolytic subtype of pancreatic cancer with poor prognosis and unique metabolic dependencies. Cell Rep 2014; 9: 2233-2249.

[12] Kroemer G and Pouyssegur J. Tumor cell metabolism: cancer's Achilles' heel. Cancer Cell 2008; 13: 472-482.

[13] Kato Y, Ozawa S, Tsukuda M, Kubota E, Miyazaki K, St-Pierre Y and Hata R. Acidic extracellular pH increases calcium influx-triggered phospholipase D activity along with acidic sphingomyelinase activation to induce matrix metalloproteinase-9 expression in mouse metastatic melanoma. FEBS J 2007; 274: 3171-3183.

[14] Kindzelskii AL, Amhad I, Keller D, Zhou MJ, Haugland RP, Garni-Wagner BA, Gyetko MR, Todd RR and Petty HR. Pericellular proteolysis by leukocytes and tumor cells on substrates: focal activation and the role of urokinase-type

plasminogen activator. Histochem Cell Biol 2004; 121: 299-310.
[15] Fukumura D, Xu L, Chen Y, Gohongi T, Seed B and Jain RK. Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumors in vivo. Cancer Res 2001; 61: 6020-6024.
[16] Chen L, Zhang J, Feng Y, Li R, Sun X, Du W, Piao X, Wang H, Yang D, Sun Y, Li X, Jiang T, Kang C, Li Y and Jiang C. MiR-410 regulates MET to influence the proliferation and invasion of glioma. Int J Biochem Cell Biol 2012; 44: 1711-1717.
[17] Visser WE, Friesema EC and Visser TJ. Minireview: thyroid hormone transporters: the knowns and the unknowns. Mol Endocrinol 2011; 25: 1-14.
[18] Friesema EC, Kuiper GG, Jansen J, Visser TJ and Kester MH. Thyroid hormone transport by the human monocarboxylate transporter 8 and its rate-limiting role in intracellular metabolism. Mol Endocrinol 2006; 20: 2761-2772.
[19] Murakami Y, Kohyama N, Kobayashi Y, Ohbayashi M, Ohtani H, Sawada Y and Yamamoto T. Functional characterization of human monocarboxylate transporter 6 (SLC16A5). Drug Metab Dispos 2005; 33: 1845-1851.
[20] Philp NJ, Wang D, Yoon H and Hjelmeland LM. Polarized expression of monocarboxylate transporters in human retinal pigment epithelium and ARPE-19 cells. Invest Ophthalmol Vis Sci 2003; 44: 1716-1721.
[21] Bergersen L, Johannsson E, Veruki ML, Nagelhus EA, Halestrap A, Sejersted OM and Ottersen OP. Cellular and subcellular expression of monocarboxylate transporters in the pigment epithelium and retina of the rat. Neuroscience 1999; 90: 319-331.
[22] Pinheiro C, Longatto-Filho A, Azevedo-Silva J, Casal M, Schmitt FC and Baltazar F. Role of monocarboxylate transporters in human cancers: state of the art. J Bioenerg Biomembr 2012; 44: 127-139.
[23] Koukourakis MI, Giatromanolaki A, Harris AL and Sivridis E. Comparison of metabolic pathways between cancer cells and stromal cells in colorectal carcinomas: a metabolic survival role for tumor-associated stroma. Cancer Res 2006; 66: 632-637.
[24] Pinheiro C, Reis RM, Ricardo S, Longatto-Filho A, Schmitt F and Baltazar F. Expression of monocarboxylate transporters 1, 2, and 4 in human tumours and their association with CD147 and CD44. J Biomed Biotechnol 2010; 2010: 427694.
[25] Pinheiro C, Penna V, Morais-Santos F, Abrahao-Machado LF, Ribeiro G, Curcelli EC, Olivieri MV, Morini S, Valenca I, Ribeiro D, Schmitt FC, Reis RM and Baltazar F. Characterization of monocarboxylate transporters (MCTs) expression in soft tissue sarcomas: distinct prognostic impact of MCT1 sub-cellular localization. J Transl Med 2014; 12: 118.
[26] Warburg O. On the origin of cancer cells. Science 1956; 123: 309-314.
[27] Williams AC, Collard TJ and Paraskeva C. An acidic environment leads to p53 dependent induction of apoptosis in human adenoma and carcinoma cell lines: implications for clonal selection during colorectal carcinogenesis. Oncogene 1999; 18: 3199-3204.
[28] Counillon L and Pouyssegur J. The expanding family of eucaryotic Na(+)/H(+) exchangers. J Biol Chem 2000; 275: 1-4.
[29] Lu H, Dalgard CL, Mohyeldin A, McFate T, Tait AS and Verma A. Reversible inactivation of HIF-1 prolyl hydroxylases allows cell metabolism to control basal HIF-1. J Biol Chem 2005; 280: 41928-41939.
[30] Esteban MA, Tran MG, Harten SK, Hill P, Castellanos MC, Chandra A, Raval R, O'Brien TS and Maxwell PH. Regulation of E-cadherin expression by VHL and hypoxia-inducible factor. Cancer Res 2006; 66: 3567-3575.
[31] Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S and Comoglio PM. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell 2003; 3: 347-361.
[32] Yang MH, Wu MZ, Chiou SH, Chen PM, Chang SY, Liu CJ, Teng SC and Wu KJ. Direct regulation of TWIST by HIF-1alpha promotes metastasis. Nat Cell Biol 2008; 10: 295-305.
[33] Sonveaux P, Vegran F, Schroeder T, Wergin MC, Verrax J, Rabbani ZN, De Saedeleer CJ, Kennedy KM, Diepart C, Jordan BF, Kelley MJ, Gallez B, Wahl ML, Feron O and Dewhirst MW. Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. J Clin Invest 2008; 118: 3930-3942.
[34] Gallagher SM, Castorino JJ, Wang D and Philp NJ. Monocarboxylate transporter 4 regulates maturation and trafficking of CD147 to the plasma membrane in the metastatic breast cancer cell line MDA-MB-231. Cancer Res 2007; 67: 4182-4189.