Int J Clin Exp Pathol 2017;10(3):3243-3250
www.ijcep.com /ISSN:1936-2625/IJCEP0034913

Original Article

MCT1 promotes tumor progression through regulating epithelial-mesenchymal transition in pancreatic cancer

Ren-Gen Xu, Bo Cai, Feng Cao, Qin-Xia Huang, Ping Mi, Hua-Ping Wang, Wei-Hong Liu, Bao-Guan Wang

Department of Laboratory, Taixing People's Hospital, Taixing 225400, P. R. China

Received February 20, 2016; Accepted August 27, 2016; Epub March 1, 2017; Published March 15, 2017

Abstract: Enhanced glycolytic flux in cancer cells, known as Warburg effect, leads to increased intracellular lactate that is exported by monocarboxylate transporters (MCT), which play critical functions in tumor initiation and progression. However, the role of MCT1 in pancreatic ductal adenocarcinoma (PDAC) is poorly explored. In this study, data from two ONCOMINE databases revealed that MCT1 mRNA was frequently up-regulated in PDAC tissues compared to normal pancreas. By immunohistochemical analysis in a PDAC tissue microarray, intense immunoreactivity of MCT1 protein was commonly observed in PDAC tissues but not normal pancreas. Kaplan-Meier survival analysis showed that high level of MCT1 was associated with poor prognosis in PDAC patients. Then genetic silencing of MCT1 in PDAC cells resulted in pronounced decrease in MCT1 protein level and lactate level in culture medium. Specially, knockdown of MCT1 markedly inhibited cell proliferation and invasive capacity as demonstrated by colony formation and transwell assay. Mechanistically, silencing of MCT1 partially compromised epithelial-to-mesenchymal transition phenotype. And suppression EMT process by a pharmacological inhibitor, SB431542, completely blocked MCT1-mediated oncogenic activities. Collectively, these findings provide evidence for use of MCT1 as potential therapeutic target in treatment of PDAC.

Keywords: MCT1, epithelial-mesenchymal transition, pancreatic cancer

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal of solid malignancies with an overall 5-year survival rate less than 6% [1, 2]. Due to failure to early diagnosis before distant metastasis and drug resistance of current therapies, the poor prognosis of PDAC poses a serious health problem at the beginning of the 21st century [3, 4]. Although genetically engineered mouse models of PDAC have better recapitulated tumor initiation and progression, as well as a multitude of oncogenic pathways, limited therapeutic agents are developed and fail to improve the prognosis of PDAC in recent three decades [5, 6]. Thus, it is imperative to fully interrogate this deadly disease.

Cancer cells exhibit a shift in glucose metabolism from oxidative phosphorylation (OXPHOS) to glycolysis, known as the Warburg effect, to facilitate tumor progression [7, 8]. Accumulating evidences suggest that PDAC cells exhibit enhanced glucose consumption as demonstrated by ^{18}F-fluorodeoxyglucose (^{18}F-FDG) accumulation [9]. This reprogrammed metabolic alternation enables PDAC cells to adapt to nutrient-deficiency environments induced by intense desmoplasia and poor vascularity, to facilitate cell proliferation through providing building blocks, to promote cell invasion through lactate-mediated acidification of tumor microenvironment, and to protect cells from oxidative stress by generating NADPH and glutathione [10]. As noted, increased glycolytic flux in PDAC cells leads to increased levels of intracellular lactate, which is exported by monocarboxylate transporters (MCTs) [11, 12]. MCTs are critical players in the maintenance of the glycolytic metabolism through transporting lactate and regulating extracellular pH homeostasis [13, 14]. Specially, MCT1-4, are known to mediate the proton-dependent transport of monocarboxylic acid across the plasma membrane in the presence of immunoglobulin-like molecule CD147 [15-17]. Increased MCT1 has been demonstrated in multiple human cancers, including colorectal cancer [18, 19], cervical cancer [20], glio-

ma [21], lung cancer [22], and melanoma [23], however, the expression profile and cellular functions of MCT1 in PDAC remain largely unknown.

Conversion of cancer cells with an epithelial phenotype into a mesenchymal phenotype, known as epithelial-mesenchymal transition (EMT), is critical for tumor progression [24, 25]. Cancer cells undergoing EMT are characterized by increased migratory and invasive capacity [25]. Transforming growth factor-β (TGF-β) has been widely used as EMT inducers [26, 27], because this extracellular mediator is overexpressed in many types of carcinomas, especially PDAC [28]. However, little is known about the correlation between glycolysis and EMT.

Given those notions, we reasoned that MCT1 would regulate PDAC progression through EMT. Here, we showed that MCT1 was widely expressed in PDAC and predicted a poor prognosis. Genetic silencing of MCT1 inhibited lactate production, cell proliferation, and invasion. And the suppressive role induced by knockdown of MCT1 might associate with EMT process.

Materials and methods

Cell culture

Human pancreatic cancer cell lines AsPC1, PANC1, BxPC-3, SW1990 and HPAC were obtained from Cell Bank of Chinese Academy of Sciences (Shanghai, China). All cell lines were routinely cultured in DMEM or RPMI-1640 medium containing D-glucose (4.5 g/l), (Invitrogen, USA), supplemented with 10% FBS (Gibco, NY, USA) and 1% penicillin/streptomycin (Invitrogen, USA), in a 37°C humidified atmosphere with 5% CO_2. SB431542 was purchased from Selleck (Shanghai, China).

Immunohistochemistry

The commercial pancreatic cancer tissue microarray (TMA) was purchased from Shanghai Outdo Biotech Inc. After deparaffinizing, rehydrating, antigen retrieval, and blocking endogenous peroxidases, the sections were washed in 1 × PBS for three times, blocked in 5% normal goat serum, followed by incubation of anti-MCT1 antibody (Abcam, USA) at 4°C overnight. After washing in 1 × PBS for three times, sections were incubated in horseradish peroxidase-conjugated secondary antibodies. Visualization was performed by 3, 30-diaminobenzidine tetrahydrochloride (DAB) and all sections were counterstained with hematoxylin. A combination of a proportion score and an intensity score was used to determine MCT1 staining. A total score was obtained by the combining both scores and divided in four levels: negative, low, moderate and high. Scoring was calculated independently by two pathologists Negative and weak staining was regarded as low expression, while moderate and strong staining was regarded as high expression.

Quantitative real-time PCR

Total RNA was extracted and reversely transcribed using PrimeScript RT-PCR kit (Takara, Japan). Measurement of gene expression was performed by quantitative real-time PCR (ABI PRISM 7700 Sequence Detector, Applied Biosystems). Relative mRNA expression levels were normalized to the β-actin mRNA levels and calculated using the $2^{-\Delta\Delta Ct}$ method. Sequences of primers are provided upon request.

siRNA transfection

BxPC-3 and SW1990 cells were transfected with 100 nmol/L siRNA against MCT1, or control siRNA from GenePharma (Shanghai, China) together with Lipofectamine 2000 (Invitrogen, USA) into a 6-well dish as recommended by the manufacturers. Cells were incubated for 24 hours before plating for cell invasion assays, and RNA isolation.

Measurement of lactate level

Lactate level in the culture medium was measured with Lactate Assay kit (Source Bioscience Life Sciences) according to the manufacturer's instruction.

Colony formation assay

A total of 1000 si-MCT1 or si-Ctrl cells were seeded into a 6-well dish, and allowed growth for 10-12 days. Culture medium was replaced every two days. Formed colonies were fixed by 4% paraformaldehyde, stained by 0.1% (w/v) crystal violet and counted. Each experiment was performed in triplicate and repeated twice.

Cell invasion assay

Cell invasion assay was performed using Transwell model according to the manufacturer's

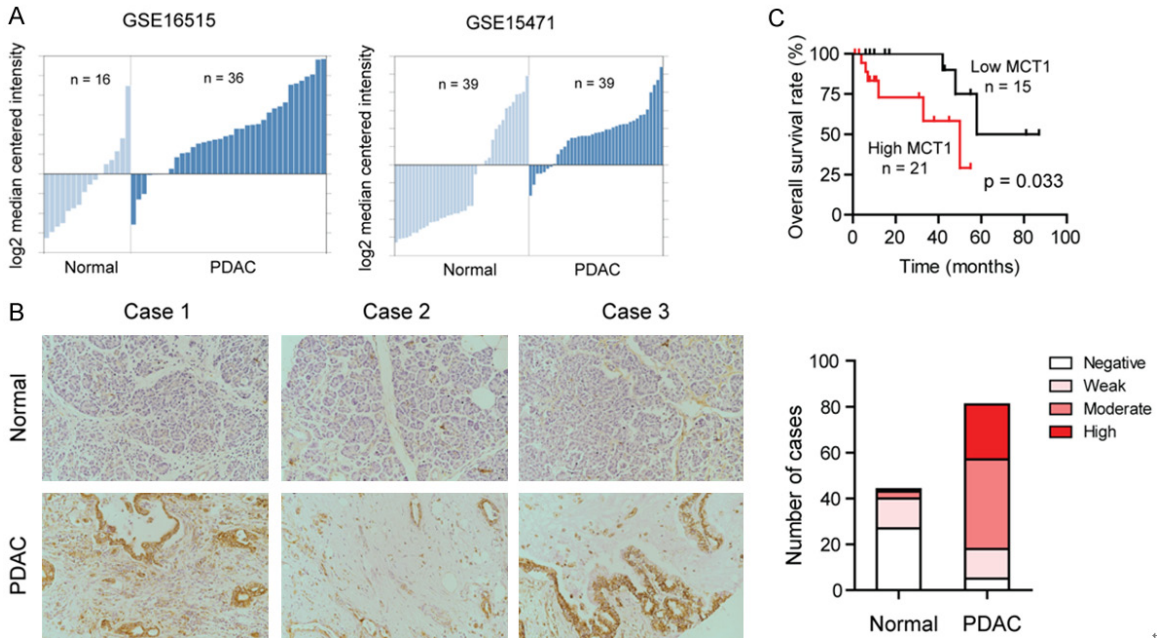

Figure 1. Up-regulated MCT1 predicts a poor prognosis in PDAC. A. Data from ONCOMINE database was analyzed by median-centered intensity, and MCT1 mRNA expression was showed in normal pancreas and PDAC. B. Representative images of MCT1 staining in normal pancreas and PDAC tissues; at × 200 magnification; statistical analysis of MCT1 expression was showed by histogram at the right. C. Kaplan-Meier curves for PDAC patients group based on MCT1 expression.

instructions (BD Biosciences, USA). Briefly, Bx-PC-3 and SW1990 MCT1-silenced and control cells were plated into matrigel-coated chambers for 48 h. Invaded cells were fixed and stained by 0.1% (w/v) crystal violet. Membranes were photographed in a stereomicroscope and invaded cells were counted.

Luciferase assays

Cells (1×10^4) were seeded in triplicate in 96-well plates and allowed to attach for 24 h. Luciferase reporter plasmids (100 ng) or 100 ng control luciferase plasmid plus 1 ng pRL-TK Renilla plasmid (Promega) were transfected into indicated cells using Lipofectamine 2000 (Invitrogen). Luciferase and Renilla signals were measured 24 h after transfection using a Dual Luciferase Reporter Assay Kit (Promega) according to the manufacturer's instruction.

Statistical analysis

All data are presented as the mean ± SD. All statistical analyses were performed using SPSS 13.0 software (SPSS Inc., USA). Kaplan-Meier method was used to evaluate survival curves and differences between survival curves were tested by the log-rank test. The student's *t*-test or one-way ANOVA was used for comparison between groups. All *P*-values less than 0.05 were considered statistically significant.

Results

Up-regulated MCT1 predicts a poor prognosis of PDAC

Data mining of ONCOMINE database revealed that MCT1 mRNA expression was significantly over-expressed in tumor tissues compared to their normal counterparts (**Figure 1A**). To further evaluate the expression pattern of MCT1 at protein level, we tested MCT1 expression in a tissue microarray, which contains 44 cases of normal pancreas and 81 cases of PDAC. As shown in **Figure 1B**, compared to normal pancreas, intense staining of MCT1 was frequently observed in PDAC tissues. By analysis of these PDAC cases with follow-ups (n = 36), we found that high MCT1 protein expression was closely associated with decreased overall survival compared those with low MCT1 expression (**Figure 1C**).

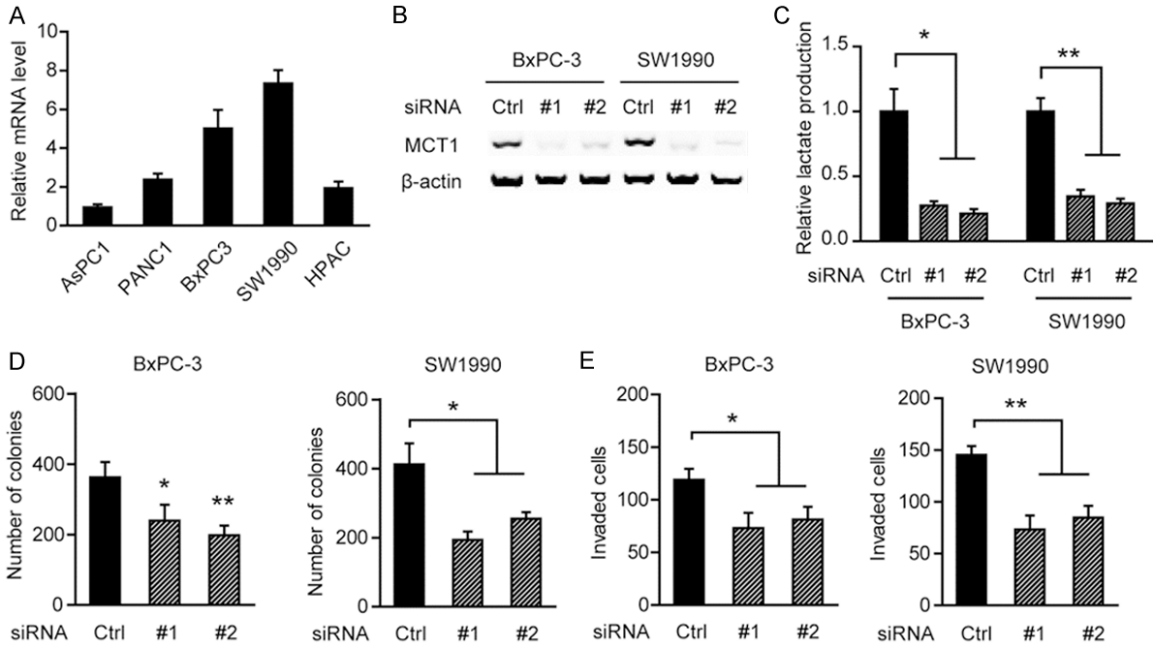

Figure 2. Genetic silencing of MCT1 inhibits the invasive capacity of PDAC cells. (A) The mRNA expression of MCT1 in PDAC cell lines was detected by qRT-PCR. (B) Silencing efficacy of MCT1 in BxPC-3 and SW1990 cells. (C) Effects of MCT1 down-regulation on lactate secretion. Cell proliferation (D) and invasive potential (E) of BxPC-3 and SW1990 cells were measured in the presence of MCT1 siRNAs. *P < 0.05, **P < 0.01.

Genetic silencing of MCT1 inhibits the invasive capacity of PDAC cells

In order to test if the oncogenic roles previously demonstrated were similar in PDAC cells, knockdown of MCT1 expression was performed using specific siRNAs in two MCT1 high expressed cells, BxPC-3 and SW1990 (**Figure 2A**). As shown in **Figure 2B**, a pronounced decrease in MCT1 expression was observed upon MCT1 siRNAs in both cell lines. Expectedly, extracellular lactate was decreased by MCT1 down-regulation (**Figure 2C**). As demonstrated by colony formation assay, si-MCT1 cells showed reduced prolific ability, especially in SW1990 cells (**Figure 2D**). Likewise, a marked decrease of the invasive capacity of PDAC cells was observed upon silencing of MCT1 (**Figure 2E**). Taken together, these data indicate that MCT1 is critically involved in development and progression process of PDAC.

MCT1 is involved in epithelial-to-mesenchymal (EMT) transition

EMT is associated with the increased metastatic invasive potential and we found a faint morphological change in SW1990 upon silencing of MCT1. Therefore, to determine the underlying mechanisms involved in MCT1-mediated invasive potential, we focused on EMT. As shown in **Figure 3A**, E-cadherin, the epithelial cell marker, was significantly up-regulated in si-MCT1 cells compared to si-Ctrl cells. Reversely, knockdown of MCT1 decreased the expression of N-cadherin, a mesenchymal cell marker, in both BxPC-3 and SW1990 cells (**Figure 3B**). And furthermore, other mesenchymal cell markers such as vimentin, snail, fibronectin and twist should also be detected. The result showed that vimentin and fibronectin were markedly reduced by silencing of MCT1, while the expression level of snail and twist was faintly affected by MCT1 alteration (**Figure 3C**). Moreover, luciferase reporter assay showed that silencing of MCT1 decreased the activity of TGF-β signaling, an indicator of EMT, in both BxPC-3 and SW1990 cells (**Figure 3D**). Importantly, pharmacological inhibition of the EMT process by SB-431542 fully blocked the oncogenic roles of MCT1 on cell proliferation (**Figure 3E**) and invasion (**Figure 3F**). Collectively, these data above suggest that MCT1 induced malignant phenotypes might be mediated by altered EMT process.

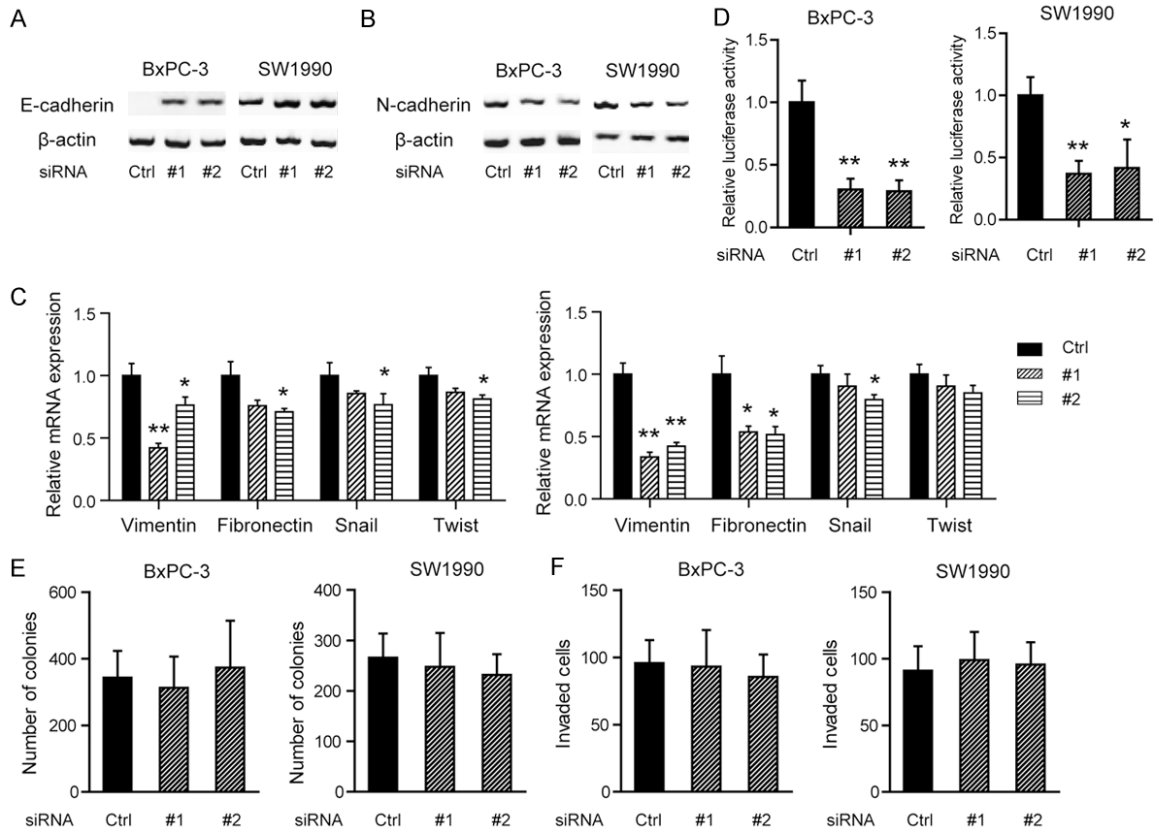

Figure 3. MCT1 is involved in epithelial-to-mesenchymal (EMT) transition. E-cadherin (A) and N-cadherin (B) expression in si-Ctrl and si-MCT1 cells was detected in BxPC-3 and SW1990 cells.by Western blotting. (C) Expression of several mesenchymal markers in si-Ctrl and si-MCT1 cells were detected in BxPC-3 and SW1990 cells by qRT-PCR. (D) Luciferase assay analyses of the BxPC-3 and SW1990 cells transfected with the TGF-β reporters in the presence of 50 nM MCT1 specific siRNAs. (E, F) Cell proliferation (E) and invasive potential (F) of BxPC-3 and SW1990 cells were measured in the presence 10 μM SB431542. *P < 0.05, **P < 0.01.

Discussion

Alternations in glucose metabolism have been emerged as a hallmark of cancer [29]. The Warburg effect, characterized by enhanced glucose consumption and lactate production, draws increasing attention in targeted therapy in recent years [30]. To protect cells from lactate-induced intracellular acidified microenvironment, lactate must be transported from the cell. MCT1, as a critical factor in facilitating the diffusion of lactate across the plasma membrane, is commonly expressed in tumor tissues compared to normal tissues and being promising therapeutic targets [31, 32]. In current study, we determined the expression profile, cellular functions and related mechanisms of MCT1 in PDAC.

By data mining of ONCOMINE database and immunohistochemical analysis of a PDAC tissue microarray, we demonstrated that MCT1 expression was upregulated in PDAC tissues at both mRNA and protein level compared with normal pancreas. Notably, patients with a higher MCT1 expression had a poor prognosis in relative to those had a lower MCT1 expression. Myc oncoproteins drive aerobic glycolysis through regulating expression of glycolytic enzymes, including lactate dehydrogenase A (LDHA) that generates lactate. It is also well revealed that Myc directly activated MCT1 transcription by binding to specific recognition sites [33, 34].

Although Myc determines the metabolic phenotype and plasticity of PDAC, however, whether Myc contributes to the elevated MCT1 in PDAC remains further investigation.

Then by genetic silencing of MCT1 in PDAC cells, we observed significant reduction in colony formation ability and invasive capacity. Consistent with previous in vitro and in vivo reports

using MCT pharmaceutical inhibitors [35, 36], our findings as a proof of principle, support the hypothesis that MCT1 could be promising therapeutic targets in PDAC. Pharmaceutical inhibition of lactate transport showed a marked decrease in glycolytic rate, cell proliferation, migration and invasion in glioma and breast cancer cells [37, 38]. Indeed, knockdown of MCT1 also led to a decrease in tumor cell aggressiveness, as well as decreased lactate transport [39, 40]. Thus, targeting tumor glucose metabolism by MCT1 blockade may become an effective therapeutic option for PDAC cells with enhanced glycolysis.

Finally, to demonstrate the mechanism underlying MCT1-mediated functions. In osteosarcoma, the antitumor effects of targeting MCT1 might be related to the NF-κB pathway [41]. In myeloma cells, targeting MCT caused downregulation of homing receptor CXCR4 and abrogated SDF-1-induced migration [42]. Here, we focused on EMT, as the morphological change observed in SW1990 cells. We found MCT1 knockdown partially compromised EMT phenotype, indicating EMT might involve in MCT1-related invasive capacity. Inconsistent with our observations that silencing of MCT1 reduced cell proliferation, however, another hallmark of tumor cells undergoing EMT is decreased proliferation [43, 44]. This discrepancy in proliferation might be explained by reduced Warburg effect, which enables cancer cells with biosynthetic building blocks for proliferation.

In conclusion, our data identify MCT1 as a key regulator of glycolysis and tumor progression, and suggest that patients with PDAC may benefit from targeted therapies focused on MCT1-related signaling pathway.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Bao-Guan Wang, Department of Laboratory, Taixing People's Hospital, 1#, Changzheng Road, Aixing 225400, P. R. China. Tel: +86-0523-87656001; E-mail: drbgwang@sina.com

References

[1] Falasca M, Kim M and Casari I. Pancreatic cancer: current research and future directions. Biochim Biophys Acta 2016; 1865: 123-32.

[2] Seufferlein T and Mayerle J. Pancreatic cancer in 2015: precision medicine in pancreatic cancer-fact or fiction? Nat Rev Gastroenterol Hepatol 2016; 13: 74-5.

[3] Del Chiaro M, Segersvard R, Lohr M and Verbeke C. Early detection and prevention of pancreatic cancer: is it really possible today? World J Gastroenterol 2014; 20: 12118-12131.

[4] Mohammed A, Janakiram NB, Lightfoot S, Gali H, Vibhudutta A and Rao CV. Early detection and prevention of pancreatic cancer: use of genetically engineered mouse models and advanced imaging technologies. Curr Med Chem 2012; 19: 3701-3713.

[5] Guerra C and Barbacid M. Genetically engineered mouse models of pancreatic adenocarcinoma. Mol Oncol 2013; 7: 232-247.

[6] Mazur PK and Siveke JT. Genetically engineered mouse models of pancreatic cancer: unravelling tumour biology and progressing translational oncology. Gut 2012; 61: 1488-1500.

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

[8] Vander Heiden MG. Targeting cancer metabolism: a therapeutic window opens. Nat Rev Drug Discov 2011; 10: 671-684.

[9] Pimiento JM, Davis-Yadley AH, Kim RD, Chen DT, Eikman EA, Berman CG and Malafa MP. Metabolic Activity by 18F-FDG-PET/CT Is Prognostic for Stage I and II Pancreatic Cancer. Clin Nucl Med 2016; 41: 177-81.

[10] Ying H, Kimmelman AC, Lyssiotis CA, Hua S, Chu GC, Fletcher-Sananikone E, Locasale JW, Son J, Zhang H, Coloff JL, Yan H, Wang W, Chen S, Viale A, Zheng H, Paik JH, Lim C, Guimaraes AR, Martin ES, Chang J, Hezel AF, Perry SR, Hu J, Gan B, Xiao Y, Asara JM, Weissleder R, Wang YA, Chin L, Cantley LC and DePinho RA. Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. Cell 2012; 149: 656-670.

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

[12] Halestrap AP. The monocarboxylate transporter family--Structure and functional characterization. IUBMB Life 2012; 64: 1-9.

[13] Halestrap AP and Meredith D. The SLC16 gene family-from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. Pflugers Arch 2004; 447: 619-628.

[14] Semenza GL. Tumor metabolism: cancer cells give and take lactate. J Clin Invest 2008; 118: 3835-3837.

[15] Wilson MC, Meredith D and Halestrap AP. Fluorescence resonance energy transfer studies on the interaction between the lactate tra-

nsporter MCT1 and CD147 provide information on the topology and stoichiometry of the complex in situ. J Biol Chem 2002; 277: 3666-3672.
[16] Kirk P, Wilson MC, Heddle C, Brown MH, Barclay AN and Halestrap AP. CD147 is tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface expression. EMBO J 2000; 19: 3896-3904.
[17] Zhu D, Wang Z, Zhao JJ, Calimeri T, Meng J, Hideshima T, Fulciniti M, Kang Y, Ficarro SB, Tai YT, Hunter Z, McMilin D, Tong H, Mitsiades CS, Wu CJ, Treon SP, Dorfman DM, Pinkus G, Munshi NC, Tassone P, Marto JA, Anderson KC and Carrasco RD. The Cyclophilin A-CD147 complex promotes the proliferation and homing of multiple myeloma cells. Nat Med 2015; 21: 572-580.
[18] 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.
[19] 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.
[20] Pinheiro C, Longatto-Filho A, Ferreira L, Pereira SM, Etlinger D, Moreira MA, Jube LF, Queiroz GS, Schmitt F and Baltazar F. Increasing expression of monocarboxylate transporters 1 and 4 along progression to invasive cervical carcinoma. Int J Gynecol Pathol 2008; 27: 568-574.
[21] 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; Discussion 1419.
[22] Koukourakis MI, Giatromanolaki A, Bougioukas G and Sivridis E. Lung cancer: a comparative study of metabolism related protein expression in cancer cells and tumor associated stroma. Cancer Biol Ther 2007; 6: 1476-1479.
[23] Su J, Chen X and Kanekura T. A CD147-targeting siRNA inhibits the proliferation, invasiveness, and VEGF production of human malignant melanoma cells by down-regulating glycolysis. Cancer Lett 2009; 273: 140-147.
[24] Thiery JP, Acloque H, Huang RY and Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009; 139: 871-890.
[25] Kang Y and Massague J. Epithelial-mesenchymal transitions: twist in development and metastasis. Cell 2004; 118: 277-279.
[26] Peinado H, Quintanilla M and Cano A. Transforming growth factor beta-1 induces snail transcription factor in epithelial cell lines: mechanisms for epithelial mesenchymal transitions. J Biol Chem 2003; 278: 21113-21123.
[27] Zhou B, Liu Y, Kahn M, Ann DK, Han A, Wang H, Nguyen C, Flodby P, Zhong Q, Krishnaveni MS, Liebler JM, Minoo P, Crandall ED and Borok Z. Interactions between beta-catenin and transforming growth factor-beta signaling pathways mediate epithelial-mesenchymal transition and are dependent on the transcriptional coactivator cAMP-response element-binding protein (CREB)-binding protein (CBP). J Biol Chem 2012; 287: 7026-7038.
[28] Gore AJ, Deitz SL, Palam LR, Craven KE and Korc M. Pancreatic cancer-associated retinoblastoma 1 dysfunction enables TGF-beta to promote proliferation. J Clin Invest 2014; 124: 338-352.
[29] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.
[30] Demetrius LA, Coy JF and Tuszynski JA. Cancer proliferation and therapy: the Warburg effect and quantum metabolism. Theor Biol Med Model 2010; 7: 2.
[31] 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.
[32] Mathupala SP, Colen CB, Parajuli P and Sloan AE. Lactate and malignant tumors: a therapeutic target at the end stage of glycolysis. J Bioenerg Biomembr 2007; 39: 73-77.
[33] Gan L, Xiu R, Ren P, Yue M, Su H, Guo G, Xiao D, Yu J, Jiang H, Liu H, Hu G and Qing G. Metabolic targeting of oncogene MYC by selective activation of the proton-coupled monocarboxylate family of transporters. Oncogene 2016; 35: 3037-48.
[34] Doherty JR, Yang C, Scott KE, Cameron MD, Fallahi M, Li W, Hall MA, Amelio AL, Mishra JK, Li F, Tortosa M, Genau HM, Rounbehler RJ, Lu Y, Dang CV, Kumar KG, Butler AA, Bannister TD, Hooper AT, Unsal-Kacmaz K, Roush WR and Cleveland JL. Blocking lactate export by inhibiting the Myc target MCT1 Disables glycolysis and glutathione synthesis. Cancer Res 2014; 74: 908-920.
[35] Baltazar F, Pinheiro C, Morais-Santos F, Azevedo-Silva J, Queiros O, Preto A and Casal M. Monocarboxylate transporters as targets and mediators in cancer therapy response. Histol Histopathol 2014; 29: 1511-1524.

[36] Kumar A, Kant S and Singh SM. Targeting monocarboxylate transporter by alpha-cyano-4--hydroxycinnamate modulates apoptosis and cisplatin resistance of Colo205 cells: implication of altered cell survival regulation. Apoptosis 2013; 18: 1574-1585.

[37] Morais-Santos F, Miranda-Goncalves V, Pinheiro S, Vieira AF, Paredes J, Schmitt FC, Baltazar F and Pinheiro C. Differential sensitivities to lactate transport inhibitors of breast cancer cell lines. Endocr Relat Cancer 2014; 21: 27-38.

[38] Miranda-Goncalves V, Honavar M, Pinheiro C, Martinho O, Pires MM, Pinheiro C, Cordeiro M, Bebiano G, Costa P, Palmeirim I, Reis RM and Baltazar F. Monocarboxylate transporters (MCTs) in gliomas: expression and exploitation as therapeutic targets. Neuro Oncol 2013; 15: 172-188.

[39] Morais-Santos F, Granja S, Miranda-Goncalves V, Moreira AH, Queiros S, Vilaca JL, Schmitt FC, Longatto-Filho A, Paredes J, Baltazar F and Pinheiro C. Targeting lactate transport suppresses in vivo breast tumour growth. Oncotarget 2015; 6: 19177-19189.

[40] Yan C, Yang F, Zhou C, Chen X, Han X, Liu X, Ma H and Zheng W. MCT1 promotes the cisplatin--resistance by antagonizing Fas in epithelial ovarian cancer. Int J Clin Exp Pathol 2015; 8: 2710-2718.

[41] Zhao Z, Wu MS, Zou C, Tang Q, Lu J, Liu D, Wu Y, Yin J, Xie X, Shen J, Kang T and Wang J. Downregulation of MCT1 inhibits tumor growth, metastasis and enhances chemotherapeutic efficacy in osteosarcoma through regulation of the NF-kappaB pathway. Cancer Lett 2014; 342: 150-158.

[42] Hanson DJ, Nakamura S, Amachi R, Hiasa M, Oda A, Tsuji D, Itoh K, Harada T, Horikawa K, Teramachi J, Miki H, Matsumoto T and Abe M. Effective impairment of myeloma cells and their progenitors by blockade of monocarboxylate transportation. Oncotarget 2015; 6: 33568-33586.

[43] Vega S, Morales AV, Ocana OH, Valdes F, Fabregat I and Nieto MA. Snail blocks the cell cycle and confers resistance to cell death. Genes Dev 2004; 18: 1131-1143.

[44] Mejlvang J, Kriajevska M, Vandewalle C, Chernova T, Sayan AE, Berx G, Mellon JK and Tulchinsky E. Direct repression of cyclin D1 by SIP1 attenuates cell cycle progression in cells undergoing an epithelial mesenchymal transition. Mol Biol Cell 2007; 18: 4615-4624.