The TKTL1 gene influences total transketolase activity and cell proliferation in human colon cancer LoVo cells

Li-Hua Hu[a], Ju-Hong Yang[a], De-Tai Zhang[a], Song Zhang[b], Lin Wang[c], Peng-Cheng Cai[a], Jian-Feng Zheng[a] and Jin-Song Huang[a]

A plasmid carrying DNA to be transcribed into a small interfering RNA against transketolase-like-1 mRNA was constructed and transfected into a human colon cancer cell line. The mRNA expression of transketolase gene family in the human colon cell line was determined by real-time polymerase chain reaction. The effect of anti-transketolase-like-1 small interfering RNA on cell proliferation and cell cycle in the human colon cancer cell line cells was detected by flow cytometry and 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide. The transketolase-like-1 gene was significantly downregulated in human colon cancer cell line cells transfected with small interfering RNA transketolase-like-1 constructs compared with the cells transfected with control vector and the cells without transfection. In addition, the anti-transketolase-like-1 small interfering RNA construct significantly decreased the level of transketolase in the transfected human colon cancer cell line cells, arrested them in G_0/G_1 phase and substantially inhibited cell proliferation. No significant difference was found in the other two genes (transketolase and transketolase-like-2 genes) between the transfected human colon cancer cell line cells and the controls ($P > 0.05$). Our data demonstrated that the transketolase-like-1 gene plays an important role in total transketolase activity and in the cell proliferation of human colon cancer. Transketolase-like-1 may serve as a target for novel anticancer therapies. *Anti-Cancer Drugs* 18:427–433

Anti-Cancer Drugs 2007, **18**:427–433

Keyword: human colon cancer cell line, small interfering RNA, transketolase activity, transketolase, transketolase-like-1

Departments of [a]Laboratory, [b]Otolaryngology and [c]Dermatology, Union Hospital of Tongji Medical College, Huazhong University of Science & Technology, Wuhan, PRC

Correspondence to Li-Hua Hu, MD, Departments of Laboratory, 1277 Jiefang Dadao Avenue, Union Hospital of Tongji Medical College, Huazhong University of Science & Technology, Wuhan, Hubei 430022, PRC
Tel: +86 27 85726311; fax: +86 27 85776343;
e-mail: hulihuajy@yahoo.com.cn

The first four authors contributed equally to this work.

Received 25 August 2006 **accepted** 7 November 2006

Introduction

In the 1930s, Warburg identified a particular metabolic pathway in carcinomas, characterized by the anaerobic degradation of glucose even in the presence of oxygen (aerobic glycolysis) that led to the production of large amounts of lactate (known as the Warburg effect) [1]. [^{18}F]Fluorodeoxyglucose (FDG) positron emission tomography (PET) imaging technology is now being used to detect an altered glucose metabolism in cancer patients and in patients with neurodegenerative diseases. An enhanced glucose usage has been found in tumors and metastases [2,3], whereas a reduced cerebral glucose metabolism has been detected in patients with Alzheimer's disease, even before the presentation of clinical symptoms [4].

Three main pathways of glucose degradation have been identified so far. The first is aerobic oxidation, in which glucose is oxidized completely to carbon dioxide and water in the presence of oxygen. The second is glycolysis, in which fructose-1, 6-diphosphate is cleaved, leading to pyruvate, and then is reduced to lactate in the absence of oxygen. The third pathway is the pentose phosphate pathway (PPP), which consists of an oxidative part and a nonoxidative part. Boros *et al.* [5] found that more than 85% of ribose recovered from nucleic acids of certain tumor cells was generated, directly or indirectly, from the nonoxidative part of the PPP. It is supposed that the nonoxidative part of the PPP plays a crucial role in cancer cell proliferation. Transketolase (TKT) is a rate-limiting enzyme in the nonoxidative part of PPP and the importance of TKT for tumor cell metabolism was underlined by the fact that application of specific TKT inhibitors to tumors induced a dramatic reduction in tumor cell proliferation [6]. Coy *et al.* [7] examined the expression of all three members of the TKT gene family in cancer and revealed that transketolase-like-1 (TKTL1) was specifically upregulated in malignancies at the mRNA level, whereas TKT and transketolase-like-2 (TKTL2) were not upregulated. Later, Langbein *et al.* [8] reported that TKTL1 expression was associated with invasive colonic and urothelial tumors, and with poor prognosis. So, it is believed that TKTL1 may be used as a target for anticancer therapies.

In this study, the expression of each member of the TKT gene family was determined by real-time quantification polymerase chain reaction (PCR) in colon cancer cells

0959-4973

(LoVo cell line). We demonstrated that TKTL1 expression was stronger than those of TKT and TKTL2 in LoVo cells. We hypothesized that TKTL1 plays a crucial role in enhanced nonoxidative glucose degradation via the PPP in tumor cells. So we designed and constructed a plasmid carrying the DNA to be transcribed into a small interfering RNA (siRNA) against TKTL1 mRNA. After anti-TKTL1 siRNA treatment, the activity of TKT was significantly reduced and the proliferation of LoVo cells was remarkably inhibited. Our results suggested that TKTL1 plays an important role in tumor cell metabolism and TKTL1 can serve as a target for anticancer therapy.

Materials and methods

Reagents and instruments

D-Ribose 5-phosphate disodium salt, xylulose 5-phosphate doium salt, triose phosphate isomerase and nicotinamide adenine dinucleotide (NADH) were obtained from Sigma (St Louis, Missouri, USA); Dulbecco's modified Eagle's medium (DMEM), Lipofectamine 2000 and Trizol were procured from Invitrogen (Carlsbad, California, USA); ReverTraAce-α (Reverse transcription kit) was from Toyobo (Osaka, Japan); Coomassie Brilliant Blue G-250 was from Amresco (Amresco, Ohio, USA); Quanti Tect SYBR Green PCR kit was from Qiagen (Hilden, Germany); FACScan Flow Cytometer was from (Becton Dickinson, Mountain View, California, USA); LightCycler Real-Time PCR Instrument was from (Roche, Switzerland); Olympus AU-2700 Autoanalyser was from (Toshiba, Japan). pEGFP-C1 plasmid was from Clontech (Palo Alto, California, USA).

Plasmid construction

The design of siRNA targeting TKTLI was as follows: The target sequences corresponding to the siRNAs for TKTL1 were selected from GeneBank (accession number BC025382) (GCAGTCAGATCCAGAGAAT, GTTG GCATGCAAAGCCAAT and CAACAGAGTCGTTGTGC TG). Synthetic sense and antisense oligonucleotides constitute the template for generating RNA composed of two identical 19-nt sequence motifs in an inverted orientation, separated by a 9-bp (TTCAAGACG) spacer to form a double-strand hairpin of siRNA. A pEGFP-C1-U6 plasmid, which had been constructed from pEGFP-C1 and U6 promoter by a cloning technique, was digested with *Bam*HI and *Hind*III. Then, the annealed oligos were ligated into the plasmid. The sequence was *Bam*HI + sense + loop + antisense + termination signal + *Sal*I + *Hind*III (e.g. 5′-GATCCGCAGTCAGATCCAGAG AATTTCAAGACGATTCTCTGGATCTGACTGCTTTT TTGTCGACA-3′). The sequence encoding siRNA with 19-nt of homology to TKTL1 was inserted down-stream of the U6 promoter between *Bam*HI and *Hind*III. Thus, the construct of pEGFP-C1-U6/TKTL1 plasmid was completed. The target sequence of unrelated siRNA controls (UC) was GACTTCATAAGGCGCATGC.

Transfection

When LoVo cells reached 30–50% confluency, they were transfected with pEGFP-C1-U6 carrying siRNA by using Lipofectamine 2000 in six-well plates by following the manufacturer's instructions. LoVo cells transfected with pEGFP-C1-U6 carrying UC plasmid DNA served as control. Transfected cells were selected by using DMEM contained the following: G418, 800 μg/ml; control siRNA, cells transfected with vector UC; and siRNA, cells transfected with siRNA TKTL1 construct. The LoVo cells were isolated 8 weeks after the selection and were detected by reverse transcription-PCR to determine the expression level of TKTL1 mRNA.

Cell culture

LoVo cells, a human colon cancer cell line, were obtained from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. Cells were cultured in DMEM supplemented with 10% fetal calf serum at 37°C with 5% CO_2.

Real-time polymerase chain reaction

A total of 10^4 cells without transfection, cells transfected with control vector or cells transfected with siRNA were seeded into a six-well culture plate. Cells of each group were harvested 72 h after the culture. Total RNA was extracted from above-mentioned cells by using TRIzol reagent (Invitrogen, California, USA) according to the manufacturer's instructions. Two micrograms of total RNA was reversely transcribed by following instruction manual. PCR was performed by using SYBR Green I dye according to the manufacturer's protocol. The PCR was carried out in a total volume of 20 μl. Real-time PCR was conducted by using the following parameters: denaturing at 94°C for 3 min, 40 cycles at 94°C for 5 s and at 57°C for 5 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an internal control and each assay included standard samples in duplicates. Data analysis was carried out by using LightCycler Data Analysis Software. To control the specificity of amplification products, a melting curve analysis was performed. In addition, PCR products were gel-separated to confirm the bands of the expected size. The relative expression amount of the target sample was expressed as the relative fold change in 2 base. $\Delta C_t = C_t$ (target) $- C_t$ (GAPDH), $\Delta\Delta C_t = \Delta C_t$ (target) $- \Delta C_t$(control), mRNA relative amount $= 2^{-\Delta\Delta C_t}$ [9]. The following primers were used for amplification of transcripts of the three known human TKT genes: TKTL1 (5′-TAACACCATGACGCCTAC TGC-3′; 5′-CATCCTAACAAGCTTTCGCTG-3′), TKT (5′-TGTGTCCAGTGCAGTAGTGG-3′; 5′-ACACTTCA TACCCGCCCTAG-3′) and TKTL2 (5′-AAACTAGGCT TATTTCTAAAAAGTCAAG-3′; 5′-GGCTTTGCTTTAA AAGAAACAG-3′). The GAPDH gene (5′-GAAGGTGA AGGTCGGATGC-3′; 5′-GAAGATGGTGATGGGATTT C-3′) was used as a control. The sequences of primers for

TKT, TKTL1 and TKTL2 were obtained by referring to Coy *et al.* [7]. The sequences of primers for GAPDH gene were designed by using Primer Premier 5.0 software package (Primer, Canada).

Measurements of transketolase activity

To prepare the extract of LoVo cells, the cells were sonicated and centrifuged. The resulting supernatant was filtered to remove the endogenous metabolites. Total transketolase (TK) activity was determined by using an enzyme-linked method [10]. Samples were added to a cuvette containing buffer (50 mmol/l Tris–HCl, pH 7.6), supplemented with 2 mmol/l ribose 5-phosphate, 1 mmol/l xylulose 5-phosphate, 5 mmol/l $MgCl_2$, 0.2 U/ml of triose-phosphate isomerase, 0.2 U/ml NADH and 0.1 mmol/l thiamine pyrophosphate (TPP). Reactions were initiated by the addition of the LoVo cell extract at 37 °C. NADH change was directly proportional to TKT activity, so TKT activity can be reflected by the decrease in absorbance of NADH at 340 nm by using a spectrophotometer. TKT activity was expressed as ng produced per min per mg protein. The protein concentration of LoVo cell extracts was determined by using the Coomassie Brilliant Blue G-250 method. Each experiment was repeated three times.

Flow cytometry

A total of 10^4 cells of each group were seeded into a six-well culture plate, harvested after cultivation for 72 h, fixed in 70% ethanol and stained for DNA content with propidium iodide. Rate of cell apoptosis and DNA content at each stage of cell cycle were determined by flow cytometry and analyzed by CellQuest software package (Beckton, Dickinson, USA).

Cell proliferation assay

To test the effect of siRNA TKTL1 on proliferation of the LoVo cell line, three kinds of cells (cells without transfection, cells transfected with control vector and those transfected with siRNA), at 2×10^3 per well, were seeded into five 96-well culture plates, with each plate having all three kinds of cells seeded separately in three rows. Absorption value of one of five culture plates was determined by 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide at 490 nm after 24-h cultivation. Then, the absorption value of one culture plate was detected on the following 4 days. The growth curve of each cell group was plotted on the basis of the absorption values.

Statistical analysis

Statistical analysis was carried out using SPSS software package (version 10.0 for Windows; SPSS, Chicago, Illinois, USA). The *t*-test was used to assess the difference in expression level of each member of the TKT gene family of LoVo cells (cells transfected with siRNA, those transfected with control vector and those without transfection). The difference in TKT activity in LoVo cells of each group was also assessed by the *t*-test.

Results

mRNA expression of transketolase family members in human colon cancer cells

We designed and constructed three plasmid vectors carrying the DNA to be transcribed into a siRNA against TKTL1 mRNA and one plasmid vector carrying the DNA to be transcribed into the sequence of UC. The plasmids were used to transfect the LoVo cell line. After 96-h transfection, cells were harvested and TKTL1 mRNA levels were analyzed by real-time PCR. Of the three vectors tested, only one gave rise to over 80% inhibition of TKTL1. UC vector did not show any significant decrease in TKTL1 level (data not shown).

The relative expression of each member of TKT gene family was determined by real-time PCR in LoVo cells. In untransfected LoVo cells, the expression level of TKTL1 gene was higher as compared with those of TKT and TKTL2 gene. The expression levels of the three genes in LoVo cells without transfection were very close to that in the cells transfected with control vector. No significant difference in expression was noted between TKT and TKTL2 genes in the three kinds of LoVo cells ($P > 0.05$). The expression level of the TKTL1 gene was, however, significantly downregulated in the LoVo cells transfected with siRNA TKTL1 construct as compared with the cells transfected with control vector and untransfected cells ($P < 0.01$) (Fig. 1). This result indicated that most TKTL1 mRNA was degraded by anti-TKTL1 siRNA in LoVo cells.

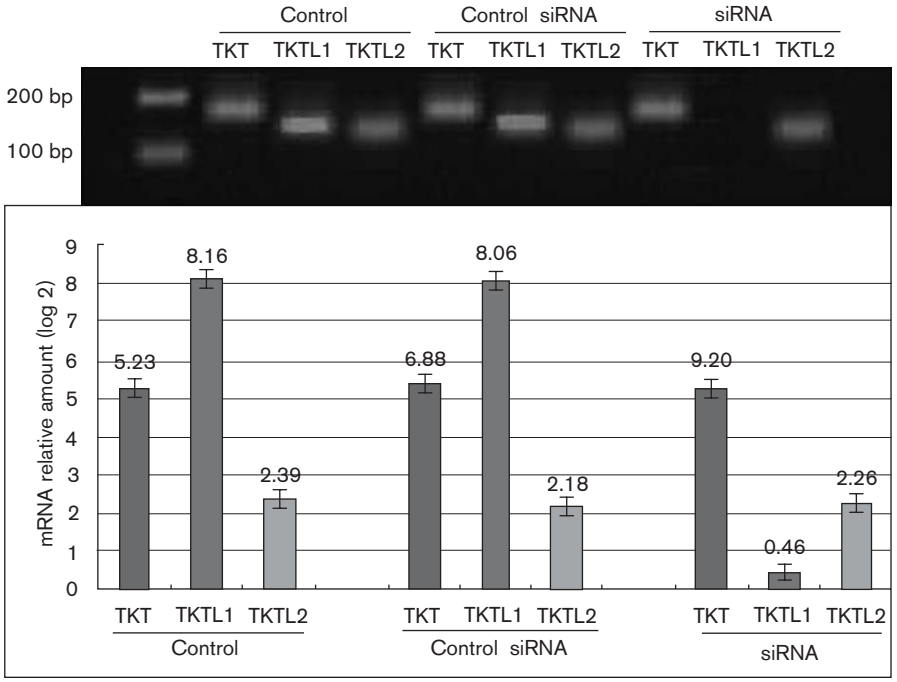
Effect of siRNA TKTL1 gene on transketolase activity in human colon cancer cell line

TKT activity was measured to determine whether TKTL1 plays an important role in total TKT activity of LoVo cells. Figure 2 shows that no significant difference existed in total TKT activity between LoVo cells transfected with control vector and cells without transfection ($P > 0.05$). In contrast, the total TKT activity was significantly decreased in the cells transfected with siRNA TKTL1 construct as compared with the cells transfected with control vector or cells without transfection ($P < 0.01$).

Arrest of human colon cancer cells into the G_0/G_1 stage by siRNA TKTL1

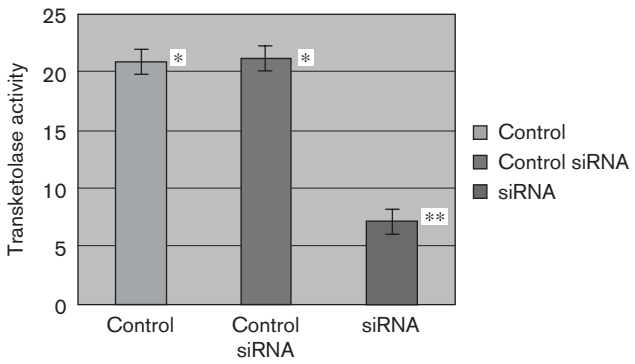
The effect of siRNA TKTL1 on the cell cycle of LoVo cells was examined and each test was repeated three times and the results were similar. Figure 3 shows one representative result of the triplicate experiments. In comparison with LoVo cells transfected with control vector or those without transfection, after transfection with the siRNA TKTL1 construct, the percentage of apoptotic cells and G_0/G_1 stage cells was increased, and

Fig. 1



Quantification of transketolase (TKT), TKTL1 and TKTL2 transcripts in human colon cancer cell line cells without transfection, cells transfected with unrelated small interfering RNA (siRNA) controls and cells transfected with siRNA. Control, cells without transfection; control siRNA, cells transfected with unrelated siRNA controls; siRNA, cells transfected with anti-TKTL1 siRNA. Expression differences of the TKT gene family among three groups cell were calculated and are shown as mRNA relative amount in control cells relative to the cell transfected siRNA. In total, 15 μl of polymerase chain reaction products to visualize the 176-bp TKT, 150-bp TKTL1 and 146-bp TKTL2 amplification product.

Fig. 2



Total transketolase activity in human colon cancer cell line cells without transfection, cells transfected with unrelated small interfering RNA (siRNA) controls and cells transfected with anti-transketolase-like-1 (TKTL1) siRNA. Control, cells without transfection; control siRNA, cells transfected with unrelated siRNA controls; siRNA, cells transfected with anti-TKTL1 siRNA. Total TKT activity was expressed as ng produced per min per mg total protein. Values are given ± SD. $^{*}P > 0.05$; $^{**}P < 0.01$.

the percentage of S stage cells showed no significant change, whereas the percentage of G_2/M stage cells was significantly reduced. These results suggested that LoVo cells were arrested at the G_0/G_1 stage by transfection with siRNA TKTL1 and that siRNA TKTL1 might inhibit the proliferation of LoVo cells.

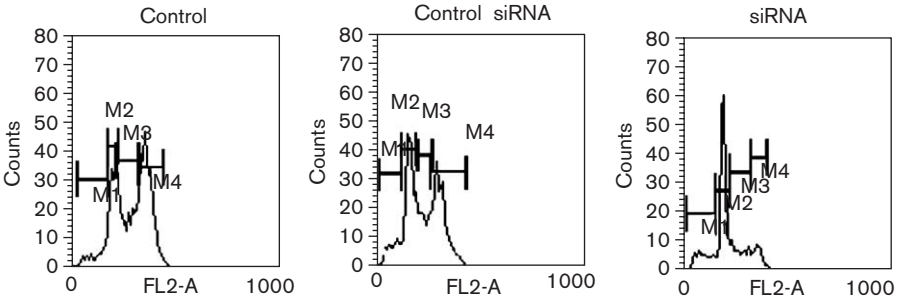
Inhibition of the proliferation of human colon cancer cells by siRNA TKTL1

To examine the proliferation of cells without transfection, cells transfected with control vector and cells transfected with siRNA, the absorption values of one culture plate from each group cells were detected by using 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide at 490 nm on daily basis for a period of 5 days. The growth curve of each cell group showed that cell proliferation was slower in the cells transfected with the siRNA TKTL1 construct as compared with the cells transfected with the control vector or cells without transfection (Fig. 4).

Discussion

Cancer is currently seen as a complicated genetic phenomenon characterized by abnormalities of cellular growth-regulating genes and their signaling pathways [11]. Genetic alterations acquired by tumors also modify their biochemical pathways, resulting in abnormal metabolism. Studies on the physiological changes in malignant conversion provided metabolic indicators for the different stages of tumorigenesis, and during tumorigenesis an

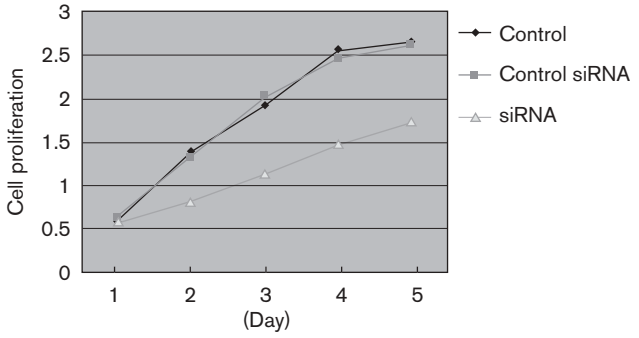
Fig. 3



Marker % gated		Marker % gated		Marker % gated	
All	100.00	All	100.00	All	100.00
M1	4.68	M1	5.61	M1	14.13
M2	35.73	M2	37.61	M2	53.86
M3	20.33	M3	20.1	M3	19.94
M4	39.26	M4	38.68	M4	12.42

The effect of anti-transketolase-like-1 (TKTL1) small interfering RNA (siRNA) on human colon cancer cell line cell cycle. Control, cells without transfection; control siRNA, cells transfected with unrelated siRNA controls; siRNA, cells transfected with anti-TKTL1 siRNA; M1, the percentage of apoptotic cells in total cells; M2, M3 and M4, the percentage of cells at of G_0/G_1, S and G_2/M stage, respectively.

Fig. 4



In-vitro proliferation of human colon cancer cell line cells without transfection, cells transfected with unrelated small interfering RNA (siRNA) controls and cells transfected with anti-transketolase-like-1 (TKTL1) siRNA. Control, cells without transfection; control siRNA, cells transfected with unrelated siRNA controls; siRNA, cells transfected with anti-TKTL1 siRNA.

increase in glucose uptake and lactate production has been observed [12]. TKT reactions of the nonoxidative part of the PPP allow oxygen-independent glucose to be degraded and play a crucial role in nucleic acid ribose synthesis that utilizes glucose carbons in tumor cells [5]. The importance of TKT for tumor cell metabolism is underlined by the fact that specific TKT inhibitors induce a dramatic reduction in tumor cell proliferation [6]. The TKT gene family includes TKT, TKTL1 and TKTL*2*. Langbein *et al.* [8] documented that TKTL1 mRNA and protein were specifically over-expressed in tumors, whereas TKT and TKTL*2* expressions were not upregulated, suggesting that TKTL1 plays an important role in tumor-specific TKT metabolism. The contribution of TKTL1 to tumor-specific TKT metabolism and tumorigenesis has not, however, been fully elucidated yet.

RNA interference (RNAi) is a post-transcriptional mechanism of gene silencing through chromatin remodeling, inhibition of protein translation or direct mRNA degradation [13]. Although the mechanism of RNAi was put forward just recently, RNAi has become one of the most popular techniques of gene silencing in laboratories worldwide. Gene knockdown by RNAi provides a very useful tool for investigating the role of various genes in pathological processes, especially in tumorigenesis. The silencing of critical gene products by RNAi has generated significant antiproliferative and/or proapoptotic effects in cell culture systems or in preclinical animal models [14,15]. To examine the relationship among TKTL1 expression, total TKT activity and growth of cancer cells, we inhibited the expression of TKTL1 by RNAi in LoVo cells. In addition, it was found that the proliferation of cancer cells was significantly inhibited, and cells were arrested at the G_0/G_1 phase in LoVo cells transfected with anti-TKTL1 siRNA. Although the detection of TKT activity is not specific with TKTL1 protein, the total TKT activity was dramatically downregulated in LoVo cells after transfection with anti-TKTL1 siRNA. Furthermore, real-time quantitative PCR showed that the expression of TKTL1 was substantially inhibited, the expression of TKT and TKTL*2* was similar in LoVo cells transfected with anti-TKTL1 siRNA as compared with

cells transfected with control vector or cells without transfection, suggesting that TKTL1 might play an important role in total TKT activity.

Anaerobic conditions are often present in tumors and limit the growth of tumors. Tumor cells, however, in which TKT is upregulated, can use glucose as an energy source through nonoxidative generation of ATP under anaerobic condition [7]. In addition, TKT reactions and other reactions of the PPP permit glucose to be converted to ribose for nucleic acid synthesis and generation of NADPH, a reducing agent required for synthesis. Both of these products of the PPP are essential for the growth of tumor cells. Using metabolic control analysis, Comin-Anduix *et al.* [16] demonstrated that TKT reactions dictate cell proliferation in Ehrlich's ascites tumor cells. We found that cell proliferation was significantly inhibited after transfection with the siRNA TKTL1 construct in LoVo cells, suggesting that TKTL1 over-expression is related to the proliferation of tumor cells.

Gatenby *et al.* [17] proposed an acid-mediated tumor invasion model. The acid-mediated invasion hypothesis provides a simple mechanism for malignant tumor growth that arises directly from the consistent adoption of the glycolytic phenotype with increased glucose flux and H^+ excretion was almost universally observed in clinical cancers by FDG-PET imaging. When nonoxidative glucose degradation takes place in tumor cells, protons will diffuse from the tumor into surrounding normal tissues, thereby subjecting nontransformed cells adjacent to the tumor edge to an extracellular pH significantly lower than normal. The acid environment leads to the death of neighboring healthy cells via *p53*-dependent apoptosis pathways, as well as degradation of the interstitial matrix, loss of intercellular gap junctions, enhanced angiogenesis and inhibition of the host immune response to tumor antigens. This enables tumor cells to remain proliferative and migrate into the surrounding normal tissues producing the invasive phenotypes. This finding demonstrated that TKT activity is correlated to tumor invasion.

The TKTL1 gene is located in Xq28, one of few chromosomal regions that are both activated in malignancies and during the cell cycle [18,19]. Coy *et al.* [7] found that TKTL1 was specifically upregulated in malignancies at the mRNA level, whereas TKT and TKTL2 were not upregulated. Langbein *et al.* [8] reported that TKTL1 expression was associated with invasive colonic and urothelial tumors, and to poor prognosis [8]. Our study demonstrated that TKTL1 plays an important role in total TKT activity and proliferation of tumor cells. Recently, PET technology has been extensively used for the detection of enhanced glucose metabolism in invasive tumors and metastases. In addition, PET is also employed for the detection of reduced glucose metabolism in clinical practice. In neurodegenerative diseases, diabetes and cancer, an altered glucose metabolism as well as altered TKT protein expression or enzyme activity have been noted. It was postulated that these diseases share a common molecular and biochemical basis. Coy *et al.* [7] suggested that there exists a novel metabolic pathway that is related to tumor metabolism, chronic diabetic complications, vascular injuries and neurodegenerative diseases [7]. TKTL1 and its enzymatic properties play crucial roles in the metabolic pathway.

To sum up, our study supported the presence of a TKTL1-based metabolic pathway in LoVo cells, and it might help to further explore the effect of anti-TKTL1 siRNA on growth of tumor cells in other cell lines and TKTL1-based anticancer therapy.

References

1 Warburg O, Posener K, Negelein E. Uber den Stoffwechsel der Carcinomzelle [Article in German]. *Biochem Z* 1924; **152**:309–344.

2 Downey RJ, Akhurst T, Gonen M, Vincent A, Bains MS, Larson S, *et al.* Preoperative F-18 fluorodeoxyglucose-positron emission tomography maximal standardized uptake value predicts survival after lung cancer resection. *J Clin Oncol* 2004; **22**:3255–3260.

3 Kumar R, Mavi A, Bural G, Alavi A. Fluorodeoxyglucose-PET in the management of malignant melanoma. *Radiol Clin North Am* 2005; **43**:23–33.

4 Reiman EM, Chen K, Alexander GE, Caselli RJ, Bandy D, Osborne D, *et al.* Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. *Proc Natl Acad Sci U S A* 2004; **101**:284–289.

5 Boros LG, Puigjaner J, Cascante M, Lee WN, Brandes JL, Bassilian S, *et al.* Oxythiamine and dehydroepiandrosterone inhibit the nonoxidative synthesis of ribose and tumor cell proliferation. *Cancer Res* 1997; **57**:4242–4248.

6 Rais B, Comin B, Puigjaner J, Brandes JL, Creppy E, Saboureau D, *et al.* Oxythiamine and dehydroepiandrosterone induce a G_1 phase cycle arrest in Ehrlich's tumour cells through inhibition of the pentose cycle. *FEBS Lett* 1999; **456**:113–118.

7 Coy JF, Dressler D, Wilde J, Schubert P. Mutations in the transketolase-like gene TKTL1: clinical implications for neurodegenerative diseases, diabetes and cancer. *Clin Lab* 2005; **51**:257–273.

8 Langbein S, Zerilli M, Zur Hausen A, Staiger W, Rensch-Boschert K, Lukan N, *et al.* Expression of transketolase TKTL1 predicts colon and urothelial cancer patient survival: Warburg effect reinterpreted. *Br J Cancer* 2006; **94**:578–585.

9 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_t}$ method. *methods* 2001; **25**:402–408.

10 Smeets EH, Muller H, de Wael J. A NADH-dependent transketolase assay in erythrocyte hemolysates. *Clin Chim Acta* 1971; **33**:379–386.

11 Weng G, Bhalla US, Iyengar R. Complexity in biological signaling systems. *Science* 1999; **284**:92–96.

12 Ramanathan A, Wang C, Schreiber SL. Perturbational profiling of a cell-line model of tumorigenesis by using metabolic measurements. *Proc Natl Acad Sci U S A* 2005; **102**:5992–5997.

13 Caplen NJ. Gene therapy progress and prospects. Downregulating gene expression: the impact of RNA interference. *Gene Ther* 2004; **11**:1241–1248.

14 Cheng SQ, Wang WL, Yan W, Li QL, Wang L, Wang WY, *et al.* Knockdown of survivin gene expression by RNAi induces apoptosis in human hepatocellular carcinoma cell line SMMC-7721. *World J Gastroenterol* 2005; **11**:756–759.

15 Uchida H, Tanaka T, Sasaki K, Kato K, Dehari H, Ito Y, *et al.* Adenovirus-mediated transfer of siRNA against survivin induced apoptosis and

attenuated tumor cell growth *in vitro* and *in vivo*. *Mol Ther* 2004; **10**:162–171.
16 Comin-Anduix B, Boren J, Martinez S, Moro C, Centelles JJ, Trebukhina R, *et al.* The effect of thiamine supplementation on tumour proliferation. A metabolic control analysis study. *Eur J Biochem* 2001; **268**:4177–4182.
17 Gatenby RA, Gawlinski ET. The glycolytic phenotype in carcinogenesis and tumor invasion: insights through mathematical models. *Cancer Res* 2003; **63**:3847–3854.
18 Glinsky GV, Ivanova YA, Glinskii AB. Common malignancy-associated regions of transcriptional activation (MARTA) in human prostate, breast, ovarian, and colon cancers are targets for DNA amplification. *Cancer Lett* 2003; **201**:67–77.
19 Glinsky GV, Krones-Herzig A, Glinskii AB. Malignancy-associated regions of transcriptional activation: gene expression profiling identifies common chromosomal regions of a recurrent transcriptional activation in human prostate, breast, ovarian, and colon cancers. *Neoplasia* 2003; **5**:218–228.