Glucose metabolism of malignant cells is not regulated by transketolase-like (TKTL)-1

ARNULF MAYER[1], ANGELIKA VON WALLBRUNN[2] and PETER VAUPEL[1,2]

[1]Department of Radiooncology and Radiotherapy, University Medical Center, Langenbeckstrasse 1, 55131 Mainz;
[2]Institute of Physiology and Pathophysiology, University Medical Center, Duesbergweg 6, 55128 Mainz, Germany

Received March 5, 2010; Accepted April 21, 2010

DOI: 10.3892/ijo_00000674

Abstract. An isoenzyme of transketolase, transketolase-like (TKTL)-1, has been hypothesized to play a pivotal role in the pathophysiology of malignant tumors. Available data are based on the detection of the putative TKTL-1 protein with one particular mouse monoclonal anti-TKTL-1 antibody, clone JFC12T10. In this study it was demonstrated that a) JFC12T10 detects multiple unspecific bands in Western blots, b) a 75-kDa band hitherto referred to as TKTL-1 corresponds to a nuclear protein and c) immunohistochemical detection of TKTL-1 in benign leiomyomas yields an expression pattern identical to that found in a variety of malignant tumors. In RT-PCR assays, using three different primer pairs for transketolase, TKTL-1 and yet another isogene of transketolase, TKTL-2, a relevant expression of TKTL-1 was not detectable in any of the 6 malignant tumor cell lines investigated (MCF-7, A549, HeLa, HT1080, M21 and TF-1). Expression levels of TKTL-1 were rather similar to those found for TKTL-2, although the latter has never been implicated in malignant disease. On the basis of these data, nutritional recommendations based on a hypothetically TKTL-1 controlled metabolism of tumor cells must be regarded as lacking scientific evidence.

Introduction

Following a publication by Coy *et al* in 2005 (1), a series of reports has been published suggesting that an isoenzyme of transketolase, transketolase-like-1 (TKTL-1), may play a critical role in the pathophysiology and progression of malignant tumors. According to the results of several clinical studies, overexpression of TKTL-1 is associated with a poor prognosis (2-5). Experimental data have been published (1,6-8) which apparently confirm the pathophysiological basis of TKTL-1-associated aggressiveness observed in clinical studies. Coy *et al* (1) hypothesized that energy production of malignant cells is crucially dependent upon TKTL-1-mediated degradation of glucose to lactate. This form of glucose degradation is supposed to be made possible by changes in the enzymatic properties of TKTL-1 compared with transketolase. The results of these studies have been used as a basis for nutritional recommendations (www.tavarlin.de) and have attracted considerable attention. The antibody which was used in the previously mentioned studies (clone JFC12T10) is sold under the trade name 'RIDA PentoCheck IHC' for TKTL-1 diagnosis in cancer patients.

Transketolase is an enzyme of the pentose-phosphate-pathway (PPP). The physiological purpose of this pathway is the generation of ribose-5-phosphate as a starting substance for purine and, hence, nucleotide biosynthesis and supply of the reduction equivalent NADPH. Transketolase catalyzes the transfer of C_2-glycoaldehyde groups from ketoses to aldoses in the so-called non-oxidative part of the PPP. Because these reactions are reversible, they provide the basis for a bidirectional connection between the PPP and glycolysis. Cells which are not engaged in intensive nucleotide biosynthesis can, with the involvement of some intermediate steps, convert unused ribose-5-phosphate to fructose and glycerinaldehyde-3-phosphate which can subsequently be used for energy production in glycolysis. The relevance of these reactions is evident from the fact that no ATP is produced in the PPP. Conversely, glycolytic intermediates can, should the need arise, be utilized for nucleotide biosynthesis while bypassing the oxidative part of the PPP. This sequence of reactions is preferred when cells have no requirement for (reduced) NAPDH, which is generated in the oxidative part of the PPP. E.g., under anaerobic conditions, $NADP^+$ cannot be regenerated, thus creating the need to circumvent the oxidative phase of the PPP. As is the case with a number of other enzymes, transketolase requires thiamine pyrophosphate (TPP) as a coenzyme. Data from the literature regarding the importance of transketolase for growth of malignant tumor cells is scarce and in addition, partially contradictory. Heinrich *et al*, in a publication from 1976 (9), found no difference in transketolase activity between subcutaneously implanted hepatomas of the rat and different metabolic states of the non-malignant rat liver. However, such a difference

Correspondence to: Dr Arnulf Mayer, Department of Radiooncology and Radiotherapy, University Medical Center, Johannes Gutenberg University, Langenbeckstrasse 1, 55131 Mainz, Germany
E-mail: arnmayer@uni-mainz.de

Key words: transketolase-like 1, tumor hypoxia, glycolysis, Warburg effect, ketogenic diet

was seen for transaldolase, for which an up to three times higher activity was found in the normal tissues. In the same year, Schwartz *et al* showed that even severe thiamine deficiency does not have a negative impact on the energy metabolism of C6 glioma cells (10). These data are in conflict with results from Boros *et al* (11), who demonstrated that the competitive transketolase-inhibitor oxythiamine can achieve a 90% inhibition of the proliferation of Ehrlich ascites cells *in vivo*. Clinical studies demonstrating a prognostic impact of different activities of transketolase in human tumors have, according to our knowledge, not been conducted or published thus far.

Reports on isoenzymes of transketolase have already been published by Iida *et al* (12) for *E. coli*, and by Schaaff-Gerstenschläger *et al* (13) for *Saccharomyces cerevisiae* (TKL2). A gene related to transketolase in humans (TKR) was first described by Coy *et al* in 1996 (14) and initially considered as being defective due to the mutation of a TPP-binding domain. The authors assumed that this gene could represent the defect gene involved in the Wernicke-Korsakoff syndrome. In 2005, Coy *et al* supplemented their initial data and hypothesized that an isoenzyme of transketolase was also present in humans, which they now referred to as TKTL-1 (1). Using quantitative real-time reverse transcriptase polymerase chain reaction (qPCR), they found a strong expression of TKTL-1 in testis, thymus and retina. However, all other normal tissues analyzed showed a low (60-1000 times lower) expression of TKTL-1 compared to transketolase. In this context, Coy *et al* (1) cite a publication by Warburg from 1924 (15), which is supposed to describe strong aerobic glycolysis in the three previously mentioned tissues and in doing so, indirectly suggests that TKTL-1 is an integral element of aerobic glycolysis (Warburg effect). In our opinion, Warburg's work has been cited incorrectly. As far as aerobic glycolysis in the thymus is concerned, Warburg literally states (15) 'Gehen wir von anaeroben zu aeroben Bedingungen über, so verschwindet für Thymus die Glykolyse fast vollständig...' (author's translation: 'If we go from anaerobic to aerobic conditions, glycolysis disappears almost completely in thymus...'). In their afore-mentioned work, Coy *et al* (1) also describe the generation of a monoclonal antibody (clone JFC12T10) against TKTL-1, which detects a 75-kDa protein (and, to a lesser extent and with more variability, some other proteins of lower molecular weight) in Western blots of lysates of 5 different established cell lines, among them MCF-7 and A549. According to Coy *et al* (1), this antibody specifically recognizes recombinant TKTL-1, but not transketolase or TKTL-2 in Western blots. However, these crucially important data were not shown in the cited study (1). Immunohistochemical studies in human tissues using the previously described antibody clone JFC12T10 showed a positivity of endothelial cells and peripheral neurons of healthy individuals (1). Notably, in addition to the expected cytoplasmic staining, a nuclear signal was also present in the former cell type. Inexplicably, data regarding the expression of TKTL-1 in testes, thymus and retina, the previously mentioned tissues with high expression of TKTL-1 at the mRNA level, are missing in the same study (1).

Using qPCR, Langbein *et al* (3) subsequently detected an increased expression of TKTL-1 mRNA in 1 out of 5 colon cancers and in 2 out of 5 lung and gastric carcinomas. Conversely, expression of transketolase was not increased in any of the examined tumors compared with the corresponding normal tissues. In the same paper, the expression of TKTL-1 was assessed using the afore-mentioned antibody clone JFC12T10 in 1030 tumors of 16 different tumor entities. The results of this extensive study, however, as was the case for the above-mentioned data regarding the validation of the antibody clone JFC12T10, were only communicated in a qualitative fashion. Accurate information concerning the fraction of positive and negative tumors as well as the intensity of positivity in individual specimens was not presented in this publication (3). Again, a nuclear expression of TKTL-1 (in a number of gastric carcinomas) is described. Langbein *et al* (3) also analyzed the prognostic significance of TKTL-1 expression, each in a smaller group of patients and only for colon and urothelial carcinomas. In both cases, the authors present their data as evidence for a strong negative impact of increased TKTL-1 expression on patient prognosis. Omissions in this communication, however, prevent an objective evaluation of this interpretation. Nevertheless, several studies using the antibody clone JFC12T10 in other tumor entities also reported an unfavorable prognostic significance of increased expression of TKTL-1 (2,4,5). In the present study, the importance of TKTL-1 for malignant tumor cells has been evaluated on the basis of our own experimental data. The starting point of our interest in TKTL-1 was the fact that to date, no studies investigating possible mechanisms of TKTL-1 overexpression in malignant cells are available. Since TKTL-1 has been suggested to have a role in energy production in malignant cells under hypoxic conditions, a series of experiments evaluating a possible induction of TKTL-1 expression by hypoxia was performed. Preliminary results of this study were presented at the 37th Annual ISOTT Meeting, Cleveland, USA (2009).

Materials and methods

Cells were cultured in MEM, Ham's F12 or RPMI-1640, supplemented with 10% (v/v) fetal calf serum, 5% glutamine and 5% penicillin/streptomycin (PAA Laboratories) under a water-saturated atmosphere with 5% CO_2 at 37˚C. The culture medium for TF-1 additionally contained 3 ng/ml GM-CSF (Biomol). Hypoxic treatment took place in an incubator with oxygen regulation (Labotect) at 1% O_2. Differential protein extraction of nuclear and cytoplasmic or membrane and cytoplasmic fractions was carried out with the commercially available NE-PER or MEM-PER kits (Pierce), respectively, according to the manufacturer's instructions. For Western blotting, identical amounts of protein lysates were separated by sodiumdodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes by semi-dry blotting. Primary antibodies (anti-TKTL-1, clone JFC12T10, Linaris; HIF-1α, clone 54, BD Transduction Labs; CA IX ab15086, Abcam; ß-actin, sc-1616-R, Santa Cruz) were detected with HRP-conjugated secondary antibodies (Santa Cruz). Bands were developed with ECL (GE Healthcare) and images were captured with a CCD camera (Fujifilm LAS-3000). ß-actin was used as a loading control.

For the immunohistochemical detection of TKTL-1 in tissue sections, retrieval of antigenic binding sites was carried out with sodium citrate (pH 6.0) in a steamer (Braun). Primary antibodies against TKTL-1 (Linaris) were detected with the micro-polymer-based, biotin-free Impress detection system (Vector). Diaminobenzidine (DAB) served as the peroxidase substrate.

RNA extraction was carried out using the peqGOLD total RNA kit (Peqlab) and DNAse I digestion (Fermentas) for the elimination of residual genomic DNA. Reverse transcription was performed using the RevertAid First Strand cDNA synthesis kit (Fermentas) and an initial quantity of 2 μg RNA. cDNA (1 μg) was amplified for each reaction in a volume of 50 μl master mix [10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3, 200 μM of dNTP and 1 U Taq (Peqlab) or Pfu (Fermentas) DNA polymerase]. Each oligonucleotide primer was added at a concentration of 0.4 μM. Polymerase chain reaction (PCR) was carried out in a Mastercycler EP thermocycler (Eppendorf) under the following conditions: initial denaturation at 95˚C for 5 min, followed by 35 cycles of a 3-step protocol consisting of denaturation at 95˚C for 30 sec, primer annealing at 60˚C for 1 min and elongation at 72˚C for 1 min. The last cycle was followed by an additional elongation step for 10 min at 72˚C. PCR products were separated in 2.5% agarose gels, visualized with ethidium bromide and documented with a CCD camera. The specificity of the amplified PCR products was confirmed in each case by subsequent sequencing (GATC Biotech). The primer sequences were designed using the software Primer3 (http://primer3.sourceforge.net/) or adopted from the publication of Coy *et al* (1). Quantitative real-time reverse transcription PCR (qPCR) was performed with SYBR-Green plus ROX (Thermo Scientific) in a volume of 25 μl on an ABI 7300 system (Applied Biosystems) with the following parameters: denaturation at 95˚C for 15 min, then 95˚C for 15 sec and 60˚C for 1 min in each of 40 cycles. ß-actin served as the house-keeping gene and all reactions were carried out in triplicate. Amplification efficiency of primers was always above 90%, the specificity of amplification was confirmed by melting curve analyses. Data analysis was performed with the ABI data analysis software according to the $\Delta\Delta$Ct method (16) and expressed as relative expression intensity relative to the calibrator (normoxic cells).

Results

Clone JFC12T10 detects non-specific bands in Western blots. Expression of TKTL-1 was investigated by Western blotting of lysates generated by differential protein extraction from cytoplasmic, nuclear and membranous fractions of HeLa and MCF-7 cells. The extraction technique employed here, using commercial kits from Pierce (NE-PER or MEM-PER), generates two different cytoplasmic extracts, each complementing the nuclear and the membranous protein lysates, respectively. Owing to these methodological details, it is reasonable that the two cytoplasmic fractions shown below do not yield identical results. From the data published by Coy *et al* (1), we expected that the monoclonal mouse anti-TKTL-1 clone JFC12T10 detects bands of different molecular weights, among which a band at 75 kDa is dominant. Our analysis indeed provided evidence of a protein with a molecular weight of 75 kDa, although mainly in the nuclear fractions, and only very weakly in the cytoplasmic lysates (Fig. 1a). In the cytoplasmic fractions JFC12T10 detected numerous other bands, which cover a wide range of molecular weights from 44 to 95 kDa (95, 66, 59, 49 and 44 kDa; Fig. 1a). As expected, membranous expression of the previously described 75-kDa band protein was not found (Fig. 1b). In the membrane-bound protein fractions, however, other bands were found (95 and 44 kDa) that may represent cytoplasmic contaminations. The correct implementation of protein fractionation (and successful induction of hypoxia) was repeatedly validated by the results of the differential expression analysis of HIF-1α (positive hypoxic nuclear fractions; Fig. 1c) and carbonic anhydrase (CA) IX (evidence of a membrane-bound isoform that is induced by hypoxia in MCF-7 cells; Fig. 1d). Since the pentose phosphate pathway and transketolase reactions take place in the cytoplasm exclusively, a 75-kDa protein detected by the antibody does not correspond to a transketolase, at least not in a functional sense. A change in the expression intensity and quality by hypoxia, as we could clearly demonstrate for HIF-1α and CA IX, does not exist for the target proteins of JFC12T10.

Using JFC12T10, benign leiomyomas show an expression pattern identical to the one described in studies of malignant tumors. Immunohistochemical studies of TKTL-1 expression have so far shown low expression in benign tissues and a strong correlation of expression intensity with the prognosis in malignant tumors. To verify the immunohistochemical expression pattern we were able to analyze two leiomyomas. Although not expected in these benign tumors, an expression of TKTL-1 was found. As shown in Fig. 2, the mixed cytoplasmic and nuclear expression patterns described in the literature could be confirmed. According to our own criteria, the intensity of the cytoplasmic component is weak. However, this weak staining is similar in intensity to the specimens depicted in the publications of Coy *et al* (1) and Langbein *et al* (2,3), which were scored as TKTL-1 positive by the latter authors.

TKTL-1 expression is absent or minimal in 6 malignant cell lines. Since the previously described results clearly speak against an expression of TKTL-1 associated with malignancy, as has been postulated by Langbein *et al* (3), we were interested in the expression of TKTL-1 at the mRNA level. Malignant cell lines (HeLa, MCF-7, A549, HT1080, M21 and TF-1) were analyzed by end-point PCR followed by electrophoretic separation of the amplicons in agarose gel. As was the case with the Western blots, this analysis was also carried out for normoxic and hypoxic cells. For the sake of comparability, this analysis included TKTL-1, transketolase and TKTL-2. For TKTL-1, we used primer sequences as published by Coy *et al* (1) as well as two additional oligonucleotide primer pairs which we had designed. For TKT and TKTL-2 three different oligonucleotide primer pairs were also used. The identity of all amplicons was confirmed by sequencing. Results were identical in all cell lines examined here: transketolase is by far the dominant isoform, while the expression of TKTL-1 or TKTL-2 at best only lies at the

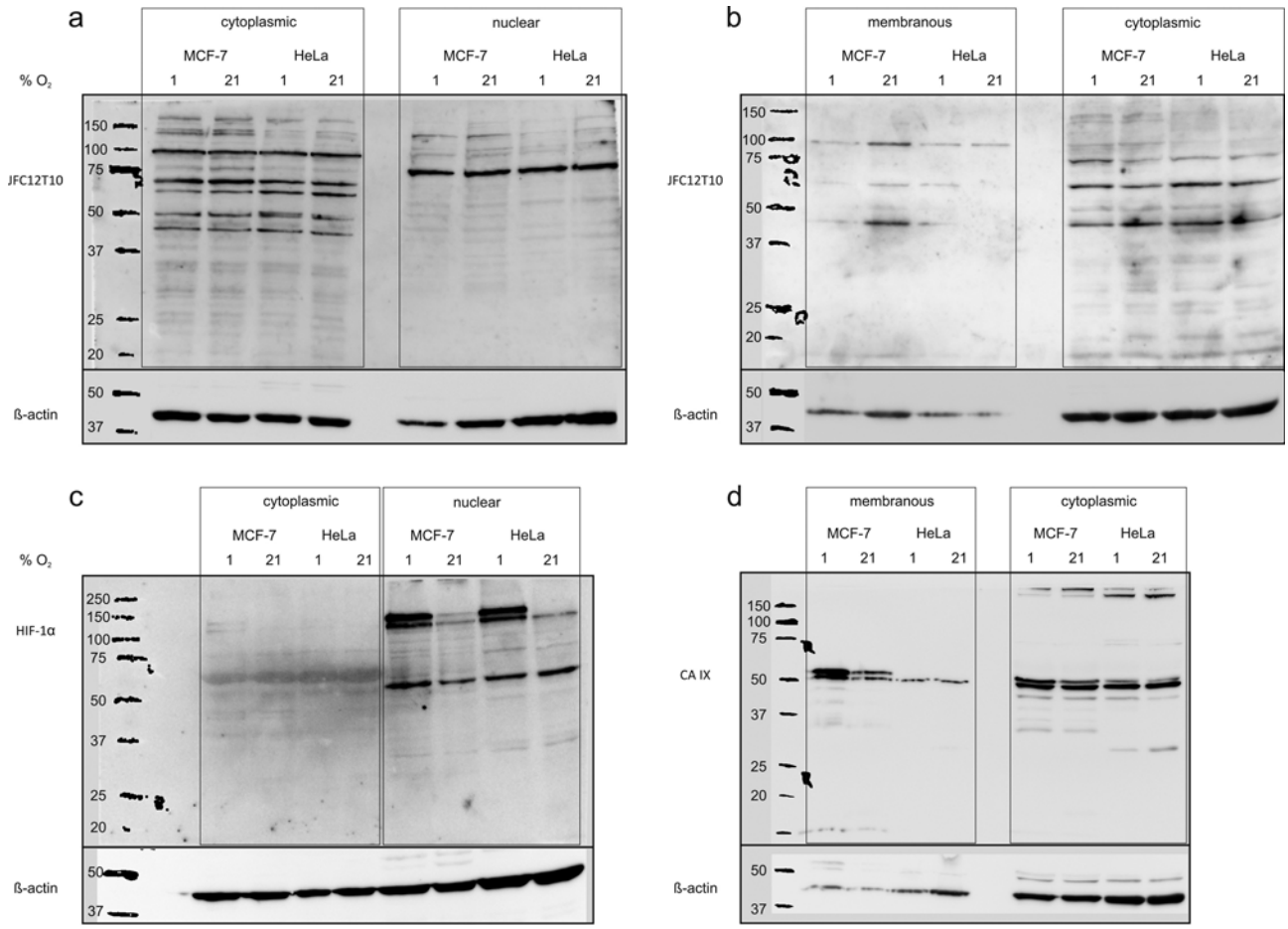

Figure 1. Western blots of protein extracts from normoxic (21% O_2) and hypoxic (1% O_2) MCF-7 and HeLa cells generated with the NE-PER kit (nuclear vs. cytoplasmic), (a and c) and the MEM-PER kit (membranous vs. cytoplasmic) (b and d). Detection with the anti-TKTL-1 antibody JFC12T10 (a and b) shows multiple bands, one of which is located at 75 kDa and is specifically detected in the nuclear extracts (b). The detection of both HIF-1α (c) and carbonic anhydrase IX (d) confirmed the successful induction of hypoxia and the proper separation of the cellular compartments.

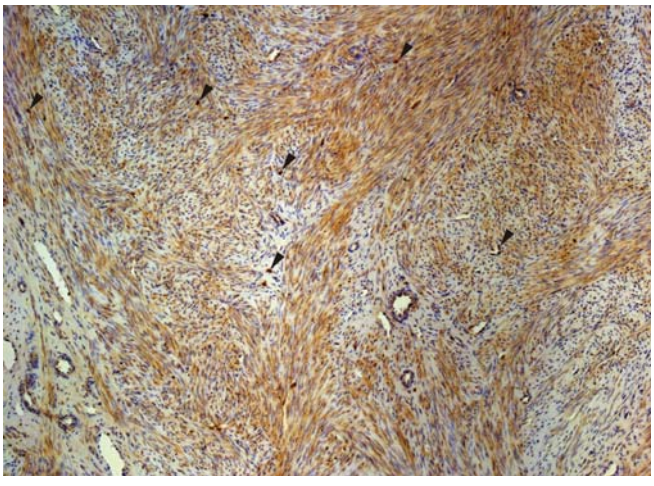

Figure 2. In a benign leiomyoma of the uterus a mixed cytoplasmic and nuclear (arrowheads) expression pattern of TKTL-1 was observed using the antibody clone JFC12T10.

limit of detection, and is even below this limit in most cases, as shown in Fig. 3, which is representative for these studies. The influence of hypoxia on the expression intensity of all three isoenzymes was also investigated, using qPCR. Fig. 4 shows data for MCF-7 and HeLa cells. In accordance with data shown before, no influence of the oxygen partial pressure on the expression intensity of any of the three genes was evident. The Ct values of the TKTL-1 and TKTL-2 isogenes were consistently 10-13 cycles higher than those for transketolase. Assuming equal amplification efficiencies of all three variants, the differences in expression levels between transketolase and TKTL-1 would be in the range of 1:1000-1:10000, and, hence, within the dimensions reported by Coy *et al* (1) for differences in the expression intensity between these two genes in normal tissues.

Discussion

The present study shows that the monoclonal antibody JFC12T10, which was generated by Coy *et al* (1), detects multiple bands in cytoplasmic, nuclear and membranous lysates of MCF-7 and HeLa cells whose molecular weights are atypical for a TKTL-1 gene product. We also detected bands of 66 kDa, which is very close to the expected molecular weight of TKTL-1 of 65 kDa (calculated according to NCBI reference sequence NP_036385.3). The assumption that these 66-kDa bands correspond to TKTL-1 is contradicted, however, by the fact that Coy *et al* (1) demonstrated

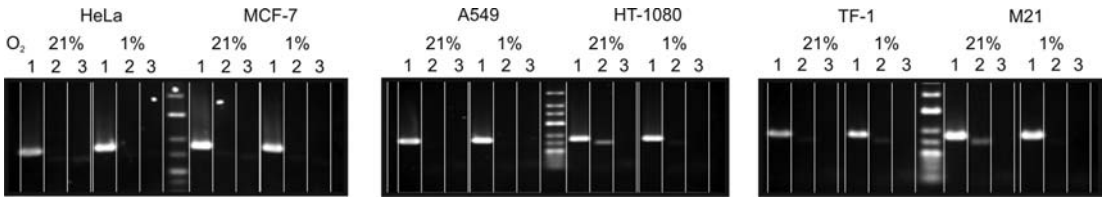

Figure 3. End-point RT-PCR of the expression of transketolase (1), TKTL-1 (2) and TKTL-2 (3) in 6 different malignant cell lines under normoxic (21% O_2) and hypoxic (1% O_2) conditions. In all cell lines examined, the expression of transketolase is clearly dominant, TKTL-1 is weakly detectable only in HT-1080 and TF-1 cells.

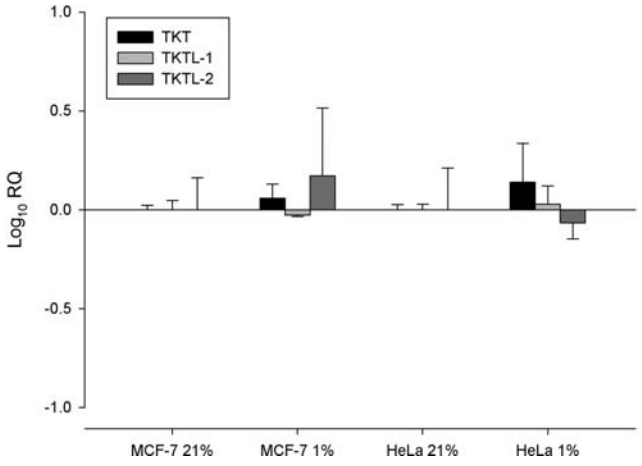

Figure 4. Quantitative real-time RT-PCR studies on the regulation of transketolase (TKT), TKTL-1 and TKTL-2 by hypoxia (1% vs. 21% O_2) in MCF-7 and HeLa cells. Regulation by the oxygenation status is not recognizable for any of the three genes (error bars = standard deviation).

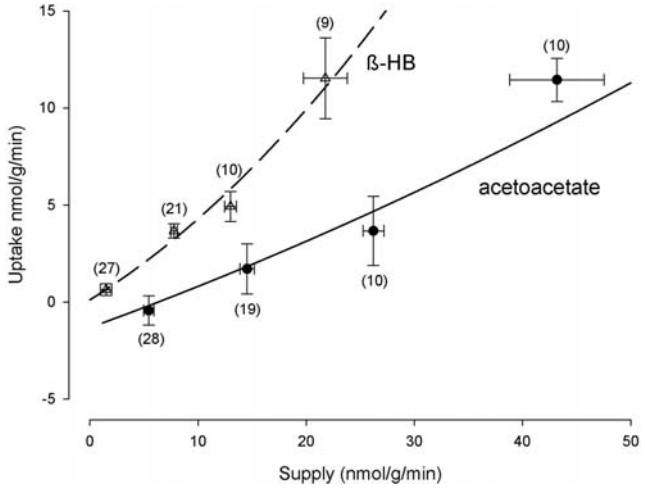

Figure 5. Uptake of ß-hydroxybutyrate (ß-HB) and acetoacetate in xenotransplanted human breast cancers as a function of arterial supply (mean ± SEM; number of tumors in parentheses).

an atypical migration pattern of the recombinant TKTL-1 protein in the SDS-PAGE with an apparent molecular weight of 75 kDa and also showed a dominant band at 75 kDa in lysates from various cell lines (including MCF-7 cells, which were also investigated in the present work). We can confirm the existence of a band of ~75 kDa in MCF-7 (and HeLa) cells, but have also clearly shown that this protein is localized in the nucleus and, therefore, not likely to be identical with a (functionally active) transketolase. Additionally, it is questionable whether 75-kDa bands found in cell lines by Coy *et al* (1) really do correspond to TKTL-1, since molecular weight alone may not be regarded as being sufficient evidence. The situation is complicated by the fact that no Western blots of the recombinant protein have been published so far and that the same authors subsequently published a paper, in which a band with a significantly lower molecular weight was accepted as proof of TKTL-1 expression [Fig. 1b, in Langbein *et al* (3)]. Results from our immunohistochemical studies in benign leiomyomas demonstrating nuclear staining with JFC12T10 are in line with our Western blot data. Since the suitability of an antibody for immunohistochemistry generally requires a separate validation on the basis of biological plausibility checks, we need to point out that such investigations are not available or at least have not been published for JFC12T10.

Doubts about the specificity of the anti-TKTL-1 antibody JFC12T10 expressed here are of crucial importance for the concept of a transketolase isoenzyme TKTL-1, since the *in vivo* detection of TKTL-1 so far has been based entirely on the detection with this antibody. Although other antibodies against TKTL-1 are commercially available, they have not been used in published studies. However, results of extensive immunohistochemical validation studies with one of these alternative antibodies (Atlas Antibodies, product number HPA000505, subsequently referred to as HPA505) in many normal tissues, tumor tissues and cells can be accessed at www.proteinatlas.org. These data are in clear contradiction to those published with clone JFC12T10. With HPA505, 9 of 12 colon and 10 of 12 urothelial carcinomas were completely negative for TKTL-1, and the remainder in each case showed only a weak positivity. As described previously, Langbein *et al* (3) obtained completely different data for these tumors using JFC12T10. The same is true for renal cell carcinoma, which was entirely negative for HPA505, whereas approximately 90% of the tumors were positive in a study using the antibody JFC12T10 (2).

Proof of the enzymatic activity of TKTL-1 is lacking, since available data are fragmentary and, importantly, based on an unreferenced enzymatic method of uncertain validity (1). Both the comparison of the activities of TKTL-1 and transketolase as well as the investigation of the influence of

TPP have not been performed in these experiments. Owing to the lack of monospecificity of JFC12T10, which is evident from the data presented in our study, additional concerns arise regarding the purity of the native TKTL-1 protein that has been extracted from K562 cells for the above-mentioned enzyme activity assays using this antibody.

Our studies of 6 different malignant cell lines yielded no evidence of a relevant expression of TKTL-1 at the mRNA level. In two of these, MCF-7 and A549, Coy *et al* (1) found the previously described 75-kDa band in Western blots, and thus, according to these authors, TKTL-1. An expression of TKTL-1 at the mRNA level was therefore considered to be present, but, nevertheless, not found. Problems with our PCR methodology can practically be ruled out as reason for the TKTL-1 negativity in the present study. We used 3 different sets of primers, including primer sequences published by Coy *et al* (1), always with the same (negative) result. Our data are also consistent with the lack of expression of TKTL-1 at the protein level, which were obtained with the afore-mentioned anti-TKTL-1 antibody HPA505 in MCF-7, A549 and HeLa, as well as in another 40 of the 47 cell lines studied. Using this antibody, only 3 cell lines showed a marginal expression and only one, THP-1 cells, was strongly TKTL-1 positive (www.proteinatlas.org).

Coy *et al* have postulated that TKTL-1 could be important for ATP production in hypoxic cells (1). Changes in enzymatic properties representing an oncogenic gain-of-function are hypothesized to enable an ATP-producing pathway (active even in the absence of oxygen), which is supposed to be analogous to the so-called phosphoketolase pathway of heterofermentative lactic acid bacteria. Beyond this hypothesis, Coy *et al* (1) also expressed doubts concerning the accuracy of the current representation of the PPP in biochemical textbooks, implying that the current paradigm underestimates the energy yield which can be derived from this pathway. Finally, the authors hypothesize that TKTL-1 is the basis for aerobic glycolysis (i.e., the Warburg effect) of tumor cells (3). The latter hypothesis implicitly assumes that, from an energetic point of view, the postulated alternative pathway is so effective that tumor cells would embark on it even in the presence of oxygen. Besides the fact that the existence of such a pathway in human tumor cells is not supported by experimental evidence, its energy output would actually be less than that of glycolysis and significantly lower than the dimension of the ATP yield of oxidative phosphorylation under aerobic conditions. In the present study we were able to exclude an upregulation of TKTL-1 under conditions of low oxygen partial pressure (i.e., hypoxia) using qPCR.

A low-carbohydrate, ketogenic diet is recommended by Coy *et al* as a therapy for a TKTL-1-controlled tumor cell metabolism, referring to the results of several studies (17-20). According to this view, low blood glucose levels lead to a reduction of the glucose supply to the tumor. The hypothesis also contains the presupposition that tumor cells cannot utilize ketone bodies for energy production. It should be remembered, however, that experimental studies from different groups have repeatedly demonstrated that tumor tissue does indeed take up ketone bodies, and may also utilize these molecules for energy production (21-23). Newly analyzed data from a previous publication (21) by one of the authors (PV) illustrate that the uptake of both acetoacetate and ß-hydroxybutyrate is a direct function of arterial supply (Fig. 5).

The data presented in this study raise reasonable doubts about the concept of the pathophysiological relevance of a transketolase isoenzyme TKTL-1 for energy metabolism, growth and progression of malignant tumors. Nutritional recommendations based on this concept are not based on resilient data.

Acknowledgements

This study has been supported by grant no. 106758 of the Deutsche Krebshilfe. We thank Dr Debra Kelleher for her excellent editorial assistance.

References

1. Coy JF, Dressler D, Wilde J and Schubert P: Mutations in the transketolase-like gene TKTL1: Clinical implications for neurodegenerative diseases, diabetes and cancer. Clin Lab 51: 257-273, 2005.
2. Langbein S, Frederiks WM, zur Hausen A, *et al*: Metastasis is promoted by a bioenergetic switch: New targets for progressive renal cell cancer. Int J Cancer 122: 2422-2428, 2008.
3. Langbein S, Zerilli M, zur Hausen A, *et al*: Expression of transketolase TKTL1 predicts colon and urothelial cancer patient survival: Warburg effect reinterpreted. Br J Cancer 94: 578-585, 2006.
4. Völker HU, Scheich M, Schmausser B, Kämmerer U and Eck M: Overexpression of transketolase TKTL1 is associated with shorter survival in laryngeal squamous cell carcinomas. Eur Arch Otorhinolaryngol 264: 1431-1436, 2007.
5. Zerilli M, Amato MC, Martorana A, *et al*: Increased expression of transketolase-like-1 in papillary thyroid carcinomas smaller than 1.5 cm in diameter is associated with lymph-node metastases. Cancer 113: 936-944, 2008.
6. Fröhlich E, Fink I and Wahl R: Is transketolase like 1 a target for the treatment of differentiated thyroid carcinoma? A study on thyroid cancer cell lines. Invest New Drugs 27: 297-303, 2009.
7. Hu LH, Yang JH, Zhang DT, *et al*: The TKTL1 gene influences total transketolase activity and cell proliferation in human colon cancer lovo cells. Anticancer Drugs 18: 427-433, 2007.
8. Zhang S, Yang JH, Guo CK and Cai PC: Gene silencing of TKTL1 by RNAi inhibits cell proliferation in human hepatoma cells. Cancer Lett 253: 108-114, 2007.
9. Heinrich PC, Morris HP and Weber G: Behavior of transaldolase (ec 2.2.1.2) and transketolase (ec 2.2.1.1) activities in normal, neoplastic, differentiating, and regenerating liver. Cancer Res 36: 3189-3197, 1976.
10. Schwartz JP and McCandless DW: Glycolytic metabolism in cultured cells of the nervous system. IV. The effects of thiamine deficiency on thiamine levels, metabolites and thiamine-dependent enzymes on the c-6 glioma and c-1300 neuroblastoma cell lines. Mol Cell Biochem 13: 49-53, 1976.
11. Boros LG, Puigjaner J, Cascante M, *et al*: Oxythiamine and dehydroepiandrosterone inhibit the non-oxidative synthesis of ribose and tumor cell proliferation. Cancer Res 57: 4242-4248, 1997.
12. Iida A, Teshiba S and Mizobuchi K: Identification and characterization of the TKTB gene encoding a second transketolase in *Escherichia coli* k-12. J Bacteriol 175: 5375-5383, 1993.
13. Schaaff-Gerstenschläger I, Mannhaupt G, Vetter I, Zimmermann FK and Feldmann H: TKL2, a second transketolase gene of *saccharomyces cerevisiae*. Cloning, sequence and deletion analysis of the gene. Eur J Biochem 217: 487-492, 1993.
14. Coy JF, Dubel S, Kioschis P, *et al*: Molecular cloning of tissue-specific transcripts of a transketolase-related gene: Implications for the evolution of new vertebrate genes. Genomics 32: 309-316, 1996.
15. Warburg O, Posener K and Negelein E: Über den Stoffwechsel der Carcinomzelle. Biochem Z 152: 309-344, 1924.

16. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative pcr and the $2^{-\Delta\Delta ct}$ method. Methods 25: 402-408, 2001.
17. Nebeling LC, Miraldi F, Shurin SB and Lerner E: Effects of a ketogenic diet on tumor metabolism and nutritional status in pediatric oncology patients: Two case reports. J Am Coll Nutr 14: 202-208, 1995.
18. Otto C, Kaemmerer U, Illert B, *et al*: Growth of human gastric cancer cells in nude mice is delayed by a ketogenic diet supplemented with omega-3 fatty acids and medium-chain triglycerides. BMC Cancer 8: 122, 2008.
19. Seyfried TN, Sanderson TM, El-Abbadi MM, McGowan R and Mukherjee P: Role of glucose and ketone bodies in the metabolic control of experimental brain cancer. Br J Cancer 89: 1375-1382, 2003.
20. Zhou W, Mukherjee P, Kiebish MA, Markis WT, Mantis JG and Seyfried TN: The calorically restricted ketogenic diet, an effective alternative therapy for malignant brain cancer. Nutr Metab 4: 5, 2007.
21. Kallinowski F, Vaupel P, Runkel S, *et al*: Glucose uptake, lactate release, ketone body turnover, metabolic micromilieu, and pH distributions in human breast cancer xenografts in nude rats. Cancer Res 48: 7264-7272, 1988.
22. Richtsmeier WJ, Dauchy R and Sauer LA: In vivo nutrient uptake by head and neck cancers. Cancer Res 47: 5230-5233, 1987.
23. Sauer LA and Dauchy RT: Ketone body, glucose, lactic acid, and amino acid utilization by tumors in vivo in fasted rats. Cancer Res 43: 3497-3503, 1983.