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Metastasis is promoted by a bioenergetic switch: New targets for progressive renal cell cancer

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Targeted therapies have demonstrated clinical benefit with limited impact on long-term disease specific survival in the treatment of renal cell cancer (RCC). New opportunities for the treatment of tumors that are resistant or have relapsed, are needed. Increased anaerobic glucose fermentation to lactate (aerobic glycolysis), leading to oxygen- and mitochondria-independent ATP generation is a hallmark of aggressive cancer growth. This metabolic shift results in increased lactate production *via* cycling through the pentose phosphate pathway (PPP), and plays an important role in tumor immune escape, progression and resistance to immune-, radiation- and chemo-therapy. This study explored the activity and impact of the oxidative and nonoxidative branches of the PPP on RCC to evaluate new therapeutic options. Activity was determined in the oxidative branch by glucose-6-phosphate-dehydrogenase (G6PD) activity, and in the nonoxidative branch by the total transketolase activity and the specific expression of the transketolase-like-1 (TKTL1) protein. Transketolase and G6PD activity were intensely elevated in tumor tissues. Transketolase, but not G6PD activity, was more elevated in metastasizing tumors and TKTL1 protein was significantly overexpressed in progressing tumors ($p = 0.03$). Lethal tumors, where surrogate parameters such as grading and staging had failed to predict progression, showed intensive TKTL1 protein expression. RCC was found to have activated oxidative and nonoxidative glucose metabolism through the PPP, displaying a bioenergetic shift toward nonoxidative glucose fermentation in progressing tumors. The coexistence of cancer cells with differentially regulated energy supplies provides new insights in carcinogenesis and novel anticancer targets.

Key words: renal cell carcinoma; transketolase-like-1 enzyme; glucose-6-phosphate-dehydrogenase; Warburg effect; pentose phosphate pathway

Worldwide, an estimated 208,000 new cases and 102,000 deaths result from kidney cancer each year. Metastatic spread is present in 30% of patients at the initial diagnosis, and will develop in 20–40% of patients with initially localized disease who have nephrectomy with curative-intent.[1,2] In summary, about 50% of patients with kidney cancer will develop metastatic disease, which has a 5-year survival of less than 10%. Treatment options in metastasized renal cell cancer (RCC) are limited. Conventional radiation and chemotherapy are of no significant benefit, and immunotherapy provides only a moderate response rate with curative results in only 5% of patients.[3] New antiangiogenic therapies, such as inhibitors of the VEGF receptor tyrosine kinase, seem promising, since they extend progression free survival time in advanced renal tumors.[4,5]

Positron emission tomography (PET) studies have unequivocally identified increased glucose uptake as a hallmark of metastatic cancer, demonstrating altered glucose metabolism in progressing tumors and in therapy response.[6,7] Tumors are characterized by specific metabolic alterations providing a metabolic signature in malignant transformation for different stages; end stage carcinomas are most dependent on anaerobic glucose degradation (aerobic glycolysis, fermentation) and least dependent on mitochondrial energy supplies.[8] The metabolic endpoint of this transformation, the anaerobic degradation of glucose even in the presence of oxygen, was first described by Nobel laureate Otto Warburg.[9] Concomitant with this metabolic switch, high lactate concentrations occur and result in immune protection of cancer cells, acid-mediated matrix degradation, invasiveness and metastasis.[10–12] Furthermore, transformation to a more malignant phenotype is associated with resistance to chemo- and radiation-therapy.[13–15]

Increased total activity of the transketolase-dependent, nonoxidative branch of the pentose phosphate pathway (PPP) in cancer cells is due to the overexpression of the transketolase-like-1 (TKTL1) protein.[16] Because TKTL1 is the only transketolase that is overexpressed in cancer cells, and since specific inhibition of TKTL1 mRNA is sufficient to inhibit cancer cell proliferation, the TKTL1 protein is the main therapeutic target in the nonoxidative branch of the PPP.[16–19] Tumor progression and patient survival of various tumor entities correlate with TKTL1 expression, but not with transketolase or transketolase-like-2 protein expression.[17,18,20–22]

The complex regulation of tumor metabolism switches from mitochondrial oxidative to nonoxidative energy production, which is dependent on the PPP. Both the oxidative and nonoxidative branches of the PPP have been described as activated in carcinogenesis.[14] It is assumed that the enzymes of the oxidative branch of the PPP [glucose-6-phosphate-dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase] are triggered by an increased need for NADPH, whereas the enzymes of the nonoxidative branch (TKTL1, transaldolases) are triggered by an increased need for ribose and energy.[14,18]

The objective of the current study was to evaluate the activity of the oxidative (G6PD) and nonoxidative branches (transketolase) of the PPP, and to determine whether anaerobic energy production is associated with renal tumor progression.

Conflict of Interest statement: Dr. J.F. Coy declares a potential conflict of interest due to the possible utilization of TKTL1 for diagnostic and/or therapeutic purposes.

Abbreviations: G6PD, glucose-6-phosphate-dehydrogenase; HIF-1, hypoxia-inducing-factor; PET, positron emission tomography; PPP, pentose-phosphate-pathway; RCC, renal cell carcinoma; ROS, reactive oxygen species; TKTL1, transketolase-like-1 protein.

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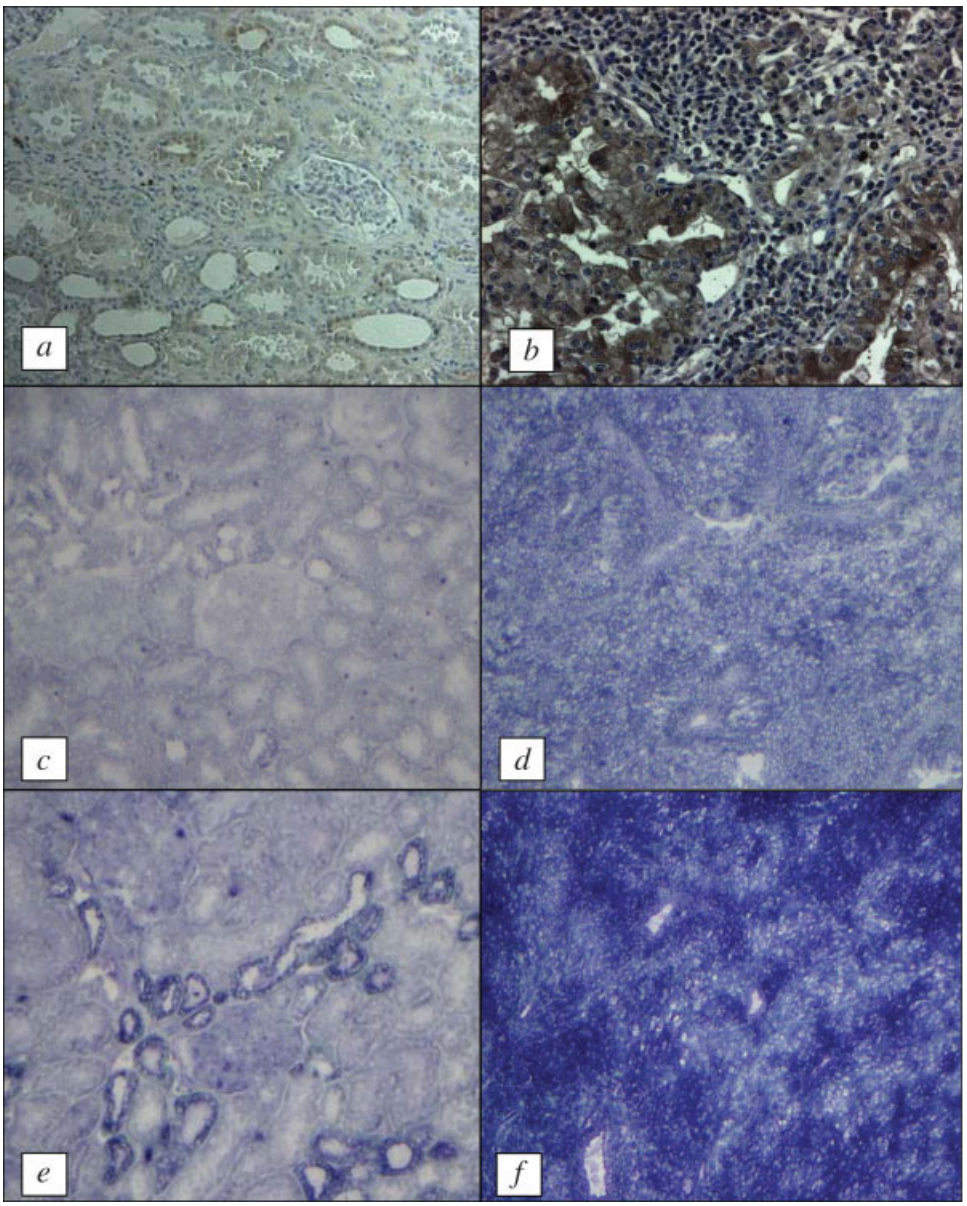

FIGURE 1 – In normal kidney tissue TKTL1 expression was detectable in epithelial cells of proximal tubules, marginal in cells of distal tubules and collecting ducts. No TKTL1 staining was detected in cells of the glomeruli (*a*). TKTL1 was intensively expressed in cells of kidney tumors (*b*). Normal kidney tissue presented TKT activity in epithelial cells of collecting ducts. Lower activity was detected in epithelial cells of lis of Henle and proximal and distal tubules. Activity was absent in glomeruli (*c*). The same tumor as on picture (*b*) showed intensive total TKT activity (*d*). Normal kidney tissue presented G6PD activity mainly in epithelial cells of distal tubules, whereas it was almost absent in cells of glomeruli (*e*). The same tumor as shown on figure (*b*) and (*d*) demonstrated extensive G6PD activity in tumor cells (*f*). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Material and methods

Patient samples

Patients with primary RCC, who received operative treatment, were included. Specimens were obtained from the Department of Pathology and Urology, University Hospital Mannheim, Faculty of Clinical Medicine of Ruprecht-Karls-University Heidelberg, Germany (approval by the Local Ethics Committee). None of the patients had received neoadjuvant treatment before nephrectomy.

Immunohistochemical staining of TKTL1

Immunohistochemical staining was described in detail previously.[18] Briefly, 3-μm thick paraffin sections were dewaxed and heated to unmask antigens. After rinsing in dH_2O, endogenous peroxidase was inhibited by 5 min incubation with 3% H_2O_2. Endogenous avidin-biotin (Vector Laboratories, Burlingame, CA) was blocked using a commercial biotin blocking system (DAKO, Glostrup, Denmark) for 10 min. After 2 washes in Tris/saline buffer (TBS), slides were incubated with 1% goat serum for 30 min. Sections were subsequently exposed to mouse anti-TKTL1 antibody (Linaris, Wertheim, Germany) overnight at 4°C. Slides were washed and incubated with biotinylated anti-mouse immunoglobulins for 30 min at room temperature and treated with streptavidin-peroxidase (DAKO). Staining was performed with 3-amino-9-ethylcarbazole (AEC) substrate and counter-staining with hematoxylin.

Alternatively, primary antibodies were visualized with avidin-biotinylated horseradish peroxidase complex (ABC) and diamino-

benzidine tetrahydrochloride (DAB) (Vector), and counter-stained with Mayer's hematoxylin.

TKTL1 expression was scored as follows: a score of 0 indicates 0–20% staining; a score of 1, 21–50%; a score of 2, 51–80%; and a score of 3 indicates >80% staining of the tumor cells. To exclude minimal staining as false positives, we assumed a score of 1 or 0 as negative. Analyses were independently performed by 2 specialists (A.Z.H. and S.L.).

Demonstration of G6PD activity

Cryostat sections of kidney tumors and their normal counterparts were allowed to dry at room temperature for 5 min and were then incubated, according to the procedure described by Van Noorden and Frederiks,[23] for the detection of G6PD activity. The method is based on the tetrazolium salt procedure in the presence of polyvinyl alcohol and 10 mM glucose-6-phosphate (Boehringer, Mannheim, Germany). Media were freshly prepared just before incubation, and nitro-BT was added after dissolution in a heated mixture of dimethylformamide and ethanol (final dilution of each solvent in the medium was 2% v/v). For the demonstration of G6PD activity, sections were incubated with the assay mixture for 10 min at 37°C. Sections were rinsed afterward with warm phosphate buffer (0.1 M, pH 5.3, 65°C) and embedded in glycerin-gelatin. Control reactions were performed in the absence of substrate and $NADP^+$. Specific activity was scored on a scale from 0 to 3 (0 = no activity, 1 = mild activity, 2 = moderate activity and 3 = strong activity).

Demonstration of transketolase activity

Transketolase activity was localized for light microscopy purposes using a modification of the dehydrogenase method with a tetrazolium salt and PVA.[24] The reaction was based on the transfer of the ketol moiety from xylulose-5-phosphate to ribose-5-phosphate, obtaining sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate. Monitoring transketolase activity was achieved by coupling the reaction to glyceraldehyde-3-phosphate dehydrogenase with the formation of NADH, which is the first product in the chain of electron transfers that leads to formazan formation precipitated at the exact place where the reaction has taken place. Incubation media were prepared using 50 mM Tris-HCl buffer, pH 7.6, containing 18% (w/v) PVA, 5 mM sodium azide, 7.5 mM NAD, 3.7 mM KH_2PO_4, 5 mM $MgCl_2$, 0.32 mM 1-methoxyphenazine methosulphate, 100 μl substrate mixture (see below) per ml of incubation medium and 5 mM nitro-BT (test reaction). The substrate mixture was prepared by dissolving 50 mM ribose-5-phosphate in 50 mM Tris-HCl, pH 7.6, and adding 0.05 IU ribulose-5-phosphate epimerase and 0.25 IU phosphoriboisomerase.

Control reactions were performed using incubation media lacking the substrate mixture, but in the presence of 10 mM ADP. Incubations were performed for 30 min at 37°C. To stop the reaction, sections were rinsed with phosphate buffer, pH 5.0, at 65°C and then mounted in glycerin-gelatin. Specific activity was scored on a scale from 0 to 3 (0 = no activity, 1 = mild activity, 2 = moderate activity and 3 = strong activity).

Statistical analysis

Immunohistochemistry and clinical data. Progression-free survival and disease-specific survival from time of surgery were defined as endpoints for this analysis. The distribution of event times was calculated separately for each of the prognostic factors with the univariate product-limit method of Kaplan and Meier.

Variables including pathological kidney tumor subtypes, tumor grading and tumor size were analyzed and correlated with TKTL1 expression. Case censoring was applied in the analysis of progression-free and disease-specific survival, when the patient had no signs of recurrence or when death related to tumor occurred during the observation period, respectively. Categorical values such as tumor size and tumor grade were assigned as score values, whereas continuous variables were entered into the model as actual values.

TABLE I – PATIENT CHARACTERISTICS

	No. of patients	% Of patients
Total	55	100
Men	39	71
Women	16	29
Grade		
1	5	9
2	39	71
3	11	20
pT category		
T1	23	42
T2	10	18
T3	20	36
T4	2	4
pN category		
pN0	48	87
pN+	7	13
cM category		
M0	51	93
M+	4	7

All reported p values were based on two-sided tests, and the threshold for significance was 0.05. Statistical analyses were performed with the SPSS software package (version 10.0; SPSS, Chicago, IL).

Results

Immunohistochemical detection of TKTL1 protein expression

In normal renal tissue, TKTL1 expression was moderately detectable in the epithelial cells of the proximal tubules and to a lesser extent in cells of the distal tubules and collecting ducts (Fig. 1*a*). Glomerular cells did not express TKTL1 in either normal or tumor tissue. Fatty and connective tissues did not express TKTL1, whereas specific cells of the immune system regularly expressed TKTL1. Subgroups of RCC showed intense staining (Fig. 1*b*). Expression of TKTL1 protein was predominantly found in the cytoplasm, and only occasionally in the nucleus. A heterogeneous staining pattern, with intensive staining alternating with areas devoid of protein expression, was detectable within the tumors.

TKTL1 protein expression and its clinicopathological associations

Patient characteristics are listed in detail in Table I. Fifty-five patients with primary RCC were included. The TNM staging system from 2002 (Union Against Cancer) was used to classify the tumors. Mean patient age was 63 years (range 37–86 years) and the mean follow-up period was 34 months (range 3–60 months). Significant differences for progression-free survival were detected depending on tumor staging ($p = 0.01$), lymph node metastases ($p < 0.001$), distant metastases ($p < 0.001$), and invasion of blood ($p < 0.001$) and lymphatic vessels ($p < 0.001$). Significant correlations between patient survival and staging ($p = 0.01$), lymph node metastases ($p < 0.001$) and distant metastases ($p < 0.001$) were found. TKTL1 protein expression pattern is summarized in Table II. Patients expressing high levels (score 3) of TKTL1 had a significantly shorter progression-free survival ($p = 0.03$) (Fig. 2). TKTL1 expression and tumor grade were strongly correlated to disease specific survival, but failed, marginally, to be statistically significant. Tumors exhibiting TKTL1 staining with a score of 3 were significantly larger in size than those with a score of 1 or 2 (Fig. 3).

TKTL1 expression was detected significantly more often in poorly differentiated tumor cells than in well or moderately differentiated tumor cells ($p = 0.03$). One pT1 tumor (6 cm), 2 pT2 tumors (7.5 and 8 cm) and 3 moderately differentiated (G2) tumors demonstrated intense TKTL1 staining. All of these tumors progressed during the follow-up period.

TABLE II – CORRELATION BETWEEN TKTL1 EXPRESSION AND PATHOLOGICAL/CLINICAL CHARACTERISTICS OF PATIENTS WITH RCC INCLUDED IN THE STUDY

Patients	TKTL1 (% (*n*))				Frequency (*n*)
	0	1	2	3	
T stage					
1	17 (4)	22 (5)	13 (3)	48 (11)	23
2	10 (1)	10 (1)	30 (3)	50 (5)	10
3	–	10 (2)	20 (4)	70 (14)	20
4	–	–	–	2	2
Tumor grading					
1	20 (1)	40 (2)	20 (1)	20 (1)	5
2	10 (4)	15 (6)	21 (8)	54 (21)	39
3	–	–	9 (1)	91 (10)	11
Nodal stage					
0	10 (5)	17 (8)	21 (10)	52 (25)	48
1	–	–	–	100 (5)	5
2	–	–	–	100 (2)	2
Distant metastases at the time of operation					
0	10 (5)	15 (8)	20 (10)	55 (28)	51
1	–	–	–	100 (4)	4
No evidence of disease during follow-up	14 (5)	16 (6)	22 (8)	47 (18)	37
Evidence of disease during follow-up		11 (2)	11 (2)	78 (14)	18

TKTL1 scoring 0: 0–20%, 1: 21–50%, 2: 51–80% and 3: > 81% of all tumor cells were TKTL1 positive.

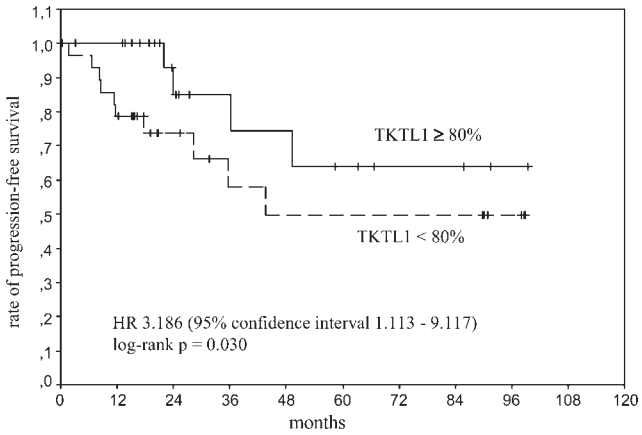

FIGURE 2 – Kaplan–Meier plot demonstrating the significant correlation between TKTL1 staining intensity and disease progression in RCC (staining intensity more than 80% = score 3 *versus* staining intensity less than 80% (score 0, 1 and 2) of tumor cells. $p = 0.03$.

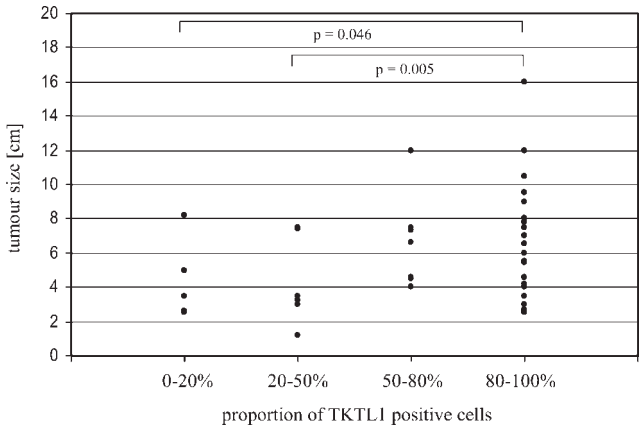

FIGURE 3 – Significant association of tumor size and immunohistochemical TKTL1 protein expression (Wilcoxon–Mann–Whitney *U*-Test). Tumors demonstrating TKTL1 expression score 3 are significantly bigger in size than tumors with score 0–1 or 2.

Transketolase and G6PD activity

Because the total transketolase activity in tumor cells consists of more than 70% TKTL1 activity, and selective determination of TKTL1 activity with histochemical methods is not possible, we determined total transketolase activity in 8 different tumors (details are listed in Table III). In normal kidney tissue, moderate transketolase activity was found in epithelial cells of the collecting ducts. Lesser activity was detected in epithelial cells of the loops of Henle and in the distal and proximal tubules. Activity was almost absent in glomeruli and connective tissue (Fig. 1*c*). Tumor tissues had higher transketolase activity (Fig. 1*d*). Heterogeneous transketolase activity patterns were detected within the tumor areas.

G6PD activity was higher in kidney tumor cells than in their normal cell counterparts (Figs. 1*e* and 1*f*). G6PD activity was localized similar to that of transketolase within the tumor areas. Table III summarizes the TKTL1 protein expression scores, and the transketolase and G6PD activities. Total transketolase activity tended to be higher in progressing than in nonprogressing tumors, whereas variable G6PD activity was detected in the tumor cells without correlation to tumor progression or tumor stage. All patients with metastasized kidney tumors had tumor tissues with intense TKTL1 protein expression, whereas nonprogressing tumors lacked strong TKTL1 expression.

Discussion

Carcinogenesis leads to enhanced aerobic glycolysis and upregulation of the PPP. The balance between the oxidative and nonoxidative branches of the PPP is critical for cancer cell survival.[14] The first regulatory enzyme of the oxidative branch, G6PD, produces ribose and NADPH and is strongly upregulated in cancer cells.[25,26] There is increasing evidence that G6PD activity is of major importance for the production of NADPH needed for biosynthesis and for the defense against oxidative stress, rather than for ribose production.[27] The results of the present study show a high increase in G6PD activity in RCC. No correlation with stage or progression was found demonstrating a constant, important role for G6PD activity in maintaining NADPH levels during carcinogenesis. In contrast, TKTL1 protein overexpression correlated significantly with transformation to a more malignant phenotype, advanced tumor stages and progression. During malignant transformation of cancer cells, a metabolic switch from mitochondria-based, oxygen-dependent ATP production (oxidative phosphorylation) to anaerobic glucose degradation (aerobic glycolysis,

TABLE III – TKTL1 PROTEIN EXPRESSION, OVERALL TRANSKETOLASE AND G6PD ACTIVITY WAS DETERMINED IN 8 TUMORS (6 CLEAR CELL CARCINOMAS AND 2 PAPILLARY RENAL CARCINOMAS)

No	Pathology	pT	G	N	M	Tumor size (cm)	Progression	TKTL1	G6PD activity	TKT activity
1	Clear cell carcinoma	1b	2	0	0	5	NO	0	3	2
6	Clear cell carcinoma	1a	2	0	0	2.7	NO	2	1	2
3	Clear cell carcinoma	3a	3	1	1	9	Yes	3	3	3
4	Clear cell carcinoma	3b	3	1	0	8	Yes	3	3	3
7	Clear cell carcinoma	3a	2	2	0	7	Yes	3	1	3
8	Clear cell carcinoma	3a	3	0	0	7	Yes	3	3	3
5	Papillary type	1a	2	0	0	3.2	NO	1	1	3
2	Papillary type	1a	2	0	0	3.5	NO	0	3	2

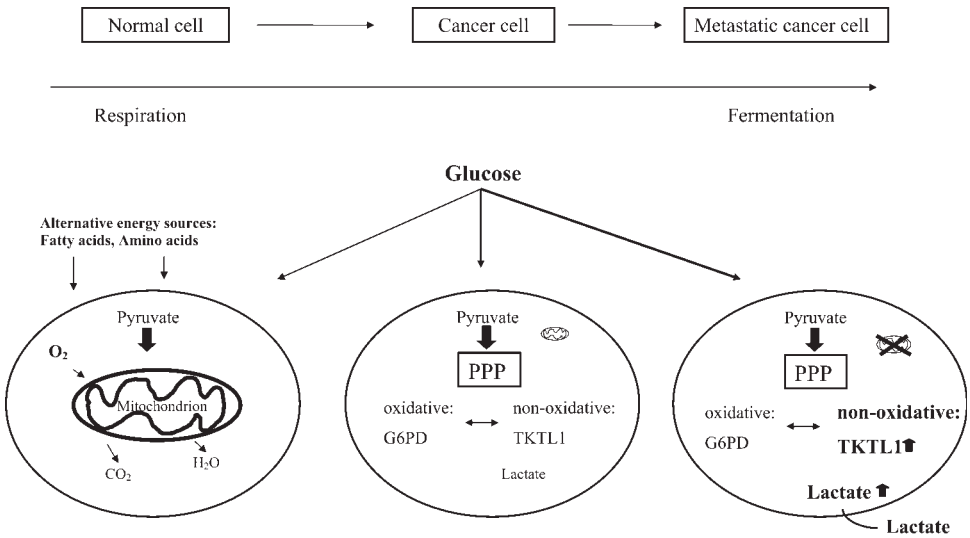

FIGURE 4 – Malignant transformation of cancer cells: a metabolic switch from mitochondria-based, oxygen-dependent ATP production to anaerobic glucose degradation (fermentation) leading to oxygen- and mitochondria-independent ATP generation takes place. An increased activity of the pentose phosphate pathway (PPP) and a shift toward anaerobic glucose degradation in metastasized kidney tumors mediated by overexpression of the TKTL1 protein was demonstrated. The oxidative and the nonoxidative branches of the PPP are differentially regulated, reflecting the coexistence of various bio-energetically controlled cancer cells within a tumor.

fermentation) leading to oxygen- and mitochondria-independent ATP generation takes place, even in the presence of oxygen.[8] Our results, demonstrating upregulation of G6PD and transketolase activity, support a finding of increased PPP activity and a shift toward anaerobic glucose degradation in metastasized kidney tumors mediated by overexpression of the TKTL1 protein (Fig. 4). We conclude that the oxidative and the nonoxidative branches of the PPP are differentially regulated, reflecting the coexistence of various bio-energetically controlled cancer cells within a tumor. These findings are important, since new antiangiogenic therapies, such as inhibitors of the VEGF receptor tyrosine kinase, are assumed to primarily target tumor cells with oxygen-dependent glucose usage. Iatrogenic selection might lead to a competitive advantage and a shift toward tumor cells using anaerobic glucose metabolism. Selective forces, such as inhibition of vascularization and oxygenation within the tumor microenvironment, would accelerate the switch to oxygen-independent and TKTL1 mediated energy production. Indeed, no long term complete remission has been described using antiangiogenic therapies in RCC; on the other hand, therapy-resistant tumors are highly positive in PET-scans, indicating high levels of anaerobic glucose metabolism.[28] Targeting both energy sources at the same time in progressive tumor cells appears promising for future therapies.

Activation of hypoxia-inducing-factor (HIF) 1, a key transcription factor that upregulates genes involved in glycolytic energy metabolism,[29] is a common feature of RCC and has been linked to malignant transformation, metastasis and treatment resistance. In the absence of a functional von Hippel-Lindau (VHL) tumor suppressor protein (70% of sporadic RCCs) irrespective of oxygen concentration, HIF-1α is not degraded and translocates to the nucleus where it dimerizes with HIF-1β to form transcriptionally active HIF. HIF-1α is increased by hypoxia, insulin, insulin-like growth factor, epidermal growth factor and angiotensin II. Lu *et al*. demonstrated the important role of glucose in the regulation of HIF-1 α in cancer cells: no increase in HIF-1 α levels occurs in glucose-free medium, but significant increases of HIF-1 α occur in human glioma cells after the addition of glucose.[30] Furthermore, it was demonstrated that the glycolytic end products lactate and pyruvate also stimulate HIF-1 α accumulation.[30,31] The glycolysis-activated accumulation of HIF-1 α protein once again stresses the crucial role of aerobic glycolysis in carcinogenesis and has been demonstrated to be a potent target in anticancer therapy.[32]

Upregulation of glucose turn-over *via* the PPP leads to enhanced TKTL1,[17,18] HIF-1α,[30] G6PD,[14,33] pAkt,[34] carbonic anhydrase enzyme activity,[35] *de novo* fatty acid synthesis,[36] lactate-dehydrogenase[37] and lactate concentrations.[38,39] All of these are biological markers that correlate with poor prognosis.

Increased lactic-acid production and excretion by fermenting tumor cells results in suppression of cytokine production, T cell inactivation, acid-mediated matrix degradation and apoptosis of surrounding healthy cells, leading to invasion and metastasis.[10–12] Thus, high lactate production results in an exceptional growth advantage for the tumor cells. The correlation between mitochondrial dysfunction and increased aerobic glycolysis in carcinogenesis has been investigated, but the competitive advantage for tumor cells is still under discussion.[8,40–42] Mitochondrial energy production is correlated with release of reactive oxygen species (ROS) that damage proteins and macromolecules such as DNA. During

proliferation, DNA is exposed to ROS that leads to severe DNA damage and mutations. Fermentative cancer cells do not produce mitochondrial ROS, thus preventing ROS-induced DNA alterations. Furthermore, as demonstrated in the present study, tumors increase G6PD activity resulting in over-production of the reducing equivalent NADPH, which protect the cell against ROS damage. Protecting and stabilizing DNA represents a strong positive selective survival advantage, and may make these cells more resistant to radiation and chemotherapeutic damage.[14,15,43,44] Inhibition of fermentation in tumor cells using antiglycolytic approaches, such as small molecules or substrate withdrawal (carbohydrate reduced/ketogenic diet), could support immune therapy and resensitize chemo- and radiation-resistant cells.[14,43–45] Substrate withdrawal seems a promising approach in RCC, since the majority of RCC tumors do have activated cytoplasmic Akt and therefore are unable to perform β-oxidation of fatty acids.[18,34,46] These tumor cells are absolutely glucose dependent, and glucose withdrawal leads to cell death.[34,47] Case reports of tumor control in patients with metastasizing tumors using a carbohydrate reduced/ketogenic diet have been published since 1995.[45,48] Taking into account the low response rate and short duration of immune and antiangiogenic therapies, a concomitant targeting of the metabolism of resistant, fermenting tumor cells seems promising.

Anaerobic glucose metabolism is believed to have a poorer energy output in relation to the energy stored in the glucose molecule. Therefore, the elevated demand for glucose is compensated by the upregulation of glucose transporters and the onset of aerobic glycolysis in a PI-3K-dependent manner, resulting in high lactate concentrations.[38,49,50] The switch to anaerobic energy production by the TKTL1-dependent, nonoxidative branch of the PPP supports the enormous demand for (ROS-free) energy and anabolic substrates, such as ribose, NADPH and acetyl-CoA. The modified, TKTL1-dependent PPP seems a general biochemical program suitable for safe and enhanced energy release, and the anabolic substrate production necessary for rapid cell proliferation.

In conclusion, renal tumors are present with an altered glucose metabolism. Enhanced glycolysis leads to increased activity of the enzymes in the oxidative (G6PD) and nonoxidative branches (TKTL1) of the PPP. Progressing tumors are characterized by specific upregulation of the nonoxidative branch of the PPP, ensuring ribose and energy, and supporting acidification of the tumor microenvironment. Acidification is a major step in invasive tumor growth, metastasis and immune escape. Additionally, upregulation of the anaerobic energy supply without ROS production, and G6PD activity for increased reducing equivalents, protects cancer cells from oxidative stress. Targeting the coexistence of aerobic and anaerobic tumor cells and the vulnerable PPP balance with tumor-specific antiangiogenic agents, and TKTL1 and G6PD inhibitors simultaneously seems promising as a novel anticancer strategy in RCC.

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