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Hypoxia Induces the Expression of Transketolase-Like 1 in Human Colorectal Cancer

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Key Words

Colorectal cancer · Aerobic glycolysis · Hypoxia · Pentose phosphate way

Abstract

Background and Aims: Transketolase-like (TKTL) 1 is one of the key enzymes for anaerobic sugar degradation even in the presence of oxygen (aerobic glycolysis). Transketolase-dependent reactions supply malignant tumors with ribose and NADPH. Therefore, TKTL1 activity could be crucial for tumor proliferation and survival. The aim of the study was to evaluate the expression of TKTL1 in colorectal cancer (CRC) and its regulation under hypoxic conditions. **Methods:** We studied TKTL1 mRNA and protein expression in CRC cell lines and human CRC biopsies by quantitative real-time PCR, Western blotting and immunohistochemistry. Regulation of TKTL1 under oxygen depletion was analyzed by cultivating cells either in a three-dimensional spheroid model or in a hypoxia incubator chamber. **Results:** TKTL1 mRNA was heterogeneously expressed in monolayers of cells with high levels in HT-29 and SW480. TKTL1 protein was also clearly detectable in HT-29 and SW480. Hypoxia-inducible factor (HIF)-1 α pro-

tein expression correlated with TKTL1 protein expression in SW480 spheroids over time. On the one hand, induction of hypoxia in T84 spheroids did not induce TKTL1; on the other hand, hypoxia by incubation at 1% O₂ in a hypoxia incubator chamber clearly showed an upregulation of TKTL1. In 50% of CRC patients, TKTL1 protein expression was upregulated in tumor compared to non-tumor tissue. The immunohistochemical staining of TKTL1 in CRC patient samples resulted in 14 positive and 30 negative samples. **Conclusions:** TKTL1 expression correlated with HIF-1 α protein expression and was induced upon hypoxic conditions which could facilitate energy supply to tumors under these circumstances.

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Introduction

Enhanced glucose consumption in cancer may be due to an extended demand of ribose for nucleic acid biosynthesis and subsequently proliferation of tumor cells. A

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special feature of colorectal cancer (CRC) cells, when compared to surrounding 'normal' cells, is an 8.5- to 15-fold enhanced glucose turnover rate [1].

The pentose phosphate pathway (PPP) enables oxygen-independent glucose degradation by transketolases and generates, among other things, riboses for nucleic acid biosynthesis. In cancer cells, transketolase-like (TKTL) 1 protein is observed to be specifically upregulated in comparison to TKT or TKTL2 [2–4].

In 1924, Otto Warburg described that cancer cells metabolize glucose to lactate even in the presence of oxygen (known as Warburg effect) [5]. Lactate promotes the expression of hypoxia-inducible factor 1 (HIF-1) and the acidification of the adjacent healthy cells [6]. The transcription factor HIF-1 represents a key regulator for the elevated fermentation of glucose [7] and is increased during colorectal carcinogenesis [8, 9]. The HIF-1 α subunit is tightly regulated at the translational level as well as its posttranslational stability. The latter is controlled by the oxygen level in the cell. Interestingly, TKTL1 is able to stabilize HIF-1 α , indicating a tight regulation and interaction [10]. Under conditions of limited oxygen supply that are frequently found in tumors, the malignant cells may switch to the nonoxidative part of the PPP. This results in a higher supply of ribose, independence of mitochondrial energy production as well as indirect generation of acetyl-CoA for fatty acid and cholesterol production [11]. Furthermore, tumor cells are protected against reactive oxygen species via NADPH production and tissue acidosis through the production of lactic acid. Whereas lactic acid normally mediates cell death via p53, tumor cells are often protected by p53 mutations [4]. Moreover, the NADPH production through the PPP seems to have a protective effect against oxidative or radical stress [12]. In breast cancer specimens, the expression of Akt [13], glucose transporters (GLUT) [14] and TKTL1 [13] was elevated compared to normal tissue. This indicates that the enhanced glucose turnover is fueled by an increased glucose uptake through GLUT and conversion by TKTL1.

Additionally, TKTL1 has been qualified as a proto-oncogene associated with demethylation of DNA [15]. This is in accordance with a significant overexpression of TKTL1 in several different tumors like nasopharyngeal [16], pulmonary [17], thyroid papillary [18, 19], gastric [20], ovarian [21, 22], cervical [23] and metastasizing renal cell carcinoma [11]. In general, increased TKTL1 expression correlates with poor patient outcome and tumor progression [20, 23–25]. TKTL1 is an independent prognostic factor for reduced survival of patients with laryn-

geal squamous cell [11, 26], urothelial carcinoma [11], colon carcinoma (CC) [27], and nonsmall lung cancer. In thyroid papillary carcinoma, there is a correlation between TKTL1 and the presence of lymph node metastases [4]. In rectum carcinoma, there is a correlation between TKTL1 expression and the presence of metastases and recurrence [24]. Inhibitors of total transketolase activity or gene expression suppressed tumor growth [16, 28–31], whereas activation led to enhanced tumor growth [28]. In addition, dietary studies indicate that the inhibition of the PPP suppresses tumor growth [32].

As CRC cells consume a high amount of glucose and TKTL1 plays an important role in its metabolism via the PPP in several malignant cells [11, 20], we assumed a potential role of TKTL in CRC physiology. However, until now only limited data on TKTL1 expression in CRC lines and CRC in humans [33, 34] are available [35].

Therefore, we investigated the expression of TKTL1 in 11 different CRC cell lines, spheroids and hypoxia chambers as well as patient specimens to elucidate the role of TKTL1 and its regulation under hypoxic conditions in CRC.

Methods

Cell Culture Conditions

Low-passage Caco-2, HT-29, WiDr, LoVo, LS 174T and T84 were maintained as monolayer in DMEM low or high glucose at 37°C and 10% CO₂. SW403, SW480, SW620, SW837 and SW948 were maintained as monolayer in RPMI 1640 at 37°C and 5% CO₂. All media were supplemented with 10% fetal calf serum and 50 U of penicillin and 50 μ g of streptomycin (all from Invitrogen, Germany).

Hanging Drop Method or Hypoxia Incubator Chamber

Cells were cultivated in the hanging drop method (spheroids) as described previously [36]. Five thousand SW480 or T84 cells per well were cultivated in 60-well Terasaki plates (Greiner Bio-One, Switzerland) for 1–5 days in 23 μ l of the corresponding media. Medium was not changed over the 5 days of cultivation.

Cells were cultivated on transwells for 2 weeks at 21% O₂ to allow the cells to form a tight monolayer. After 2 weeks, the medium was removed and either medium with or without glucose was applied. Cells were transferred to a hypoxia incubator chamber (In-Vivo400, UK) and cultivated at 37°C, 5% CO₂ and 1% O₂. Control samples were kept under normal conditions. After 4 h, 1 and 3 days, cells were harvested and RNA and protein were collected.

Patient Material – Protein Analysis and Immunohistochemistry

Twenty surgically resected specimens (10 tumor and matching non-tumor) were obtained from the Institute of Surgical Pathology of the University Hospital Zurich. The study was approved by the local ethical committee (Ethical Committee for Surgery, Anes-

Table 1. Patient characteristics

ID	Gender	Age, years	G
<i>Patient samples used for Western blotting</i>			
G05.89	female	40	2
G05.167	male	60	2
G05.220	male	76	2
G04.258	female	77	2
G05.274	female	43	2
G05.286	female	60	2
G04.541	male	88	2
G04.623	female	84	2
Neuroendocrine carcinoma			
G08.148	female	75	
Extra-abdominal fibromatosis (desmoid tumor)			
G05.586	male	62	
<i>Patient samples used for immunohistochemical staining</i>			
1	male	65	2
2	male	74	2
3	male	58	2
4	female	80	2
5	male	76	2
6	female	56	2
7	male	82	3
8	female	88	3
9	female	64	2
10	male	69	3
11	female	78	2
12	male	71	3
13	male	68	2
14	female	90	3
15	male	67	2
16	female	68	2
17	male	65	2
18	male	77	2
19	female	60	2
20	female	65	2
21	female	86	3
22	female	50	2
23	male	70	2
24	female	86	2
25	male	56	2
26	male	85	2
27	male	76	2
28	male	93	2
29	female	87	2
30	male	77	3
31	male	69	2
32	female	56	2
33	female	92	2
34	female	67	3
35	female	53	1
36	male	53	3
37	male	75	2
38	female	69	3
39	female	52	2
40	female	80	3
41	male	77	2
42	male	76	1
42	male	73	2
44	male	60	2

Classification by the tumor grading system (G) in patients suffering from CRC.

thesiology and Pathology at the University Hospital Zurich). Each participant gave written consent.

Forty-four patient samples were stained for TKTL1 by immunohistochemistry. Paraffin-fixed sections were rehydrated and heated for antigen unmasking in 10 mmol/l of sodium citrate buffer (pH 6.0, Sigma-Aldrich, USA). Peroxidases were inhibited by incubating the sections for 10 min in 3% hydrogen peroxide in methanol. Slides were blocked with 1% goat serum (Sigma-Aldrich, USA) in 1× PBS for 15 min and incubated with the monoclonal mouse anti-TKTL1 antibody (RIDA® PentoCheck® IHC, clone 1210-4, R-Biopharm AG, Germany) and a peroxidase-conjugated secondary anti-mouse antibody (Santa Cruz Biotechnologies, USA). Staining was developed by adding 3,3-diaminobenzidine (DAKO, Denmark) with subsequent counterstaining using hematoxylin. Afterwards, sections were dehydrated by washing in xylene and graded ethanol and embedded in Pertex (Calbiochem, Norway). The staining intensity was classified as weak, moderate, or strong. The percentage of positively stained tumor cells was assessed semiquantitatively. All negative cases were confirmed by a second staining. Staining was scored by the immunoreactive score (IRS or Remmele score). The IRS considers the percentage of positive tumor cells and the staining intensity. Staining intensity ≥ 2 and $>10\%$ positive stained cells resulted in an IRS of ≥ 4 . Only samples with an IRS ≥ 4 were evaluated as positive staining result. For clinical correlation, file records of the patients were investigated (table 1). Cancer stage was evaluated by the tumor node metastases (TNM) staging system (6th edition).

RNA Extraction and Quantitative Real-Time PCR

Total RNA was extracted using the RNeasy Mini Kit and the automated sample preparation system Qiacube according to manufacturer's instructions (Qiagen, Switzerland). cDNA was synthesized with High-Capacity cDNA Reverse Transcription Kit according to the manufacturer's instructions (Applied Biosystems, USA). qRT-PCR was performed under the following cycling conditions: 20 s at 95°C, then 45 cycles at 95°C for 1 s and 60°C for 20 s with the TaqMan Fast Universal Mastermix and the following primers: hTKTL1 sense 5'-CGCCGAGCACTGCATAAAC-3', antisense 5'-CCACATAAGTGTTCACCCAAA-3' and fluorescent probe 6FAM-TCTATCAGAGGCGCTGCGAAGC-MGB with nonfluorescent quencher in the 7500 Fast Thermal Cycler. Glut1, HkII and ACTB mRNA levels were determined using gene expression assays (HKII: Hs00606086_m1; Glut1: Hs00892681_m1; ACTB: 4310881E) (all from Applied Biosystems, USA). Each sample was analyzed in triplicate, and the ΔCt method was applied.

Protein Extraction and Western Blotting

Cells or tissue were resuspended in M-PER lysis buffer (Thermo Fisher Scientific Inc., USA), sonicated, centrifuged for 5 min at 13,000 g and the supernatant was further processed. Protein was separated on 4–12% Bis-Tris gradient gels with MOPS SDS running buffer and transferred onto a nitrocellulose membrane (Invitrogen, USA). After blocking with 5% milk powder, 3% BSA and 0.1% Tween 20 (Roth, Germany) in 1× PBS, proteins were labeled with primary antibodies (TKTL1: 1:100 dilution, clone 1210-4, R-Biopharm, Germany; HIF-1 α : 1:1,000, Novus Biologicals, USA) and peroxidase-conjugated secondary goat anti-mouse or goat anti-rabbit antibody (1:3,000, Santa Cruz, USA). Protein bands were visualized using a commercial chemiluminescence detection kit (ECL Plus; Amersham Biosciences, USA) according to the manu-

facturer's protocol and exposed on a film. Equal loading of the samples was demonstrated by reprobing with anti- β -actin (Ms X Actin, Chemicon, Switzerland).

Immunocytochemical Staining

Cells were grown in transwell plates and fixed on day 3 in 4% PFA in 1 \times PBS. Cells were permeabilized with 0.3% Triton X 100 in 1 \times PBS, blocked with 1% BSA in 1 \times PBS in a humidified chamber and incubated with the primary anti-human TKTL1 antibody (1:100) at 37°C for 1 h, Cy3-labeled anti-mouse antibody as a secondary antibody (1:500, Jackson ImmunoResearch, USA) and mounted using the Hard Set Mounting Medium (Vector Laboratories, USA).

Data Analysis and Statistics

The level of protein expression in patient samples was quantified by the OptiQuant analysis software.

Statistical analysis was carried out using Mann-Whitney test with SPSS. Data are shown as mean value \pm standard error. Statistical significance was based on a p value <0.05 .

Results

TKTL1 mRNA and Protein Expression in vitro

TKTL1 mRNA expression was analyzed in 11 cell lines by qRT-PCR. All cell lines expressed TKTL1 mRNA except WiDr and SW620 (fig. 1a). The highest TKTL1 mRNA expression was observed in HT-29 cells. High mRNA expression of TKTL1 was also detected in Caco-2, T84, SW480 and SW837 cell lines. All other investigated cell lines, namely LoVo, LS 174T, SW403 and SW948, showed lower mRNA levels of TKTL1.

Western blotting was performed in order to demonstrate the presence of TKTL1 protein. HT-29 and SW480 cells showed high expression of TKTL1 protein (fig. 1b) corresponding to the qRT-PCR data (fig. 1a). In all other 9 cell lines, TKTL1 protein was barely detectable.

For TKTL1 localization within the cells, we performed immunocytochemistry in HT-29, SW480 and T84 cells (fig. 2a–f). All three cell lines expressed TKTL1 mainly in the cytoplasm (fig. 2a–f). To account for a difference in expression level by prolonged cultivation time, SW480 cells were grown over 10 days. No difference in expression pattern over time was detected (data not shown).

TKTL1 and HIF-1 α Protein Expression in Spheroids

In silico analysis (MatInspector) verified the presence of a transcription factor binding site for HIF-1 in the TKTL1 promoter (data not shown). To mimic oxygen-limiting conditions in tumor cell aggregates as found in vivo, the three-dimensional spheroid model was applied. Upon forming large cellular aggregates with low oxygen

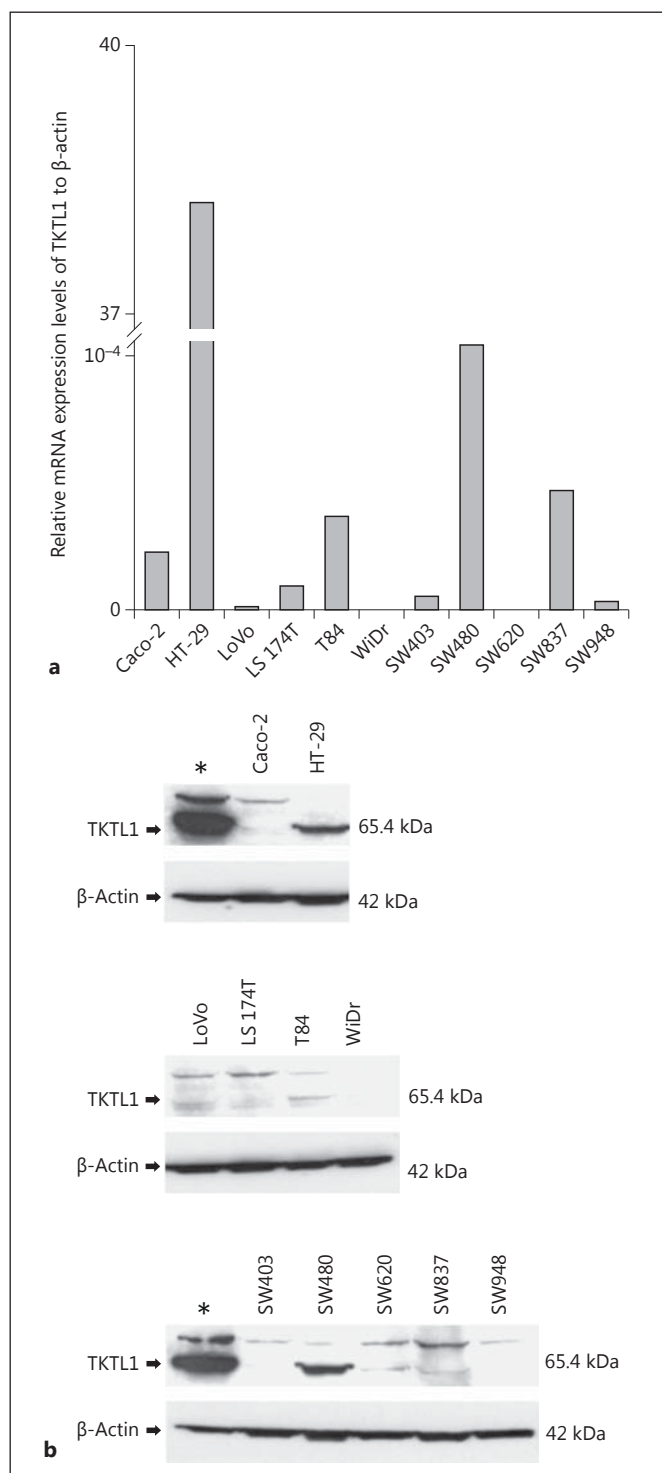


Fig. 1. TKTL1 expression in cell lines. **a** mRNA expression of TKTL1 relative to β -actin in 11 cell lines. High TKTL1 mRNA expression was found in monolayers of HT-29 compared to Caco-2, T84, SW480, SW837, LoVo, LS 174T, and SW948. No mRNA expression was found in SW620 and WiDr. **b** TKTL1 protein was detectable in HT-29 and SW480. Positive control: TKTL1 stable transfected HEK293T cells. * $p < 0.05$.

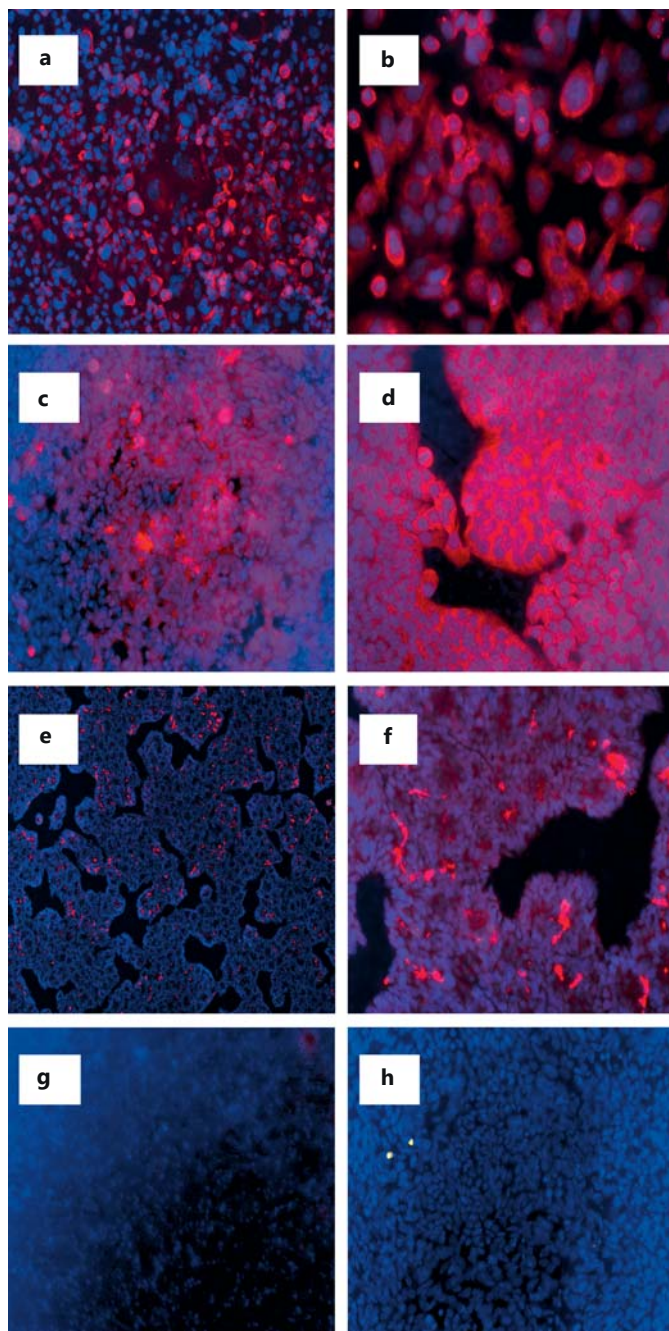


Fig. 2. Immunocytochemical staining of CRC cell lines. Cells were stained with TKTL1 (red) and DAPI (blue). TKTL1 was expressed in the cytoplasm of SW480 (a, b), HT-29 (c, d) and T84 (e, f; all magnification $\times 50$ and $\times 200$). Highest expression was seen in HT-29, followed by SW480, and weaker expression in T84. Staining without any antibody (g) and staining with secondary antibody (h) only as negative controls.

supply in the center of the spheroids, malignant cells may switch to anaerobic metabolism, i.e. the nonoxidative part of the PPP [4]. As mentioned, hypoxic conditions mainly occur in the center of the spheroid structure, especially in cell lines that form large spheroids [37]. Spheroids of cell lines forming large spheroids subsequently also show a trend for necrosis in the center of the cell aggregates.

Spheroids of T84 cells which are relatively small (online suppl. fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000355015) weakly expressed TKTL1 protein over the 5 days of culture (fig. 3a). They did not upregulate HIF-1 α protein expression over time, indicating that there was no significant hypoxia (fig. 3a, b). In contrast, in spheroids of SW480 spheroids which are larger (online suppl. fig. 1) HIF-1 α protein expression increased over time. This was accompanied by an increasing protein expression of TKTL1 over time as well as in comparison with spheroids of T84 cells (fig. 3a, c).

HIF-1 α protein expression correlated well with TKTL1 protein expression over 5 days of culture in SW480 spheroids, indicating that hypoxia influences the expression of TKTL1.

TKTL1, Glut1 and HkII Expression under Hypoxic Conditions

To control for the influence of hypoxia on the expression of TKTL1, we cultured cell lines also in hypoxia incubator chambers for 4 h, 1 and 3 days, respectively, at 1% O₂ to have more reproducible hypoxic conditions. Additionally, cells were kept in medium without glucose (and normal content of amino acids) to evaluate the possible changes of TKTL1 expression in the absence of glucose.

Under normoxic conditions, only minor changes in pH were seen between cells cultivated with or without glucose (fig. 4a, left panel). Induction of hypoxia by applying 1% O₂ resulted in a decrease in medium pH (fig. 4a, right panel; 1% O₂ with glucose). This was more pronounced in T84 cells as compared to Caco-2 cells. Without glucose, pH was higher, indicating either production of alkalines or less lactate formation (fig. 4a, right panel; 1% O₂ without glucose).

TKTL1 mRNA levels were elevated upon hypoxia in T84 and Caco-2, whereas depletion of glucose counteracted the effect of hypoxia (fig. 4b).

The expression of genes known to be elevated under hypoxia and involved in glucose degradation, Glut1 (fig. 4c) and HkII (fig. 4d), was highly induced after 1 or

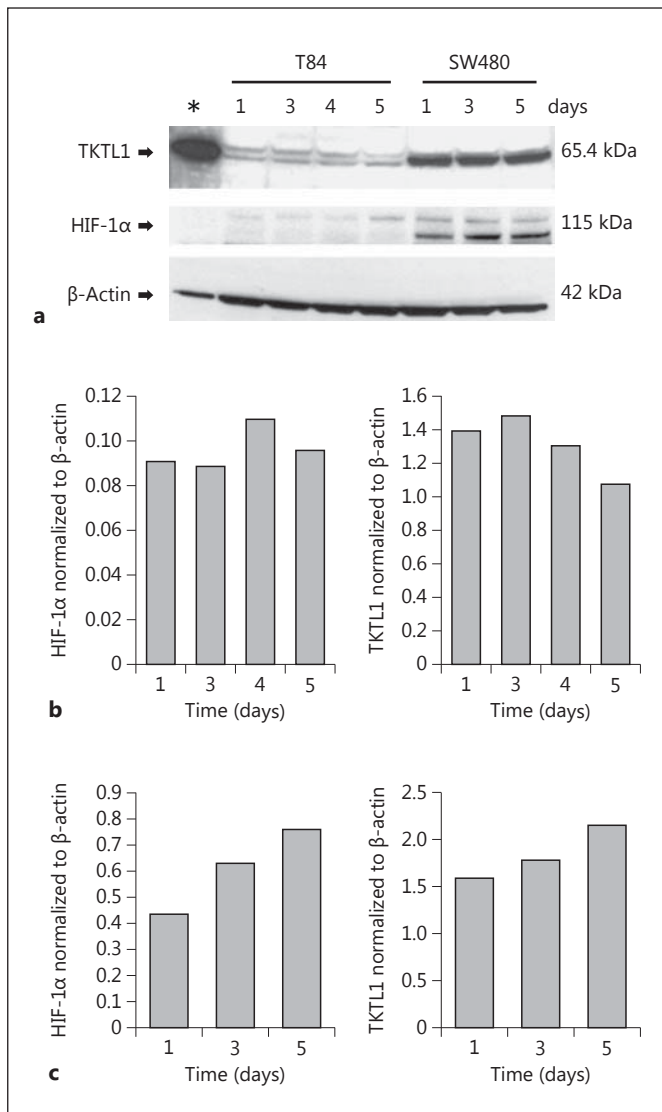


Fig. 3. TKTL1 expression in spheroids. **a, b** Spheroids of T84 showed no upregulation of HIF-1 protein expression; correspondingly, TKTL1 expression remained unaffected. **a, c** Spheroids of SW480 expressed HIF-1α protein time dependently. HIF-1α protein expression increased in the same manner as the TKTL1 protein expression over 5 days of culture. Positive control: TKTL1 stable transfected HEK293T. * $p < 0.05$.

3 days of cultivation upon hypoxic conditions. Consistent with TKTL1 mRNA levels, the expression of Glut1 and HkII was reduced in glucose-starved hypoxic cells (fig. 4c, d, right part of graph, without glucose) as compared to hypoxic cells with glucose (fig. 4c, d, left part of graph).

In contrast to the mRNA expression, only minor changes for TKTL1 protein expression levels were detect-

able during incubation in the hypoxia chamber (fig. 4e). However, these results have to be interpreted with care as upon incubation of cells without glucose and oxygen for 1 day and 3 days, also β -actin was affected, indicating necrosis and cell death. When cell viability was tested, no changes could be observed for all cell lines and conditions on day 1. However, on day 3, the cell viability of Caco-2 cells was strongly reduced upon combination of hypoxia and glucose starvation. T84 cells already showed a reduction in cell viability under hypoxic conditions or glucose depletion alone, but the effect was much more pronounced when both conditions were combined (online suppl. fig. 2).

Expression of TKTL1 in Patient Samples

TKTL1 protein expression was assessed by Western blotting in specimens of 10 patients that had undergone surgery due to CRC. The 6 female and 4 male patients were 66.5 ± 16.3 years old (table 1). All patients suffered from an invasive CRC (TNM classification pT3) except 2 non-CRC patients with a neuroendocrine carcinoma and desmoid tumor. The protein expression of TKTL1 was heterogeneous among the 8 CRC and 2 control patients (fig. 5a). In 4 of the 8 CRC patients, the TKTL1 protein expression was higher in tumor as compared with corresponding normal tissue (fig. 5b). The remaining 4 patients showed lower TKTL1 protein expression in tumor as compared with normal tissue. Patients G04.89 and G05.274 were excluded from total statistical analysis due to weak β -actin expression reflecting degradation processes (fig. 5c). However, the difference for all remaining 6 patients failed to be statistically significant ($p = 0.3$, Mann-Whitney test). The control patient G08.148 who suffered from a neuroendocrine carcinoma also had a higher TKTL1 protein expression in tumor as compared with normal tissue. Patient G05.589 had a slightly higher expression in normal in contrast to tumor tissue (fig. 5a). No clear trend could be found between the level of TKTL1 expression and clinical parameters such as tumor grading, status of lymph nodes and presence of metastases. TKTL1 expression was further assessed in 44 CRC patients by immunohistochemistry. The cancer stage was evaluated by the TNM staging system.

The patients had moderately differentiated tumors. The average age of the patients was 71.3 ± 11.6 years. A nuclear as well as a cytoplasmic expression of TKTL1 was observed in testis tubuli cells, which served as control (fig. 6a, b). Surrounding stroma cells and Leydig cells stained very weak.

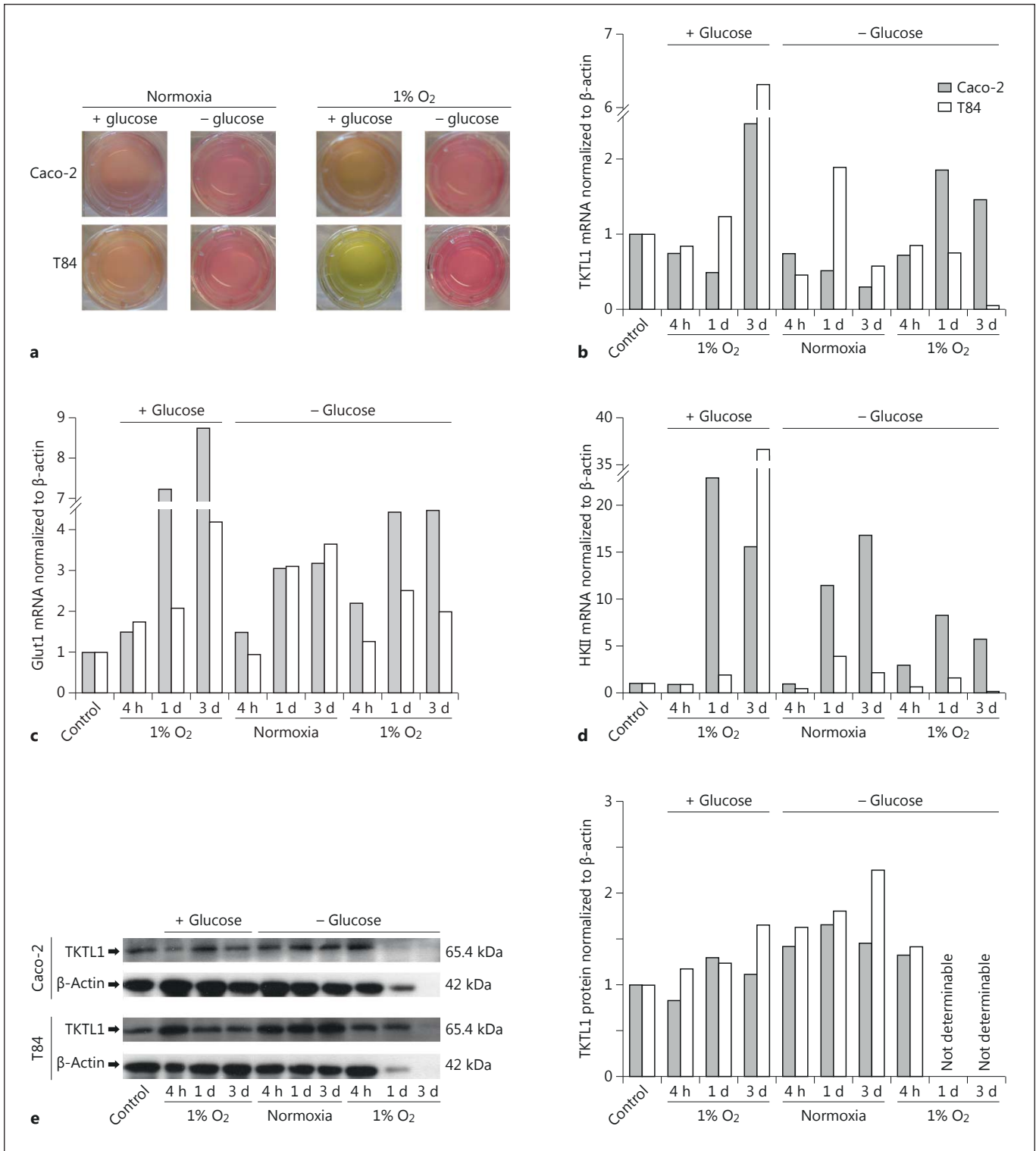


Fig. 4. TKTL1 expression under hypoxic conditions. Minor changes in medium color under normoxic conditions (**a**), whereas pronounced differences under hypoxia indicated lower pH (**b**). TKTL1 expression was induced upon hypoxia on day 3 (with glucose), diminished under glucose starvation in T84 and completely

prevented in Caco-2. Target genes of hypoxia Glut1 (**c**) and HKII (**d**) were massively upregulated upon hypoxia, which was reduced when glucose was absent. **e** Protein levels of TKTL1 were changed to a lesser extent upon hypoxia or additional glucose starvation.

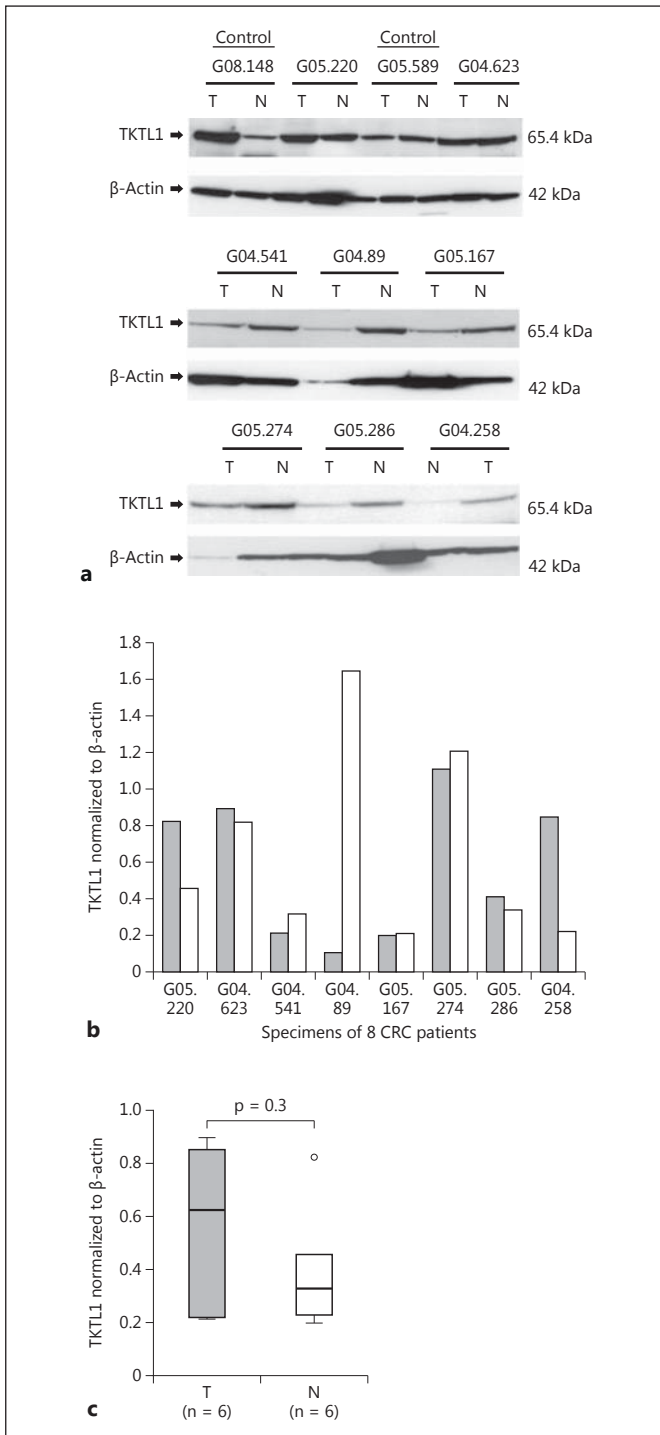


Fig. 5. TKTL1 protein expression in tissues from patients with CRC. TKTL1 protein expression in tumor tissues (T) and non-tumor tissues (N) of 8 CRC patients and 2 non-CRC patients. **a** All expressed TKTL1 protein. **b** In 4 of 8 patients, TKTL1 expression was upregulated in T compared to N (when normalized to β -actin). **c** Exclusion of slightly degraded samples. No significant difference was found between TKTL1 protein expression in T and N (Mann-Whitney test, $p = 0.3$).

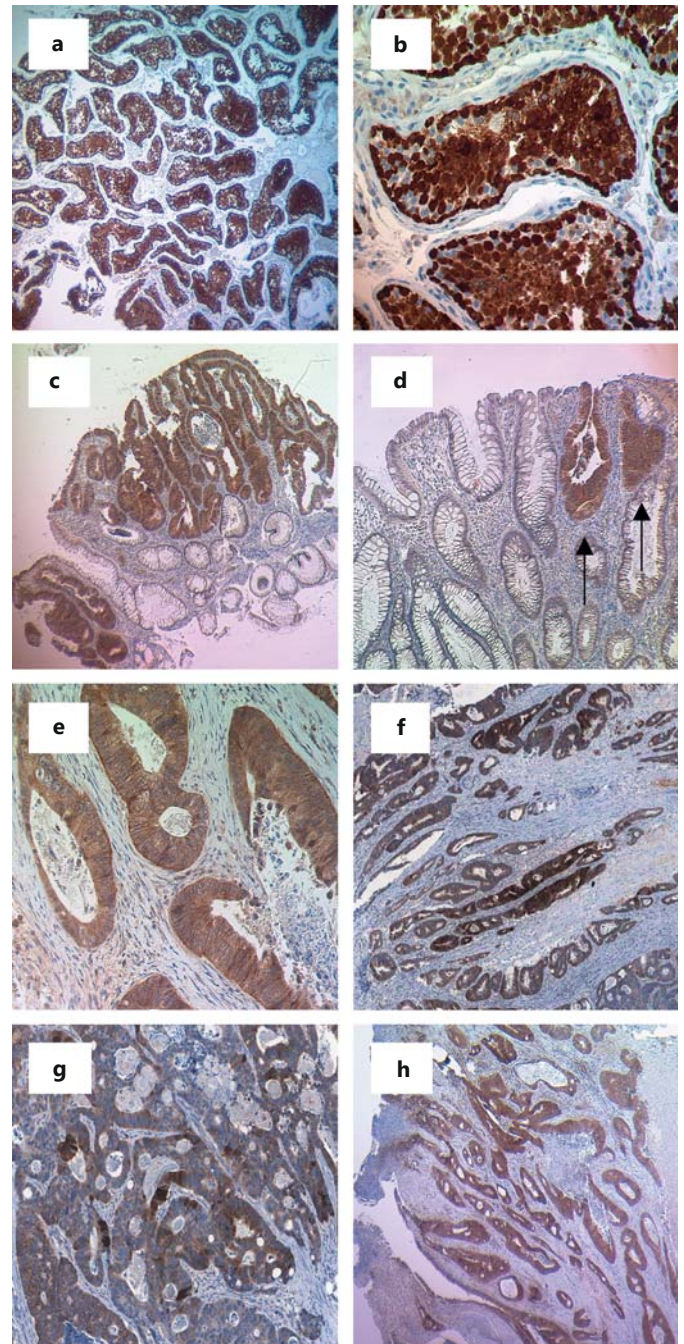


Fig. 6. TKTL1 in patient samples. **a, b** Nuclear and cytoplasmic expression of TKTL1 in tubuli cells of the testis (positive control). **c, d** Staining of TKTL1 in specimens with intraepithelial neoplasia as parts of the peripheral border area of a CC (**e-h**) and CC tissues and their surrounding tissue (all magnifications $\times 40$ – 250). The immunohistochemical staining of TKTL1 resulted in 14 positive and 30 negative samples.

TKTL1 positively stained carcinomas are shown in figure 6c–h. TKTL1 was only expressed in 2 crypts of an intraepithelial neoplastic epithelium; the surrounding epithelium appeared negative (fig. 6d, indicated with arrows). This is also represented in figure 6c. The non-neoplastic crypt epithelium demonstrated no or lower TKTL1 expression. Overall, 31.8% (14) of the samples were highly TKTL1 positive (IRS ≥ 4), and 68.2% (30) were stained TKTL1 negative (IRS < 4).

Discussion

Based on the role of TKTL1 described for other tumors, we investigated its expression in CRC. TKTL1 was highly expressed in HT-29 as well as in SW480 cells. Cultivation in the in vitro spheroid model showed that hypoxia did influence the expression of TKTL1 and correlated with HIF-1 α expression in SW480. TKTL1, Glut1 and HkII mRNA were reduced in glucose-starved, hypoxic cells compared to hypoxic cells with glucose. Four of 8 CRC patients had a higher TKTL1 protein expression in tumor than in matching normal tissue. Moreover, immunohistochemistry demonstrated that 14 CRC patients showed increased TKTL1 expression, whereas 30 had no detectable TKTL1 expression.

In contrast to our data, Langbein et al. [34] found strong TKTL1 protein expression in CRC which was correlated with invasive behavior. They concluded that CRC tumorigenesis involves energy production by aerobic glycolysis (Warburg effect) in malignant tumors and that TKTL1 is a key enzyme specific for glucose metabolism in CRC tumor cells and contributes to a malignant phenotype [34, 38]. Interestingly, anaerobic conditions are often present in malignant tumors [39, 40] and may be causative for the combined expression of TKTL1 and HIF-1 α protein in our spheroid model. It is already known that hypoxia mostly appears in the center of the spheroid structure [37]. Sun et al. [10] postulated that TKTL1 expression in head and neck cancer cells contributes to HIF-1 α accumulation, and moreover, HIF-1 α upregulates glycolytic target genes as well as indirectly increases the substrates of TKTL1. HIF-1 α inhibits the Krebs cycle by upregulating pyruvate dehydrogenase kinase 1 concomitant with an inhibition of pyruvate dehydrogenase enzyme complex. This leads to an increased conversion of pyruvate to lactate even under normoxic conditions in cancer cells [41].

It has already been shown that the TKTL1 metabolite glyceraldehyde-3-phosphate significantly increases with

the transformation of benign to malign prostate cancer [42]. These findings are supported by Sun et al. [10]. They could demonstrate that in head and neck cancer cells with exogenous TKTL1 overexpression, fructose-6-phosphate and glyceraldehyde-3-phosphate were increased and consequently an increased aerobic glycolysis, the Warburg effect, occurred. On the other hand, Xu et al. [12] demonstrated by TKTL1 knockdown that a significantly reduced glucose consumption and lactate production occurs in HCT116 CC cells.

A specific upregulation of TKTL1 in a subset of CRCs could be demonstrated in our study. 31.8% of the analyzed tumors had an IRS ≥ 4 , whereas 68.2% of tumor samples had an IRS < 4 .

As already mentioned, the PPP is controlled by thiamine-dependent transketolases [43]. In 1976, Basu and Dickerson [44] claimed an important role of nonoxidative TKT reactions in cancer cell proliferation in patients with breast and bronchial carcinoma. Hence, the thiamine status of patients with CRC should be considered when interpreting the results.

Conclusion

TKTL1 expression in CRC is induced by oxygen-limiting conditions. The role of TKTL1 during tumor progression in CRC patients and factors that induce TKTL1 expression have to be further elucidated.

Acknowledgement

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Disclosure Statement

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

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Erratum

In figure 1c of the article by Amanzada et al. [2012;86:218–227] entitled ‘High predictability of a sustained virological response (87%) in chronic hepatitis C virus genotype 1 infection treatment by combined IL28B genotype analysis and γ -glutamyltransferase/alanine aminotransferase ratio: a retrospective single-center study’ an error occurred.

Different from what is stated in the Results section on page 222, left column, line 9, the text should read: ‘C homozygotes of IL28B with low pretreatment γ -GT/ALT ratio showed an OR of 5.5 (95% CI: 2.2–13.7, $p = 0.0001$) compared to CT and TT genotypes with low γ -GT/ALT ratio. C homozygotes of IL28B with low pretreatment γ -GT/ALT ratio showed an OR of 26.6 (95% CI: 10–71.1, $p < 0.0001$) compared to CT and TT genotypes with high (>0.70) pretreatment γ -GT/ALT ratio (fig. 1c).’ Figure 1c should read as follows:

