Transketolase protein TKTL1 overexpression: A potential biomarker and therapeutic target in breast cancer

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Abstract. Malignant tumors degrade glucose to lactate even in the presence of oxygen via the pentose phosphate pathway (ppp). The non-oxidative part of the ppp is controlled by thiamine-dependant transketolase enzyme reactions. Over-expression of the transketolase-like-1-gene (TKTL1) in urothelial and colorectal cancer is associated with poor patient outcome. We analyzed the expression of the TKTL1 protein in a retrospective institution-based patient cohort with invasive breast cancer by immunohistochemical analysis of 124 paraffin-embedded breast cancer tissues. Our study revealed TKTL1 expression in 86% of breast cancer specimens with 45% showing high expression levels. In contrast, only 29% of corresponding non-neoplastic breast tissues were TKTL1 immunopositive, including 9% with high expression levels. High expression levels of TKTL1 correlated significantly to Her2/neu overexpression (p=0.015). However, TKTL1 expression failed to reach statistical significance for other common prognostic parameters. In contrast to recent data for e.g. colorectal cancer TKTL1 overexpression did not correlate to patient outcome and survival. However, in the context of novel insights into TKTL1-related tumor metabolism and the high proportion of TKTL1 overexpressing breast cancers, this enzyme represents a potential candidate for targeted inhibition of tumor growth in this tumor entity.

Introduction

Malignant cells show complex genetic alterations, which determine their abnormal cellular proliferation, differentiation and survival. Changes in gene expression profiles in malignant cells also modify biochemical pathways, which result in abnormal tumor metabolism.

In most non-neoplastic tissues, glucose is degraded via the Embden-Meyerhof pathway, in case of sufficient oxygen supply and physiological mitochondria activity. If oxygen is absent, healthy cells switch to a fall back reaction in which glucose is fermented to lactate. If oxygen is available, the fermentation is blocked by oxygen, and the intact mitochondria are used for oxidative phosphorylation. This regulation ensures the efficient glucose usage by mitochondrial oxidative phosphorylation in the presence of sufficient oxygen supply.

In contrast, especially in carcinoma tissues glucose is fermented to lactate even if oxygen is present (aerobic glycolysis; 'Warburg effect') (1). Although this particular metabolism in cancer was identified 80 years ago, the relevance of aerobic glycolysis for cancer cell biology is still controversial (2,3). A mutated transketolase enzyme (transketolase-like-1/TKTL1) has been suggested to be a basis of a mammalian glucose fermentation pathway (4).

Transketolase enzyme reactions enable an oxygen-independent glucose degradation and play a crucial role in nucleic acid ribose synthesis utilizing glucose carbons in tumor cells (5). As a consequence, the treatment of tumor cells with specific transketolase inhibitors led to a reduction in tumor cell proliferation (6), and activation of transketolases by application of thiamine stimulated tumor growth (7).

Three human transketolase genes have been recognized: TKT, TKTL1 and TKTL2. TKT is known to encode an active transketolase enzyme (8), and TKTL2 is also likely to encode an active transketolase enzyme. TKTL1 has been assumed to be a pseudogene; however, it has been previously shown that TKTL1 could encode a transketolase-like protein (9). The relative contributions of TKT, TKTL1 and TKTL2 to tumor-specific transketolase metabolism have been investigated in a recent study which found a specific TKTL1 overexpression in urothelial and colon carcimomas at the mRNA and protein level, whereas TKT- and TKTL2-expression are not up-regulated. Overexpression of TKTL1 protein predicted poor patient survival (10).

Evidence that breast carcinomas also exhibit the Warburg effect can be derived from a series of previous studies (11,12).

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However, the expression of TKTL1 protein in breast cancer has not yet been investigated.

In the present study, we examined 124 breast cancer samples for specific TKTL1 protein expression by immuno-histochemistry. A marked subset of breast cancers showed a TKTL1 overexpression which correlated significantly with Her2/neu overexpression. However, in contrast to colon and urothelial cancer, TKTL1 overexpression was not associated with a poor patient outcome in the examined study population. Due to the high rate of TKTL1 overexpression, we assume that TKTL1 can be viewed as a novel metabolic biomarker in breast cancer, and a possible future target for mechanism-driven drug development.

Materials and methods

Patient sample and histopathological criteria. A total of 124 breast cancer patients without metastasis or second malignancies treated for invasive breast cancer at the Department of Obstetrics and Gynaecology, University Hospital Freiburg, Germany, between 1990 and 1999 were included into this retrospectively designed study. Breast conserving surgery or mastectomy was performed without prior radio-, chemo- or endocrine therapy.

Adjuvant systemic therapy was given according to the international recommendations of the time. After breast conserving surgery radiation was performed with 52 Gy and a 10 Gy boost to the tumor area. After mastectomy radiation was given only to advanced tumors. In hormone receptor-positive patients tamoxifen was recommended for a period of 2-5 years. Systemic chemotherapy was mostly given to lymph node positive patients and was mainly CMF (cyclophos-phamide, methothrexate, 5-fluorouracil)-based, only in some patients anthracyclines were used for adjuvant treatment.

Immunohistochemistry. For immunohistochemistry (IHC) 3- to 5-μm paraffin sections of tissue microarrays of the 124 breast cancers and corresponding non-neoplastic tissues were independently analyzed by two experienced pathologists (G.K., A.z.H.). Antigen demasking was performed by heating dewaxed sections in 10 mM sodium citrate (pH 6.0) in a microwave oven for 1 min at 450 W followed by 5 min at 100 W. Inhibition of endogenous peroxidase was performed by a 5 min incubation with 3% H_2O_2. Endogenous avidin-biotin was blocked by the use of a commercial biotin blocking system (Dako) for 10 min. After two washes in Tris-saline buffer (TBS), slides were incubated with 1% goat serum for 30 min to block unspecific staining. Sections were sub-sequently exposed to mouse anti-TKTL1 (clone JFC12T10; mouse IgG2b) antibody (15 μg ml^{-1}) for 1 h. The monoclonal anti-TKTL1 antibody JFC12T10 has been described (4,10). Slides were washed in TBS and incubated with biotinylated anti-mouse immunoglobulins for 30 min at room temperature and treated with streptavidin-peroxidase (Dako). Staining was revealed using 3-amino-9-ethylcarbazole (AEC) substrate and counter-stained with haematoxylin.

The obtained tissue underwent routine staining for the standard histopathological and prognostic parameters, such as tumor type, tumor size (T), nodal status (N), estrogen/progesterone receptor expression (ER/PR), grading (G) and

Table I. Histopathological characteristics of included tumors and level of TKTL1 expression.

			TKTL1 expression		
		N	Low (%)	High (%)	p-value
T stage	1	53	22 (41.5)	31 (58.5)	0.052
	2	51	34 (66.6)	17 (33.3)	
	3+4	20	12 (60.0)	8 (40.0)	
Nodal status	0	68	33 (48.5)	35 (51.5)	0.12
	1	56	35 (62.5)	21 (37.5)	
Grading	1+2	63	32 (50.8)	31 (49.2)	0.38
	3	61	36 (59.0)	25 (41)	
ER	Pos	83	45 (54.2)	38 (45.8)	0.843
	Neg	41	23 (56.1)	18 (43.9)	
PR	Pos	90	52 (57.8)	38 (42.2)	0.285
	Neg	34	16 (47.1)	18 (52.9)	
Her2/neu	0+1	111	65 (58.6)	46 (41.4)	0.015
	2+3	13	3 (23.1)	10 (76.9)	

Her2/neu score (Her2; Dako HercepTest™). To facilitate statistical analysis of TKTL1 expression, these criteria were categorized into expression groups as shown in Table I. In brief, TKTL1 expression was scored on a scale ranging from 0 to 3: score 0, 0-20%, score 1, 21-50%, score 2, 51-80%, and score 3 >80% of the tumor cells stained for TKTL1.

Hormone receptors and Her2/neu expression. Immunohisto-chemical staining was quantitatively evaluated as follows: For the estrogen- and progesterone-receptor proteins the percentage of tumor cells with nuclear immunostaining was calculated. Additionally, we applied a staining score according to its intensity: 0, no staining; 1, weak staining intensity; 2, moderate staining intensity; 3, strong staining intensity. For Her2/neu, we followed the recommended HercepTest guidelines: score 0, no membrane staining in tumor cells; 1, incomplete membrane staining in >10% of tumor cells; 2, weak to moderate complete membrane staining in >10% of tumor cells; 3, complete membrane staining in >10% of tumor cells with strong staining intensity.

Statistical analysis. To facilitate statistical analysis, criteria were categorized into expression groups as shown in Table I. TKTL1 scores of 0 and 1 were summarized as low and scores of 2 and 3 were categorized as high expression. To compare both groups the Chi-square test and Fisher's exact test were used. The significance level was set at 5%, and each p-value was two tailed.

Overall survival was defined as time from surgery to death, or the date last known to be alive. Overall survival rates were estimated by the Kaplan-Meier product limit method. The SPSS-software package version 13.0 was used (SPSS for windows, SPSS, Chicago, IL, USA).
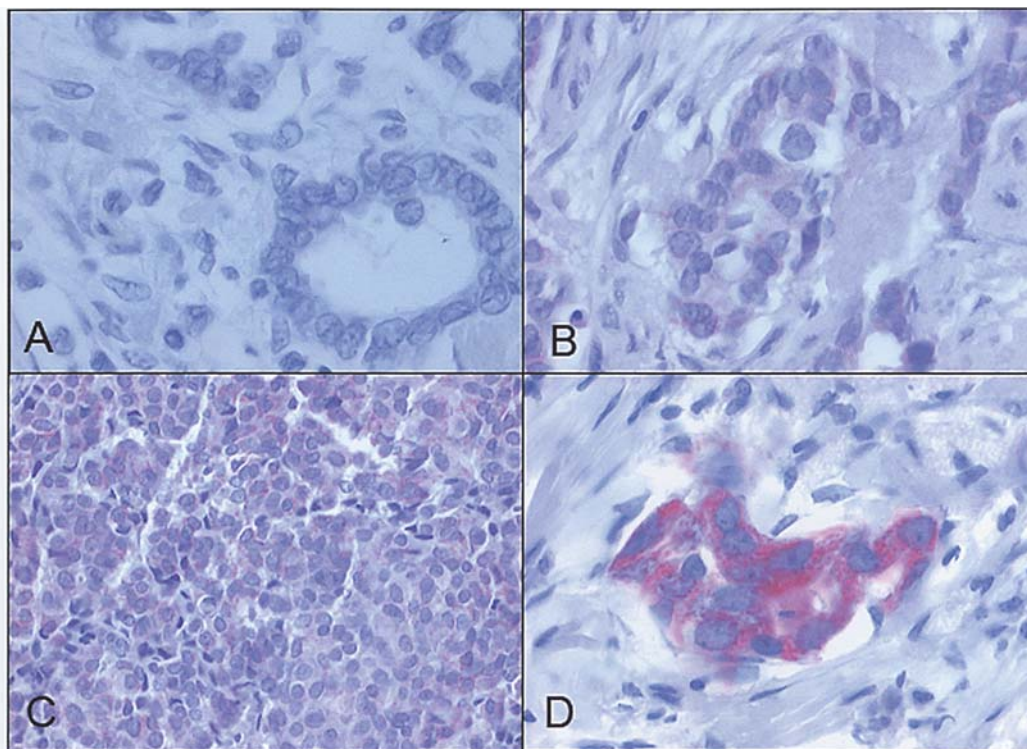
Figure 1. (A) No staining of TKTL1 in epithelial cells of mammary tissue (score 0). (B) Weak cytoplasmic expression (faint red) of TKTL1 in invasive breast carcinoma (score 1). (C) Moderate, specific cytoplasmic expression in an invasive breast carcinoma (score 2). (D) Strong, specific cytoplasmic (red) expression of TKTL1 in an invasive breast carcinoma (score 3).
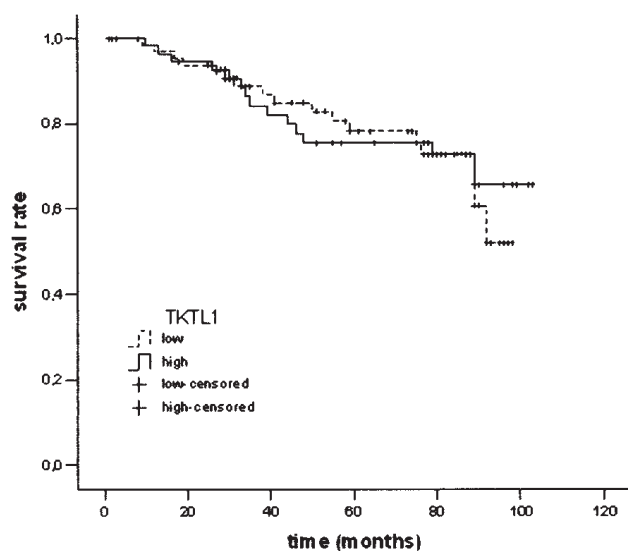

Figure 2. Estimated survival rates by TKTL1 overexpression. Kaplan-Maier curves on overall survival for TKTL1 low (score 0 and 1) and high expression (score 2 and 3).

Results

TKTL1 expression in non-neoplastic tissue and breast cancer. Tumor tissue of 124 patients and corresponding adjacent non-neoplastic breast tissue (n=41) were tested for TKTL1 expression by immunohistochemistry. Analysis of non-neoplastic breast tissue revealed no to weak expression of TKTL1 in 37 samples (90.2%) with 29 (70.7%) samples showing score 0 and 8 (19.5%) samples, score 1, respectively. Only 4 specimens of corresponding normal breast tissues showed high expression levels of TKTL1 (9.8%).

In contrast, a total of 107 of 124 breast cancers (86%) expressed TKTL1 specifically in tumor cells. Seventeen specimens (13.7%) were TKTL1 negative, whereas 51 (41.1%) showed score 1. Fifty-six (45.1%) breast cancers exhibited a high expression level of TKTL1 with 37 (29.8%) tumors expressing score 2 and 19 (15.3%) tumors, score 3 (Fig. 1).

TKTL1 expression and clinico-pathological parameters. We were able to detect a significant positive correlation between Her2/neu status and TKTL1 expression levels: breast cancers with a Her2/neu score of 2 and 3 displayed a significantly increased TKTL1 expression whereas Her2/neu scores of 0 and 1 correlated with low TKTL1 staining intensity (p=0.015; Table I). However, no significant correlation could be found for TKTL1 expression levels and T stage (p=0.052), nodal status (p=0.12), grading (p=0.358) or hormone receptor expression (ER/PR; p=0.843 and 0.285, respectively; Table I).

TKTL1 expression and patient outcome and survival. In order to examine a possible impact of the level of TKTL1 over-expression on patients' overall survival, a Kaplan Meier analysis was performed. We found no correlation of TKTL1 expression to treatment modalities. There was no significant correlation to patients age (p=0.346), surgical procedure (p=0.199), adjuvant radiation therapy (p=0.849), adjuvant chemotherapy (p=0.773) or endocrine treatment (p=0.206). Moreover, our analysis did not reveal a correlation of TKTL1 overexpression to overall survival (Fig. 2).

Discussion

This study analyzing the expression of TKTL1 in breast cancer revealed an induction of TKTL1 expression in breast cancer from 29% in corresponding non-neoplastic versus 86% in neoplastic tissue. The highest score level was determined in 9% of non-neoplastic and 45% of breast cancer tissue, respectively.

Our data on TKTL1 expression in non-neoplastic breast tissue are supported by a study which determined TKTL1 expression in various non-neoplastic tissues, especially in those exhibiting increased aerobic glycolysis (4). Since the non-neoplastic tissue specimens are derived from adjacent areas of the invasive breast cancer the observed high expression levels of TKTL1 in a small subset of these non-neoplastic specimens might reflect ongoing alterations in gene expression patterns which accompany the initial steps of breast cancer tumorigenesis. These effects have been described for other genes such as p53, FAS or GLUT1 (13,14).

In the investigated patient cohort we detected a significant positive correlation of TKTL1 and Her2/neu overexpression. In breast cancer, the Her2/neu gene is amplified in 25-30% of the cancers and is in these cases a negative predictor for patient prognosis (15,16). In our study cohort TKTL1 over-expression failed to show a significant correlation with clinico-pathological parameters, other than Her2/neu, such as T stage, nodal status or grading. These results are in contrast to data on colorectal and urothelial cancer where TKTL1 overexpression is associated with aggressiveness of tumors and a poor patient outcome (10). In the present breast cancer patient cohort TKTL1 failed to be a valuable predictive marker. However, it is of importance to note that also Her2/neu overexpression in our patient cohort also failed to be a negative prognostic predictor (data not shown). However, in the context of metabolic changes observed in malignant tumors and metastases, TKTL1 protein overexpression might be of potential biological, diagnostic and therapeutic significance in breast cancer. Tumor cells with intact mitochondrial oxidative phosphorylation have been experimentally converted to respiration-defect clones by damaging the mtDNA. The altered tumor cells performed aerobic glucose fermentation and were less sensitive to chemotherapeutic agents commonly used in the clinical treatment of cancer, but significantly responded to inhibition of glucose fermentation (17). Activation of the oncogenic kinase Akt has been shown to stimulate glucose uptake and metabolism in cancer cells, and renders these cells susceptible to death in response to glucose withdrawal (18). Such tumor cells have been shown to be dependent on glucose because the ability to induce fatty acid oxidation in response to glucose deprivation is impaired by activated Akt (19). This might explain why ketogenic diet has been successfully applied to children with aggressive brain tumors (20,21).

Metabolic changes in tumor cells might in future be exploited for the development of novel drugs for the inhibition of glucose fermentation. Similar to the relevance of Her2/neu as a 'drugable' target for an individualized anti-cancer therapy (22-25), TKTL1 might also be a future target for small molecule drug design and individualized treatment strategies in breast cancer: metabolic control analysis and inhibition of transketolase enzyme reactions have shown *in vitro* as well as *in vivo* that tumor proliferation can be inhibited by anti-transketolase approaches (5-7,26-29).

In summary, TKTL1 might represent a novel biomarker and a 'drugable' target in breast cancer. Determination of metabolic changes in tumors via TKTL1 might lead to aprogress in individualized diagnostic and anti-cancer treatment strategies.

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