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TKTL1 as a prognostic marker in pancreatic ductal adenocarcinoma and its correlation to FDG-PET-CT

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Short title: TKTL1 in PDAC and its correlation to FDG-PET-CT

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Abstract

INTRODUCTION: Glucose metabolism in cancer cells differs from noncancerous cells. The expression of transketolase-like protein 1 (TKTL1), a key enzyme in the glucose metabolism of cancer cells, predicts poor prognosis in several cancer types. We studied TKTL1 as a prognostic tool and whether TKTL1 expression correlates with 18F-FDG-PET-CT among patients with pancreatic ductal adenocarcinoma (PDAC).

METHODS: This retrospective study examined two PDAC patient cohorts: 168 patients operated on at Helsinki University Hospital between 2001 and 2011, and 20 patients with FDG-PET-CT results available from the Auri Biobank. We used immunohistochemistry (IHC) for TKTL1 expression, combining results with clinicopathological data.

RESULTS: Five-year disease-specific survival (DSS) was slightly but not significantly better in patients with a high versus a low TKTL1 expression, with DSS of 28.0% vs 17.3%, respectively ($p = 0.123$). TKTL1 served as a marker of a better prognosis in patients over 65 years old ($p = 0.012$) and among those with TNM class M1 ($p = 0.018$), stage IV disease ($p = 0.027$), or perivascular invasion ($p = 0.008$).

CONCLUSIONS: Our study shows that TKTL1 cannot be used as a prognostic factor in PDAC with the exception of elderly patients and those with advanced disease. The correlation of TKTL1 with 18F-FDG-PET-CT requires further study in a larger patient cohort. (210 words)

INTRODUCTION

Globally, nearly half a million (458,918) new cases of pancreatic cancer occur annually resulting in nearly as many deaths (432,242), the incidence of which is rising [1]. While cancer prognosis in recent decades has generally improved, that for pancreatic cancer has not. Many patients present with advanced disease upon diagnosis, whereby only a minority are eligible for curative surgery. Perioperative chemotherapy is offered to patients with locally

advanced disease, and trials remain ongoing to determine whether all patients might benefit from neoadjuvant chemotherapy [2]. Chemoradiotherapy pre operatively compared to surgery alone offers no overall survival benefit, but leads to a larger rate of R0 resection in patients with borderline disease [3].

In cancer cells, glucose metabolism increases compared to that in normal cells. This offers a novel target for chemotherapy but can also be used to diagnose and monitor tumor growth with positron emission tomography (PET). Nobel laureate Otto Warburg described an increased glycolysis in an aerobic environment via the pentose phosphate pathway (PPP), a typical attribute of cancer cells [4]. Transketolases control the nonoxidative part of PPP. Transketolase-like protein 1 (TKTL1), represents one transketolase isoform that causes rapid tumor-cell growth, while also leading to reduced glucose consumption when suppressed [5]. TKTL1 expression is elevated in a number of cancers, and serves as a marker of poor prognosis in colorectal and gastric cancer among many others [6-16].

¹⁸F-fluorodeoxyglucose (FDG), a glucose analog and a marker for glucose uptake in the tissue, is used to diagnose and monitor cancer growth through PET. In pancreatic cancer, ¹⁸F-FDGPET-CT is effective in detecting malignant masses of the pancreas, and superior in detecting lymph node metastases when compared to ultrasound and computed tomography (CT) [17].

In a cohort of 56 pancreatic cancer patients without suspected metastasis in other preoperative imaging, PET-CT identified metastases in the liver, lymph nodes, and bone in 9 patients, ultimately altering the treatment plan [18]. The advantages of PET-CT appear in the detection of distant metastasis. But, when differentiating inflammatory lymph nodes from

metastatic nodes or determining cancer growth around critical arteries and veins, PET-CT has not proved sufficiently sensitive nor specific [19,20]. PET-CT is, however, valuable in detecting recurrent pancreatic cancer with a 96% detection rate compared to a 39% detection rate for CT or magnetic resonance imaging (MRI) [21]. In addition, immunostaining with Ki-67 has been compared to the FDG uptake of pancreatic tumors in PET-CT, but no correlation was found between the proliferation index and FDG uptake [22]. To our knowledge, no tumor markers have been compared to the FDG activity in PET-CT. Yet, a high compared to low FDG uptake represents an independent predictor of survival in pancreatic cancer [23,24]. The National Comprehensive Cancer Network (NCCN) guidelines from 2017 do not recommend PET-CT in the preoperative setting [25]. In the updated guidelines from 2019, imaging was not discussed at all [26] nor did it appear in the European Society for Medical Oncology (ESMO) guidelines from 2015. In ESMO's updated version of the guidelines in 2019 (ePub), only new chemotherapy recommendations were listed [27].

The tumor marker currently used to monitor the course of PDAC disease is CA19-9, despite proving less useful during the initial or primary diagnosis [27]. Other serum biomarkers examined are not yet used in clinical practice. Quite modest steps have been taken to personalize treatment among pancreatic cancer patients, only a few mutations were identified as targets for chemotherapy treatment. We still need better biomarkers to evaluate disease progression and prognosis among PDAC patients and to predict the treatment response for chemotherapy regimens.

Therefore, this study aims to determine whether TKTL1 could serve as a prognostic biomarker in pancreatic cancer and to evaluate the correlation between TKTL1 expression and 18F-PET-CT findings.

MATERIALS AND METHODS

Patients and tissues

This study comprised 168 pancreatic cancer patients surgically treated in the Department of Surgery at Helsinki University Hospital between 2001 and 2011. For 166 patients, TKTL1 expression could be determined from tumor tissue microarray (TMA) slides. Among these, 93 (55.4%) were men, and 80 (47.6%) were older than 65 years. The tumor was located at the head of the pancreas in 124 patients (73.8%), the tail in 9 (5.4%), the body in 11 (6.5%), and the entire pancreas in one patient (0.6%). No patient received neoadjuvant therapy (data missing for 15.5% of patients), and 73 (43.5%) patients received postoperative adjuvant chemotherapy. Disease-specific survival (DSS) at 5 years for all patients was 21.3% [95% confidence interval (CI) 14.6–28.0]. The median follow-up time was 1.96 years [interquartile range (IQR) 0.80–3.74].

Surgical tumor samples were fixed in formalin for more than 24 hours, embedded in paraffin, and stored in the archives of the Department of Pathology. All specimens underwent reevaluation by an experienced pathologist. Representative tumor areas were marked on hematoxylin eosin (HE) slides. Thereafter, TMA blocks including three 1.0-mm-diameter punches of tumor tissue were constructed [28,29].

Our second cohort consisted of 20 patients with pancreatic adenocarcinoma with pre- or postoperative FDG-PET scans. Clinical data and tissue samples were supplied by the Auria

Biobank (Turku, Finland). These samples were whole-tissue slides and were analyzed separately from the Helsinki cohort.

Immunohistochemistry

The TMA blocks were cut into 4- μ m-thick sections, fixed on slides, and dried for 12 to 24 hours at 37°C. Sections were subsequently deparaffinized in xylene and rehydrated through graded ethanol and distilled water. Immunohistochemistry for both TMA and whole-tissue samples was completed in a similar fashion. For antigen retrieval, the slides were treated with Tris-HCl (pH 8.5) in a PreTreatment module (LabVision Corp.) for 20 minutes at 98°C. The staining of sections took place in an Autostainer 480 (LabVision) with an antihuman TKTL1 antibody (Rida Pentocheck IHC, Clone JFC12T10, R-Biopharm AG) diluted to 1:200 with Dako REAL Antibody Diluent S2022 (Dako). The primary antibody was kept on glasses overnight (O/N) followed by 30-minute incubation with the secondary peroxide-conjugated rabbit/mouse ENV (K5007) Dako REAL Envision/HRP antibody (Dako). The slides were finally visualized using the Dako REAL DAB+Chromogen kept on glasses for 10 minutes. Between each step in the staining procedure, slides were washed with PBS-0.04% Tween20. The slides were counterstained using Meyer's hematoxylin, washed in tap water for 10 minutes, and finally mounted in an aqueous mounting medium (Aquamount, BHD). We used a gastric and a colon cancer specimen known to be TKTL1-positive as the positive control in each staining.

Scoring

Samples were scored independently by KA and JH. Cytoplasmic positivity of ductal epithelial cells was scored on a four-grade scale, where the absence of staining was scored as 0, mild staining as 1, moderate staining as 2, and strong staining as 3. The highest score out of three was selected to represent the tumor, since high TKTL1 appears to associate with poor survival

in other gastrointestinal cancers. Samples with no tumor tissue or with too few cells for adequate evaluation were excluded. Samples receiving different scores from the two researchers were discussed in order to reach consensus. For analysis, samples were regrouped into two groups: low expression (scores 0 and 1) and high expression (scores 2 and 3).

Statistical analysis

We assessed the associations between TKTL1 and clinicopathological variables using the chisquare test. Disease-specific survival (DSS) was calculated from the date of surgery through the date of death from pancreatic adenocarcinoma or until the end of follow-up. We constructed survival curves based on the Kaplan–Meier method and compared them using the log-rank test. For the univariate and multivariate survival analyses, the Cox proportional hazard model entered the following covariates: age, gender, grade, TNM grade, tumor location (head, body, or tail of the pancreas), perineural and perivascular invasion, and TKTL1 expression. TNM grade, grade, histological type, and TKTL1 expression were entered as categorical covariates.

We considered $p < 0.05$ statistically significant. All statistical analyses were performed using IBM's SPSS Statistics for Mac, version 25.0 (IBM Corporation).

The Surgical Ethics Committee of Helsinki University Hospital (Dnro HUS 226/E6/06, extension TMK02 §66 17.4.2013) and the National Supervisory Authority for Welfare and Health (Valvira Dnro 10041/06.01.03.01/2012) approved the study protocol and granted us license to study the archived tissue samples without requiring specific individual consent.

RESULTS

Immunohistochemistry

We analyzed the cytoplasmic TKTL1 staining of tumor cells. In two patients, all TMA spots lacked tumor tissue for adequate evaluation and were excluded. Thus, 166 of 168 patients were included in the analyses. Among these, 13 patients (7.8%) had a score of 0, 80 (48.2%) a score of 1, 57 (34.3%) a score of 2, and 16 (9.6%) a score of 3 (Fig. 1). For the subsequent analysis, scores 0 and 1 were combined to indicate negative staining and scores 2 and 3 represented positive staining.

The patient series from the Auria Biobank (n = 20) was too small for statistical analyses. Among these, 6 had disseminated disease and did not undergo pancreatic resection. The remaining 14 patients underwent surgery, either a pancreaticoduodenectomy (11 patients), pancreatic tail resection (2 patients), or removal of the entire pancreas (1 patient). There were 2 patients with a score of 0, 7 patients with a score of 1, 3 patients with a score of 2, and 2 patients with a score of 3. Furthermore, 13 patients had an FDG-PET-CT prior to biopsy or surgery. Among these, 11 exhibited an increased FDG uptake around the pancreatic tumor; surprisingly, those two patients who did not, had unresectable disease.

Associations

We found no associations between the TKTL1 score and the covariates entered: age, gender, TNM classification, stage, or perivascular invasion (Table 1) nor between grade, American Society of Anesthesiologists (ASA) class, or perineural invasion (data not shown).

Survival

DSS at 5 years among all patients was 21.3% [95% confidence interval (CI) 14.6–28.0]. DSS was 17.3% (95% CI 9.5–25.1) among the low expression group and 28.0% (95% CI 16.4–

39.6) among the high expression group, although this was not statistically significant ($p = 0.123$; Fig. 2). In the univariate analysis, patients with perivascular invasion experienced a better survival than those with no perivascular invasion [hazard ratio (HR) 0.55, 95% CI 0.37–0.81; Table 3].

In the subgroup analysis, a high TKTL1 score marked a better prognosis among patients over 65 ($p = 0.012$; Fig. 3a) as well as among patients with advanced disease: TNM class M1 ($p = 0.018$), perivascular invasion ($p = 0.008$; Fig. 3b), and stage IV disease ($p = 0.027$) (Table 2).

In the multivariate survival analysis, perivascular invasion, tumor location, and the TKTL1 score represented independent risk factors. Patients with a high TKTL1 expression exhibited a better prognosis than those with a low TKTL1 expression (HR 0.61, 95% CI 0.38–1.00; Table 3).

DISCUSSION

We previously showed that a high TKTL1 expression in cancer cells correlates with a poor prognosis in gastric and colorectal cancer. TKTL1 and its prognostic value in PDAC have, to the best of our knowledge, not previously been examined. Here, we show in a rather large patient cohort that the prognostic value of TKTL1 in PDAC remains less clear compared to other cancers studied. However, in certain subgroups, a high TKTL1 expression served as a marker of better prognosis. This represents a slightly confusing finding since previous studies showed that a high TKTL1 expression typically associates with a poor prognosis. The difference between various cancers is difficult to explain. It is possible that the overall aggressive nature of PDAC in some way renders it different compared to other cancers.

In a previous study, TKTL1 could be detected using the epitope detection in monocytes (EDIM) blood test in all 34 pancreatic cancer patients studied, whereas a healthy control group exhibited a negative EDIM TKTL1 score [30]. Circulating TKTL1 is, however, not specific to pancreatic cancer, but also accompanies colorectal and biliary cancer. In another study, transketolase, TKTL1, and TKTL2 all expressed in all pancreatic cancer cell lines tested. The mRNA levels of transketolase were significantly higher than those for TKTL1 and TKTL2 [31]. Normal pancreatic ductal cells express transketolase, whereas cancer cells exhibit higher TKTL1 levels. In our material, TKTL1 was expressed in most tumors (n = 153 or 92.2%).

In our previous studies, TKTL1 tissue expression associated with poor survival in colorectal and gastric cancer [6,32]. Other groups have reported similar results in other cancer types, such as urothelial, non-small cell lung cancer, oral squamous cell carcinoma, and tumors of the ocular adnexa [7,10,15,16].

TKTL1 appears to play a contradictory role in PDAC. Tumors invading the celiac axis, common hepatic, or superior mesenteric arteries (T4), and those with distant metastasis (M1) carry a poor prognosis. Our patient cohort was carefully selected with patients operated on with a curative intent, whereby only a few presented with advanced disease. Interestingly, in this very small subgroup of patients with pT4 or c/pM1, a high TKTL1 expression predicted a better prognosis. This tendency was similar across the entire cohort, although this finding was not significant. We, thus, must exercise caution in drawing conclusions based on this result. A similar effect was, however, seen in Diaz–Moralli’s work on metastasized colorectal cancer, where TKTL1 expression in the primary tumor diminished in stage IV disease when examining expression levels using computational image analysis [33]. One possible

explanation for this is that TKTL1 is necessary for tumor progression, but becomes unnecessary when the tumor progresses and metastasizes. Alternatively, tumors that do not strongly express TKTL1 are no longer able to grow locally, but have metastasized instead. Because PDAC is often advanced at the time of diagnosis, this may explain why TKTL1 is not a prognostic marker in this cancer type.

PET-CT represents a valuable diagnostic tool, but its value in the staging of PDAC remains vague at best [34]. In our small series, 18F-FDG-PET was positive in all of the PDAC surgical patients who underwent a preoperative scan. The patient series received from the Auria Biobank was too small to allow for adequate statistical analyses. We could, however, see that 18F-FDG-PET-CT was positive both for patients with a low and a high TKTL1 expression. A positive postoperative FDG-PET-CT signaled either a R1 resection or disease progression and was, thus, a sign of poor prognosis (data not shown). Using PET-CT in planning treatment for PDAC has thus far been limited to those with ambiguous CT scan findings and to those with a negative CT finding, but a rising tumor marker level. Unfortunately, the patient material with tissue specimens and PET results available for this study was too small to draw definitive conclusions. However, the associations between the tissue TKTL1 expression and glucose metabolism tumor markers, as well as the 18F-FDG-PET findings, remain interesting and require further exploration.

To conclude, here we show that TKTL1 cannot serve as a prognostic marker in pancreatic ductal adenocarcinoma with the exception of elderly patients and those with advanced disease. The correlation between TKTL1 and 18-FDG-PET-CT needs further study. The larger question remaining is how we can better treat our patients with PDAC.

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STATEMENT OF ETHICS

The Surgical Ethics Committee of Helsinki University Hospital (Dnro HUS 226/E6/06, extension TMK02 §66 17.4.2013) and the National Supervisory Authority for Welfare and Health (Valvira Dnro 10041/06.01.03.01/2012) approved the study protocol and granted us license to study the archived tissue samples without requiring specific individual consent.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to declare.

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AUTHOR CONTRIBUTIONS

The histological scoring was done by KA and JH, data collection by KS, HS and SK, statistical analyses by KA and CB, and study design by CB and CH. All authors contributed to writing of the manuscript.

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FIGURE LEGENDS

Fig. 1. TKTL1 immunohistochemical staining of PDAC samples. Cytoplasmic intensity was scored along a four-grade scale. A) negative, B) mild, C) moderate, and D) strong. Original magnification: 400x.

Fig. 2. Disease-specific survival in PDAC according to TKTL1 expression. No significant difference between the low and high expression groups was found ($p = 0.123$).

Fig. 3. A high TKTL1 expression served as a marker of a better prognosis in a) patients older than 65 years ($p = 0.012$) and b) patients with perivascular invasion ($p = 0.008$).

Figure 1

Figure 2

Patients at risk

TKTL1 high expression:	73	10	2
TKTL1 low expression:	93	15	5

Figure 3

Patients at risk

TKTL1 high expression:	39	21	5	0	0
TKTL1 low expression:	39	12	5	4	1

Patients at risk

TKTL1 high expression:	18	8	1	1	0
TKTL1 low expression:	31	4	1	0	0

Table 1. Associations between TKTL1 and clinicopathological data in PDAC.

Clinicopathological		TKTL-1 low (0-1) expression		TKTL-1 high (2-3) expression		<i>P</i> -value*
		<i>n</i>	%	<i>n</i>	%	
variable	<i>n</i>	<i>n</i>	%	<i>n</i>	%	

						0.141
Age, years						
< 65						
≥ 65	88	54	61.4	34	38.6	
	78	39	50.0	39	50.0	0.526
Gender						
Male						
Female	91	53	58.2	38	41.8	
	75	40	53.3	35	46.7	0.591
TNM Classification						
T1						
T2	11	5	45.5	6	54.5	
T3	42	27	64.3	15	35.7	0.573
T4	106	57	53.8	49	46.2	
N0	5	3	60.0	2	40.0	
N1	48	25	52.1	23	47.9	1.000
N2	116	66	56.9	50	43.1	
M0						
M1	157	87	55.4	70	44.6	
	7	4	57.1	3	42.9	0.774
Stage						
IA						
IB	9	3	33.3	6	66.7	
IIA	17	9	52.9	8	47.1	
IIB	20	12	60.0	8	40.0	
III	105	61	58.1	44	41.9	
IV	3	2	66.7	1	33.3	
	8	4	50.0	4	50.0	0.135
Perivascular invasion						
Yes						
No	49	31	63.3	18	36.7	
	88	44	50.0	44	50.0	0.231
Location						
Head						
Body	122	75	61.5	47	38.5	
Tail	11	7	63.6	4	36.4	
Whole pancreas	9	3	33.3	6	66.7	
	1	0	0	1	100.0	

Abbreviations: TKTL1 = transketolase-like protein 1

Table 2. Kaplan-Meier analysis for disease-specific survival stratified for subgroups of pancreas.

	5-year cumulative survival (95% CI)
Subgroup	

	All patients	TKTL-1 low	TKTL-1 high	<i>P</i> -value
TKTL-1	21.3 (14.6-28.0)	17.3 (9.4-25.1)	28.0 (16.4-39.6)	0.123
Age, years				
<= 65	19.9 (11.3-28.5)	19.3 (8.7-29.9)	22.3 (7.8-36.8)	0.739
> 65	22.8 (12.6-33.0)	14.7 (3.3-26.1)	31.3 (13.9-48.7)	0.012
Gender				
Male	18.9 (10.1-27.7)	15.8 (5.8-25.8)	29.4 (12.7-46.1)	0.521
Female	23.5 (13.5-33.5)	19.7 (7.4-32.0)	28.0 (12.3-43.7)	0.147
Stage *				
IA	25.9 (-4.9-56.7)	33.3 (-20.2-86.6)	22.2 (-15.4-59.8)	0.413
IB	55.6 (32.7-78.5)	55.6 (23.1-88.1)	62.5 (29.0-96.0)	0.936
IIA	22.9 (3.7-42.1)	16.7 (-4.5-37.9)	37.5 (4.0-71.0)	0.582
IIB	14.7 (7.4-22.0)	11.5 (3.5-19.5)	22.1 (8.6-35.6)	0.228
III	0	0	0	0.225
IV	12.5 (-10.0-35.4)	0	25.0 (-17.5-67.5)	0.027
Perivascular spreading				
yes	8.2 (-0.0-16.4)	3.2 (-3.1-9.4)	17.8 (-2.2-37.8)	0.008
no	26.0 (16.2-35.8)	21.4 (9.1-33.7)	31.7 (16.4-47.0)	0.724
Perineural spreading				
yes	20.5 (12.7-28.3)	14.3 (5.7-22.9)	30.3 (16.0-44.6)	0.070
no	22.4 (6.1-38.7)	15.0 (-4.0-34.0)	28.0 (2.9-53.1)	0.304
Location				
head	18.8 (11.5-26.1)	19.6 (10.6-28.6)	18.3 (5.76-30.8)	0.895
body	0	0	0	0.140
tail	37.0 (0.3-73.7)	33.3 (0-86.6)	44.4 (1.0-87.9)	0.177

Abbreviations: TKTL1=transketolase-like protein 1; CI=confidence interval

Table 3. Cox regression analysis for DSS of PDAC patients.

Variable	Univariable survival analysis			Multivariable survival analysis		
	Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Age, years						
<=65	1.00			1.00		
>65	0.99	0.70-1.40	0.959	0.86	0.55-1.36	0.526
Gender						
Male	1.00			1.00		
Female	0.978	0-69-1.38	0.899	1.13	0.73-1.74	0.583
Stage						
IA	1.00			1.00		
IB	0.66	0.24-1.82	0.417	0.91	0.19-4.29	0.904
IIA	1.11	0.43-1.88	0.828	0.54	1.60-1.83	0.324
IIB	1.58	0.69-3.61	0.283	0.58	0.19-1.83	0.354
III	3.92	0.97-15.94	0.056	0.83	0.30-2.27	0.716
IV	1.89	0.63-5.65	0.254	1.82	0.41-8.18	0.432
Perivascular invasion						
yes	1.00			1.00		
no	0.55	0.37-0.81	0.002	0.63	0.39-1.00	0.048
Perineural invasion						
yes	1.00			1.00		
no	0.74	0.47-1.15	0.182	0.89	0.52-1.54	0.679
Location						
head	1.00			1.00		
body	0.01	0-0.15	0.001	0.003	0-0.07	<0.001
tail	0.01	0-0.16	0.001	0.004	0-0.07	<0.001
whole pancreas	0.02	0-0.18	0.001	0.005	0-0.09	<0.001
TKTL1						
low	1.00			1.00		

high	0.76	0.53-1.08	0.124	0.61	0.38-1.00	0.048
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