

Transketolase-like 1 (TKTL1) expression correlates with subtypes of ovarian cancer and the presence of distant metastases

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Abstract

Tumorbiology of ovarian cancer remains unclear. However it is known, that ovarian tumors, especially carcinomas show elevated expression of glucose membrane transporters for facilitated glucose uptake. It can be assumed, that increased glucose uptake leads to higher glucose metabolism. The energy resources of fully malignant transformed carcinomas are mainly supplied by aerobic glycolysis for which several pathways are known. A key role in aerobic glycolysis is described for the transketolase enzymes. Recently a novel transketolase-like enzyme called transketolase-like 1 (TKTL1) has been described that links aerobic glycolysis to the synthesis of fatty acids via production of acetyl-CoA. In order to investigate the role of TKTL1 for the progression of ovarian carcinomas, we examined paraffin sections of normal ovarian tissues, ovarian borderline tumors and mucinous or serous-papillary ovarian adenocarcinomas with respect to their expression of TKTL1. We identified a significantly elevated expression of TKTL1 in serous-papillary ovarian adenocarcinomas which correlates with poor prognostic parameters in the examined study group. Therefore it can be assumed, that TKTL1 plays a crucial role in ovarian cancer metabolism and that its expression predicts poor prognosis. Further investigations should be performed in order to evaluate whether this new enzyme is important for ovarian cancer tumorbiology and to analyze the potential role of TKTL1 as new target for specific antitumoral therapy.

Introduction

Ovarian Cancer is one of the most common malignant gynecological tumors usually bearing a poor prognosis ⁽¹⁾. The most important therapeutical option is surgery especially if disease is limited, but also as tumor debulking or after neoadjuvant chemotherapy. Chemotherapy is also of great value in patients with ovarian cancer ⁽²⁾. There are multiple data dealing with the link between ovarian cancer and immunity, and even new therapeutical implications like anti tumoral vaccination have been evaluated ⁽³⁻⁵⁾. The biology of ovarian cancer has so far virtually escaped the academic attention. However, almost 80 years ago Otto Warburg described a particular metabolic pathway in carcinomas which is characterized by glycolytic degradation of glucose even under normal oxygen supply, known as Warburg effect.

Increased glucose consumption is a characteristic of malignant cells ⁽⁶⁾. It is known, that a facilitated glucose transport via non ATP-dependent glucose transporters exists in ovarian cancer cells ⁽⁷⁾. Therefore several authors have investigated glucose uptake of ovarian cancer cells and found a positive correlation between the grade of malignancy and the expression pattern of glucose transporters. For example Rudlovski and coworkers found an elevated expression of glucose transporter 1 (GLUT1) membrane molecules in invasive ovarian cancer when compared to benign ovarian neoplasms and borderline tumors ⁽⁸⁾. Cantuaria et al. even described a positive correlation between GLUT1 expression and poor outcome ⁽⁹⁾. Other authors also found significantly elevated expressions of a different member of the GLUT supergene family, GLUT4, in ovarian malignancies ⁽⁷⁾.

Therefore it seems to be a fact, that in ovarian malignancies the expression of glucose transporters that facilitate glucose uptake is induced. So it can be assumed that ovarian cancer cells also have an increased glucose uptake that is beyond doubt used for cellular metabolism and anabolism. But is there a link between facilitated glucose uptake and advantages for tumor cell growth?

According to Ramanathan et al. increased glucose uptake during tumorigenesis results in increased lactate production ⁽¹⁰⁾. In a fully malignant transformed state, tumors are mostly dependent on aerobic glycolysis and least dependent on the mitochondrial generation of ATP ⁽¹¹⁾. Therefore in this stage, glucose is the most important source of energy for tumor cells.

Until now, two possible pathways of glucose degradation have been described. In the Embden-Meyerhof pathway cleavage of fructose-1,6-diphosphate leads to pyruvate, which can be reduced to lactate in the absence of oxygen ⁽¹¹⁾. The second pathway of non-oxidative glucose degradation is the non-oxidative part of the pentose phosphate pathway. The latter is

controlled by thiamine-dependent transketolase enzyme reactions ⁽¹¹⁾. These transketolase enzyme activities enable oxygen-independent glucose degradation. A potential role of transketolases in tumor glucose metabolism has been demonstrated by the application of transketolase inhibitors resulting in a dramatic reduction of tumor growth ^(12,13). Comin-Anduix et al. showed that on the other hand the activation of transketolase enzymes by application of thiamine stimulates tumor growth ⁽¹⁴⁾.

Recently a novel transketolase-like gene and protein (TKTL1) has been described that is found at elevated levels of mRNA and protein in malignant cells ^(15,11). TKTL1 is postulated to be the key enzyme of a recently described metabolic pathway that links the pentose phosphate pathway and the Embden-Meyerhof pathway together. In addition this enzyme provides a link in between anaerobic glucose degradation and the production of fatty acids by usage of acetyl-CoA. The pathway also facilitates anaerobic glucose degradation. The clinical importance of the increased expression of TKTL1 has been demonstrated in invasive colon and urothelial cancers. In both cancer entities there was a strong correlation between high TKTL1 expression in tumor tissue and high tumor stages as well as poor patients' survival ⁽¹¹⁾.

In order to investigate the role of TKTL1 in ovarian cancer, we performed a study that compares the TKTL1 protein expression in ovarian carcinomas (serous-papillary and mucinous adenocarcinomas), borderline tumors (serous-papillary and mucinous) and normal ovarian tissues.

Materials and Methods

Immunohistochemical staining

Routinely paraffine embedded tissue samples of 20 serous-papillary and 21 mucinous adenocarcinomas of the ovary, 18 borderline tumors (11 serous-papillary and 7 mucinous) and 24 normal ovaries were cut at 2 μm , placed on slides (Superfrost, Langenbrinck, Emmendingen, Germany) and deparaffinized with xylene. Slides were then rinsed in decreasing concentrations of ethanol before being heated for antigen unmasking in 10 mM sodium citrate buffer in a microwave oven at 600 W for 5 minutes. After rinsing in distilled H_2O , inhibition of endogenous peroxidases was performed by incubation of the sections for 10 min in H_2O_2 (3% in methanol). Slides were washed in PBS and incubated with 1% goat serum in PBS for 15 min. Subsequently, slides were incubated with the monoclonal mouse anti-TKTL1 antibody (clone JFC12T10), previously described by Coy et al. diluted in commercial antibody diluent (DAKO, Hamburg, Germany) at 1:200-1:400 depending on tissue conditions⁽¹⁵⁾. After 45-60 min of incubation at room temperature slides were washed in PBS and incubated with biotinylated anti-mouse immunoglobulins (DAKO) and treated with streptavidin-peroxidase (DAKO) according to the manufacturers protocol. Staining was developed by adding 3,3'-diaminobenzidine (DAB; DAKO) with subsequent counterstaining using haematoxylin. Sections were then dehydrated in graded ethanol and embedded in Vitro Clud (Langenbrinck).

Statistical analysis and clinical correlation

TKTL1 expression was evaluated by two independent observers. In normal ovaries, the expression in the epithelium of the surface was analyzed (coelomic epithelium). In borderline tumors and fully malignant transformed ovarian tumors the expression of TKTL1 was evaluated in tumor tissue. For semiquantitative evaluation of TKTL1 expression, a scoring scale from 0-3 was defined: score 0 indicates 0 to 25%, score 1 indicates 26 to 50 %, score 2 indicates 51 to 75% and score 3 indicates that over 75% of the tumor cells were stained. Staining intensities in scores 0 and 1 were faint, in score 2 medium to high and at score 3 always very high. No significant differences between the scores of the two observers were evident. For clinical correlation file records of the patients were investigated. Matters of

particular interest were initial FIGO stage, histological grading, initial ascites and age of patients at the time of diagnosis, representing important prognostic factors of ovarian cancer^(16, 17). Statistical analysis was performed using Statistica[®] software (version 6.0 for windows; StatSoft Inc., Tulsa, USA)

Results

Immunohistochemistry

The score data of TKTL1 expression are summarized in table 1. Typical patterns of TKTL1 expression in normal ovarian tissue, borderline tumors and ovarian carcinomas are shown in figure 1. By immunohistochemical staining, a significantly elevated expression of TKTL 1 could be detected in serous-papillary carcinomas in comparison to mucinous carcinomas, borderline tumors and normal control ovarian tissue (Fig. 2).

Statistical analysis

Mann-Whitney U-Test was used for statistical analysis. Analysis of the scores obtained from the evaluation of the biopsies revealed a significantly higher expression of TKTL1 in serous-papillary ovarian carcinomas when compared to mucinous ovarian carcinomas ($p=0,05$), borderline tumors ($p=0,002$) and normal ovarian tissues ($p=0,0004$, Fig. 2). Among the borderline tumors that were examined no difference in TKTL1 expression of the serous-papillary and the mucinous subtype was evident ($p=0,54$).

Langbein et al. describe a positive correlation between TKTL1 expression and poor prognosis in colon and urothelial carcinoma patients⁽¹⁰⁾. However in our collective of ovarian carcinoma patients also a strong correlation between TKTL1 expression and poor prognosis was evident. The patients suffering from serous papillary ovarian carcinomas, the subtype with the poorest prognosis, were examined respective to FIGO stage, histological grading, initial ascites and age of patients at the time of diagnosis. Data on median survival and resistance to carboplatin were not available in this group.

Of the serous papillary carcinomas, 60% revealed TKTL1 expression with scores of 2 or 3 (25% score 2, 35% score 3). The patients bearing these highly TKTL1 positive tumors presented with initial FIGO stage IV in 16,7%, and initial FIGO stage III in 41,7%. 42% presented with initial ascites and the mean histological grading in this group was 1,9. The mean age of these patients was 61,8 years.

Within the group of serous-papillary carcinomas with TKTL1 expression of scores lower than 2, no patient presented with initial FIGO stage IV and 37,5% with initial FIGO stage III, but more than 70% suffered of ovarian cancer in initial FIGO stage I or II.

Initial ascites was found in 37,5% of the patients in this group and the median histological grading of these tumors was 1,5. The median age of affected patients was 57,9%.

The data given in table 2 indicate, that poor prognostic parameters (high FIGO stage, initial ascites and high histopathological grading) of serous-papillary ovarian carcinoma are more common when TKTL1 expression status was 2 or 3.

The same criteria were examined in mucinous ovarian carcinomas, although these tumors displayed significantly lower levels of TKTL1 than serous-papillary ovarian carcinomas. In this group no correlation of poor prognostic parameters of ovarian carcinomas to TKTL1 expression could be observed (Table 3).

Discussion

Focusing on the special metabolic situation of tumors, some authors believe in a crucial role of glycolysis in tumor metabolism. Other than in benign cells, glycolysis persists even in the presence of sufficient oxygen supply (aerobic glycolysis; Warburg, 1924)⁽⁶⁾. An aerobic glycolysis can either be blocked by application of enzyme inhibitors or promoted by experimental co-factor activation of transketolase enzymes^(11,13). Therefore, transketolases are assumed to be key enzymes for this specific tumor metabolism. Recently, a novel transketolase like enzyme (TKTL1) was found to be expressed at high levels in several malignancies, but ovarian cancer has not been investigated in this respect yet.⁽¹¹⁾ Our data on ovarian carcinoma are based on immunohistochemistry and reveal significant increased expression of TKTL1 in ovarian carcinoma compared to normal ovaries and borderline tumors of the ovary. Interestingly, the serous-papillary subtype of ovarian cancer showed a notably higher expression of TKTL1 than the mucinous subtype. Serous-papillary ovarian carcinomas have the worst prognosis when compared to other histological subtypes^(16,17). Thus, we evaluated whether TKTL1 is correlated with factors predicting an unfavorable outcome in general or just for the serous-papillary subtype. We found that high TKTL1 expression correlates with factors of poor prognosis like initial high FIGO stage, initial ascites, high histological grading and increased age of patients in this subtype. In contrast, the mucinous ovarian carcinomas, which have a more favorable outcome, showed no correlation of TKTL1 expression to the prognostic factors investigated. Therefore, a role for TKTL1 in tumor progression is very likely and TKTL1 expression might serve as a prognostic marker in this disease. According to other authors, the changes on the path toward tumorigenic conversion can be interpreted as a Darwinian process⁽¹⁸⁾. Gatenby and Gillies suggest, that in tumors anaerobic conditions are often present⁽¹⁸⁾. Therefore non-oxidative glucose metabolism can be of advantage for proliferation and survival of tumor cells. The relevance of this finding was recently confirmed by Fantin et al., who demonstrated that the inhibition of glycolysis results in a decline of tumor growth⁽¹⁹⁾. However, non-oxidative degradation of glucose is less efficient in energy production than the oxidative phosphorylation pathway in mitochondria. Despite this disadvantage, the non-oxidative pathway is much faster, independent of mitochondrial function and results in generation of high amounts of lactate which is secreted into the tumor stroma⁽¹⁹⁾. According to Park et al. and others the tissue acidosis generated by

the high lactate concentration may lead to a p53 mediated cell death of healthy cells, whereas tumor cells with mutated p53 can easily survive.^(20,21,22) The energetic benefit for tumor cells, that produce the required ATP via non-oxidative pathways is underlined by Ramanathan et al. who showed that the complete inhibition of mitochondrial, and therefore oxidative ATP production, does not result in significant decrease of ATP levels in tumor cell lines⁽⁹⁾.

The elevated levels of TKTL 1 in ovarian cancer may therefore be a selection advantage, because transketolase activities enable oxygen-independent glucose degradation⁽¹²⁾. As shown by Comin-Anduix et al. the induction of transketolase enzyme activities by application of thiamine stimulates tumor growth⁽¹³⁾. In addition some authors found an increased expression for GLUT transporter proteins in ovarian cancer, enabling the tumor cells to easily require large amounts of glucose⁽⁶⁻⁸⁾. Both glucose usage and lactate production represent markers, indicating poor prognosis in different malignant tumor types⁽²³⁻²⁶⁾.

The fact that ovarian carcinomas display increased expression of TKTL1, can be seen as potential contribution to the aggressive behavior of these tumors. It can be suggested that the described facilitated glucose uptake by GLUT-transporter enzymes in combination with the advantage in non-oxidative glucose metabolism may be of favorably for in tumor growth and tumor progression.

TKTL1 could thus have a possible value as target for non-toxic antitumoral therapeutic approaches especially in advanced ovarian cancer, a disease where really efficient treatment options are limited.

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Table 1

Scores	0	1	2	3
numbers (n) of serous-papillary carcinomas	4	4	5	7
(n) mucinous carcinomas	15	0	4	2
(n) borderline tumors (serous-papillary/mucinous)	7/7	3/1	0/0	0/0
(n) normal ovaries	21	3	0	0

Table 1: Scores of immunohistochemical staining in ovarian carcinomas (serous- papillary and mucinous), borderline tumors and normal ovaries

Table 2

TKTL1 score	FIGO I	FIGO II	FIGO III	FIGO IV	Initial ascites	Histological Grading	Age of patients
2/3	24,9 %	8,3 %	49,9%	16,7%	41,7 %	Mean: 1,9	Mean: 61,8 years
0/1	50 %	12,5%	37,5%	none	37,5 %	Mean: 1,5	Mean: 57,9 years

Table 2: Analysis of prognostic parameters in patients suffering of serous-papillary ovarian carcinomas in correlation to TKTL1 expression

Table 3

TKTL1 score	FIGO I	FIGO II	FIGO III	FIGO IV	Initial ascites	Histological Grading	Age of patients
2/3	100 %	none	none	none	none	Mean: 1,0	Mean: 54,5 years
0/1	63,6%	9,1 %	27,3%	none	36 %	Mean: 1,5	Mean: 44,5 years

Table 3: Analysis of prognostic parameters in patients suffering of mucinous ovarian carcinomas in correlation to TKTL1 expression

Figure legend 1:

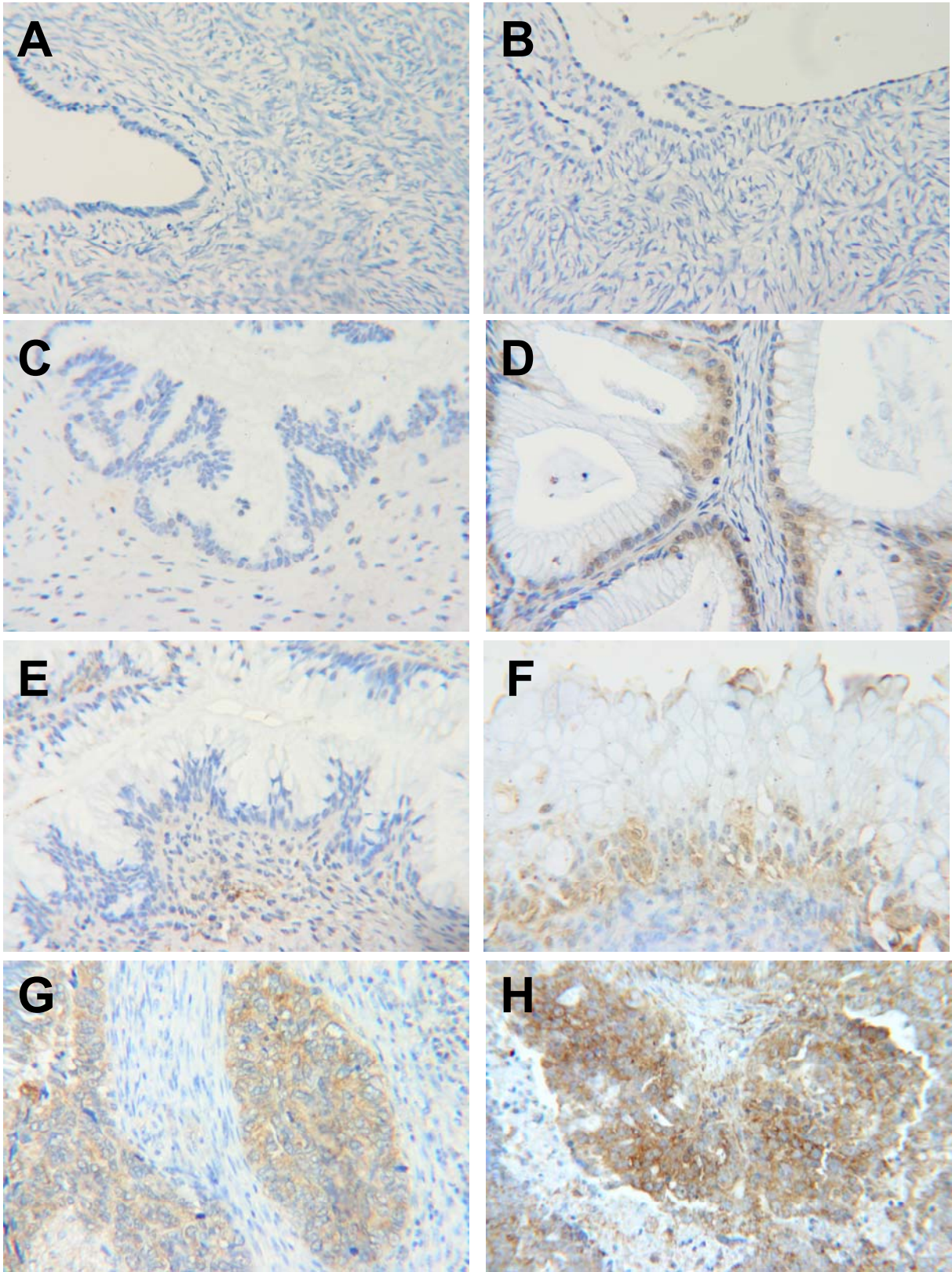


Figure 1: Expression of TKTL1 in normal ovaries (A, B score 0), borderline tumors (C score 0, D score 1), mucinous (E, F score 2) and serous-papillary (G, H score 3) ovarian

carcinomas. Expression of TKTL1 is localized in the cytoplasm. Original magnification is x 250.

Figure legend 2

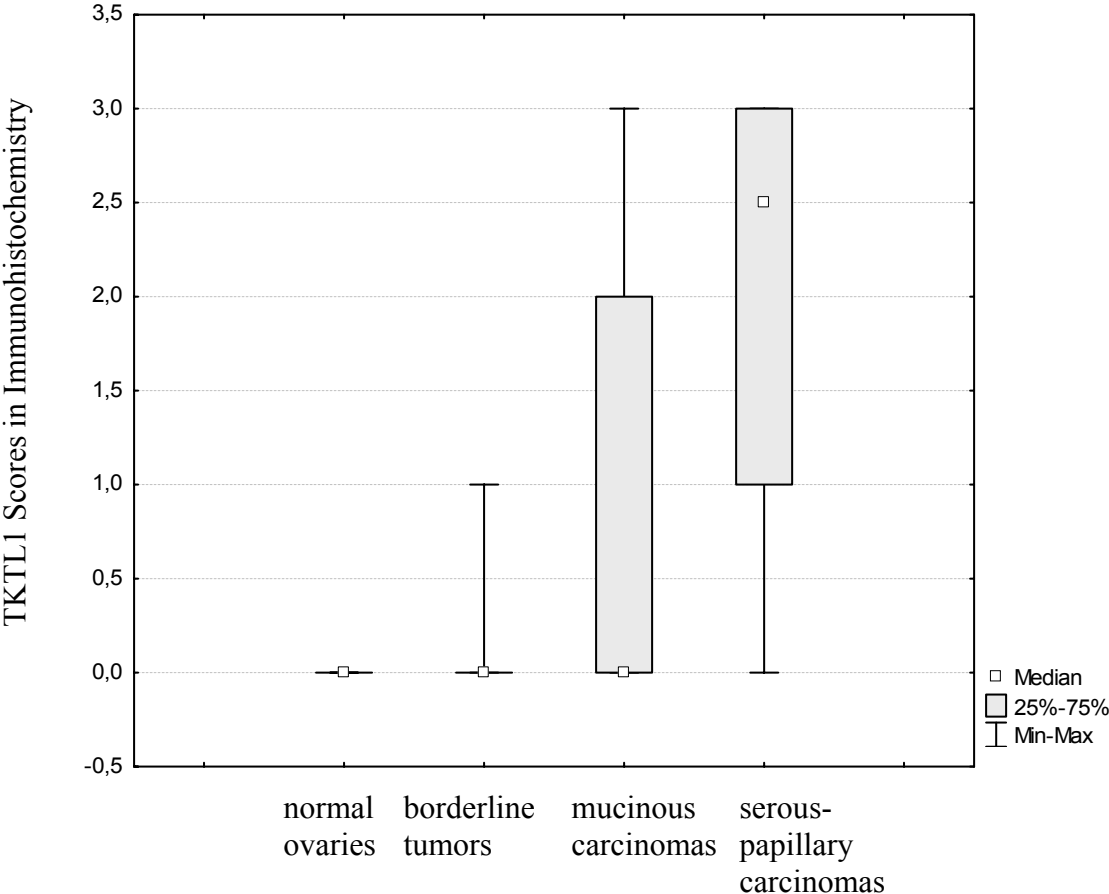


Figure 2: Expression of TKTL1 detected by immunohistochemistry in paraffin sections of normal ovaries, borderline tumors (mucinous and serous-papillary subtype), mucinous and serous-papillary ovarian carcinomas