

# Expression of cancer testis genes in gastric neoplasms — a preliminary study

Michał Czerewaty<sup>1</sup>, Maciej Tarnowski<sup>2</sup>, Krzysztof Safranow<sup>3</sup>, Elżbieta Uraśińska<sup>4</sup>,  
Bernardeta Chajnowska<sup>4</sup>, Andrzej Pawlik<sup>1</sup>

<sup>1</sup>Department of Physiology, Pomeranian Medical University, Szczecin, Poland

<sup>2</sup>Department of Physiology in Health Sciences, Pomeranian Medical University, Szczecin, Poland

<sup>3</sup>Department of Biochemistry and Medical Chemistry, Pomeranian Medical University, Szczecin, Poland

<sup>4</sup>Department of Pathomorphology, Pomeranian Medical University, Szczecin, Poland

**Introduction.** Scientists are currently searching for new and improved diagnostic markers and treatment modalities for gastric cancer. One putative target are the cancer/testis genes (CTGs), whose expression is restricted to male germ cells, trophoblasts and ovaries. CTGs are also aberrantly expressed in several types of cancers. In healthy somatic tissues, CTGs expression is either not detected or present at low levels. About 270 CTGs have been described thus far. The aim of this study was to investigate the expression levels of CTGs in stomach tissue samples from patients with gastric neoplasm, in relation to selected clinical and pathomorphological parameters.

**Material and methods.** 28 patients with histologically confirmed gastric neoplasms were included in this study. Total RNA was extracted from homogenates using the RNeasy Fibrous Tissue Mini Kit in accordance with the manufacturer's protocol. A quantitative assessment of mRNA levels for 35 genes was performed using real-time RT-PCR.

**Results.** We report that 11 out of 35 CTGs tested (*ATAD2*, *FBXO39*, *HORMAD1*, *IGSF11*, *IL13RA2*, *KIF2C*, *LDHC*, *OIP5*, *PLU1*, *SPAG9* and *TTK*) were significantly ( $p \leq 0.05$ ) overexpressed in tumour tissue compared with healthy stomach samples isolated from the same patients. Additionally, our results indicated that overexpression of *OIP5* was associated with gastric adenocarcinoma in women. Moreover, two of the tested CTGs (*HORMAD1*, *TTK*) were significantly overexpressed in tubular gastric adenocarcinoma. Additional analysis showed a correlation between *KU-CT-1* expression in gastric adenocarcinoma and patient age at diagnosis.

**Conclusions.** Our results suggest that the overexpression of CTGs may be specific for gastric neoplasms, but it should be confirmed in larger numbers of patients.

**Keywords:** gastric neoplasm, genes, biomarkers, CTGs, expression

## Introduction

For over a century, medical researchers have been searching for tumour markers. An ideal tumour marker is a marker with expression limited to the tumour tissue and, thus, could play a key role in the development of new methods aimed at

stimulating the body's immune response against cancer cells. Over 160 years ago, Rudolf Virchow hypothesised that a certain pool of embryonic cells remains dormant in adult tissues and, upon reactivation, gives rise to various tumours [1]. In 1902, John Beard put forward a theory that all tumours originate from

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embryonic cells, basing his theory on observations of early placental development during pregnancy. Beard noticed that the formation of the placenta in the uterus resembles carcinogenesis, and that this process stops when enzymes produced by the foetus appear; otherwise, choriocarcinoma occurs [2]. In the 1970s, long-term research on this issue resulted in the discovery of cancer/testis antigens (CTAs) encoded in the human body by a heterogeneous group of cancer/testis genes (CTGs) [3]. According to the Ludwig Institute for Cancer Research Database, more than 270 CTGs have been identified thus far [4]. Some CTGs are located on the X chromosome and these encode the most immunogenic CTAs. These CTGs constitute more than 10% of the genes on the X chromosome, where they form so-called "gene families". Additional CTGs are located on the autosomes as well as the Y chromosome and most often occur in the form of single gene copies [5]. The high expression of CTGs is limited to multiple tumour types and their function is still largely unknown. Their limited expression in healthy tissue has made them potential candidates for biomarkers of gastric neoplasms. The function of the proteins encoded by CTGs is largely unknown, however, most often they are associated with meiosis and gametogenesis [6, 7]. Cancer/testis gene overexpression plays a key role in the processes of angiogenesis, metastasis, inhibition of apoptosis and cell proliferation in tumour tissues [8]. Moreover, overexpression of certain CTGs may produce differing effects in individual cells and tumour lines [9]. Cancer/testis genes encode surface antigens that can potentially be presented to the immune system with, among others, Class I and II human leukocyte antigens (HLAs). This process may lead to humoral responses as well as anti-tumour cytotoxic T cell effects against cancer cells [10]. Therefore, finding further immunodominant determinants for CTAs may be particularly important clinically [11]. Regulation of CTAs is associated with epigenetic mechanisms that either lead to changes in methylation of promoter regions or changes within histones. These mechanisms are part of a larger program of gene changes during carcinogenesis [10, 12, 13]. A large role in the carcinogenesis process is currently attributed to CTAs, particularly those encoded by CTGs located on the X chromosome. Expression of these CTAs is often characterized by high immunogenicity and is limited to malignant lesions [2].

Certain MAGE-A antigens may either regulate ubiquitin E3 ligase activity or disrupt cancer cell apoptosis via binding to procaspase-12 [8, 14]. Additional oncogenic functions have also been associated with cell proliferation through excessive levels of cyclins D1 and E. CTAs also affect genome instability resulting from chromosomal aberrations occurring during mitosis. CTAs may also be important in angiogenesis, a key process in metastasis [8, 15].

Gastric cancer is most often diagnosed at an advanced stage, which makes it one of the most common causes of death among cancer patients. More than half of all cases of gastric cancer occur in underdeveloped countries; the highest rates

are in Eastern Europe, South America and East Asia, while the lowest incidence rates occur in Western Europe and North America [16]. Cancer diagnosis is difficult and involves a wide range of tests for accurate confirmation. Up to 75–85% of patients diagnosed with gastric cancer die within 5 years of disease onset [17]. Surgery is the most common treatment method, giving the best chance of recovery if the disease is diagnosed at an advanced stage. Chemotherapy and radiation therapy often constitute supplementary treatment, usually in a palliative manner. Early diagnosis is extremely important in disease management, and such diagnosis can be achieved by gastroscopy and histopathology. Many studies on new treatment methods have been conducted to investigate their effectiveness, including the use of molecularly-targeted drugs and CTGs may constitute one of the objectives [17, 18].

In the present study, the expression levels of 35 CTGs were determined in gastric neoplasm tissue from patients. The gene panel was determined based on the available literature, however, a decision was made to also include several new CTGs that are potential gastric tumour marker candidates. Our objective was to answer the question of whether certain CTGs may fit the previously mentioned biomarker specifications for specific gastric cancers. The results of our study suggest that expression levels of certain CTGs correlate with an increased risk of this disease. Our findings indicate that the research is still in its early phase. Our preliminary results are the first step in our research process. They show which genes should be confirmed in larger numbers of patients.

## Material and methods

### Patients

All samples were collected at the Department of Gastroenterology, Pomeranian Medical University in Szczecin. 28 patients with newly diagnosed gastric neoplasms were included in the study. The median age of the patients was 68 years (range 33–82 years) and 57% of the patients were male (Tab. I). All patients gave written informed consent to participate in the study. The study conforms with The Code of Ethics of the World Medical Association (Declaration of Helsinki), printed in the British Medical Journal (18 July 1964). 19 samples (68%) were taken from the proximal part of the stomach and 9 samples (32%) were taken from the distal part of the stomach. The entire material collected during the research process included: 16 adenocarcinomas (14 intestinal-type, 2 diffuse type), 6 adenomas, 4 lymphomas [anaplastic large cell lymphoma (ALCL), diffuse large B-cell lymphoma (DLBCL), mucosa-associated lymphoid tissue (MALT), Burkitt's lymphoma) and 2 neuroendocrine tumours (G1 and G2). No patients included in the study had co-existing tumours and none of the patients received radiotherapy or immunotherapy before biopsy. The final diagnosis was made by the gastroenterologist after histopathological and gastroscopic examination. Two samples were taken from each patient at the time of the gastroscopy;

one sample from the gastric neoplasia and the other from normal stomach mucosa, located 5 cm away from the tumour's edge. Patient data are summarized in Table I.

### RNA isolation

Tissue fragments were cut into small fragments and immediately stored in RNAlater® Solution (Invitrogen) at -80 °C until the time of genetic analysis. Samples were homogenized with the Ultra-Turrax T-10 basic (IKA®) dispersing tool in 600 µl RLT buffer (Qiagen) for 4 min at 30,000 rpm/min. Total RNA was extracted from homogenates using the RNeasy Fibrous Tissue Mini Kit (Qiagen) in accordance with the manufacturer's protocol. The concentration and purity of RNA samples was determined by measuring the absorbance using

a spectrophotometer Perkin Elmer Lambda Bio+ (PerkinElmer). The obtained RNA was used for the reverse transcription reaction. 0.5 µg of RNA from each sample was reverse transcribed into cDNA with the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) according to the manufacturer's instructions. The total reaction volume for each sample was 20 µL cDNA.

### Real-time quantitative reverse transcription PCR (RQ-PCR)

Quantitative expression analysis of the selected genes, as well as the beta2-microglobulin reference gene, was performed using real-time reverse-transcription polymerase chain reaction (RT-PCR) on an ABI PRISM® Fast 7500 Sequence Detection System (Applied Biosystems). Real-time conditions

**Table I.** Clinicopathological characteristics of patients enrolled in the study

Feature	N
<b>Age</b>	Median value = 68 (range 33–82)
Gender	
Female	12
Male	16
Location	
Proximal	19
Distal	9
<b>Types of neoplasia</b>	
Adenocarcinomas	16
WHO classification (2019)	
Tubular	11
Papillary	1
Mucinous	3
Tubular/mucinous	1
Laurén classification	
Intestinal	14
Diffuse	2
Grading	
G1	5
G2	2
G3	1
Unknown	8
Gastritis	
Positive	5
Negative	11

Feature	N
Intestinal metaplasia	
Present	5
Absent	11
Peptic ulcer disease	
Present	3
Absent	13
Adenomas	
WHO classification (2019)	6
Tubular (minoris)	4
Tubular (minoris et majoris)	1
Tubular/villosum (minoris)	1
Neuroendocrine tumors	
Histologic grade (2019)	
NET G1	1
NET G2	1
Gastric lymphoma	
WHO classification (2019)	4
Anaplastic large-cell lymphoma	1
Diffuse large B-cell lymphoma	1
Mucosa associated lymphoid tissue lymphoma	1
Burkitt's lymphoma	1

WHO — World Health Organization; NET — neuroendocrine tumor

were as follows: 95°C (15 sec), 40 cycles at 95°C (15 sec) and 60°C (1 min). Melting point analysis confirmed only one PCR product under these conditions. To normalise mRNA levels between different samples, we used  $\beta$ -2 microglobulin as a reference gene. Each sample was analysed in two technical replicates. To calculate the values, two methods were used. Absolute expression ( $2^{-\Delta Ct}$  method) and relative expression ( $2^{-\Delta\Delta Ct}$  method).

### Statistical analysis

Statistical analysis was performed with STATISTICA Version 12.5 data analysis software system. Data were analysed as gastric neoplasms absolute expression (AE) and gastric neoplasms relative expression (RE) to normal tissue in the same patient, calculated as the ratio of expression levels: neoplastic tissue/normal tissue. The Mann-Whitney test was used to compare CTG expression between tumour types. Cancer/testis gene expression data

**Table II.** Comparison between cancer/testis genes (CTGs) expressions in normal and adenocarcinoma tissue obtained during gastroscopy. RNA was isolated from cancer tissue and normal tissue located ~5 cm away and reverse transcribed and CTG expression was analyzed by real-time polymerase chain reaction (PCR). Differences with p value  $\leq 0.05$  (Wilcoxon signed-rank test) are in bold

	CTGs	Median	IQR	p value
Adenocarcinoma tissue versus normal	<i>CAGE1</i>	1.617	2.582	0.88
	<i>MAGEA2</i>	16.101	447.765	1
	<i>MAGEA1</i>	2.294	395.437	0.17
	<b><i>TTK</i></b>	2.13	6.367	<b>0.003</b>
	<i>NY-ESO-1</i>	7.122	279.465	0.33
	<i>MAGEA3</i>	2.309	451.499	0.17
	<i>CXorf48</i>	1.5	6.538	0.79
	<i>DKKL1</i>	2.847	10.939	0.22
	<b><i>OIP5</i></b>	1.781	3.654	<b>0.002</b>
	<i>KU-CT-1</i>	0.265	0.984	0.18
	<b><i>FBXO39</i></b>	3.305	7.021	<b>0.039</b>
	<i>CAGE</i>	1.652	6.062	0.5
	<i>HAGE</i>	1.434	5.883	0.39
	<i>RGS22</i>	0.965	2.676	0.38
	<i>SSX4</i>	1.901	18.467	0.37
	<b><i>PLU1</i></b>	2.021	1.551	<b>0.001</b>
	<i>PLAC1</i>	1.969	3.312	0.2
	<b><i>LDHC</i></b>	3.586	20.314	<b>0.007</b>
	<i>CTAGE1</i>	1.966	37.56	0.93
	<i>SPAG4</i>	1.567	0.924	0.14
	<i>CCDC110</i>	0.673	1.2	0.26
	<i>SPA17</i>	1.385	1.957	0.08
	<b><i>SPAG9</i></b>	1.962	2.429	<b>0.01</b>
	<i>MAGEB6</i>	1.561	18.177	0.23
	<i>MAGEA11</i>	2.556	11.463	0.46
	<b><i>HORMAD1</i></b>	6.755	8.353	<b>0.001</b>
	<i>PRSS55</i>	0.805	1.727	0.73
	<b><i>IL13RA2</i></b>	1.785	1.231	<b>0.003</b>
	<i>HORMAD2</i>	1.713	201.877	0.24
	<b><i>KIF2C</i></b>	2.958	8.916	<b>0.001</b>
	<b><i>IGSF11</i></b>	0.478	0.575	<b>0.035</b>
	<i>SYCP1</i>	1.617	8.081	0.64
<i>CALR3</i>	1.324	2.666	0.64	
<i>SPAG1</i>	1.076	1.484	0.54	
<b><i>ATAD2</i></b>	3.712	5.838	<b>0.002</b>	

Differences with p value  $\leq 0.05$  (Wilcoxon signed-rank test) are in bold; IQR — interquartile range

were compared between samples from adenocarcinoma tissue and normal stomach mucosa with the Wilcoxon signed-rank test. Correlations between CTG expression and patient age were analysed using the Spearman rank correlation coefficient ( $R_s$ ). The relationships between CTG expression in patients with gastric adenocarcinoma and other clinical data were analysed using the Mann-Whitney test, and  $p \leq 0.05$  was considered statistically significant.

## Results

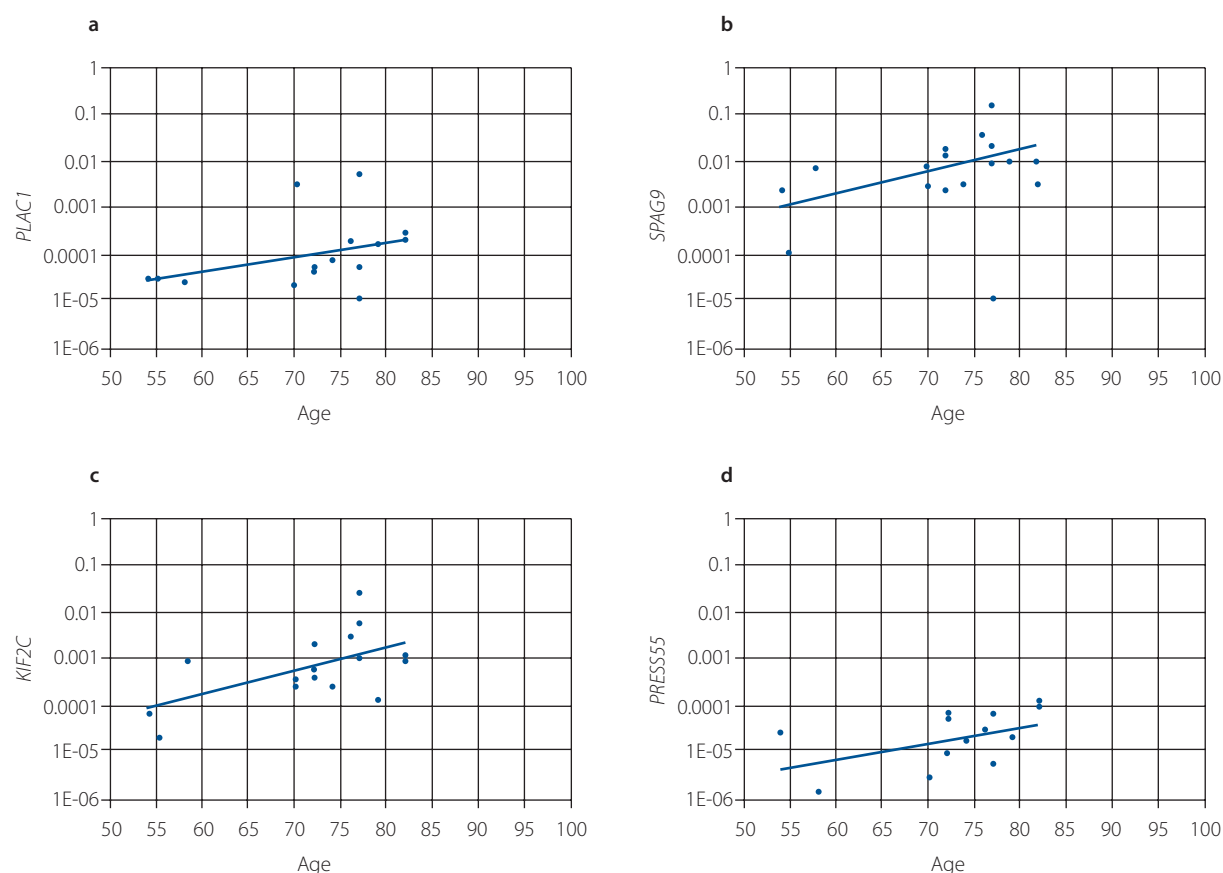
### Cancer/testis gene expression in clinical samples

Sixteen adenocarcinomas, 6 adenomas, 4 lymphomas and 2 neuroendocrine tumours were confirmed histologically from the collected samples (Tab. I). We designed a panel of CTG candidates, including those previously shown to be expressed in gastric cancer [19–24] as well as some promising new targets, known to be expressed in various cancers [25–29]. A detailed statistical analysis was made only for adenocarcinoma, as this was the largest group.

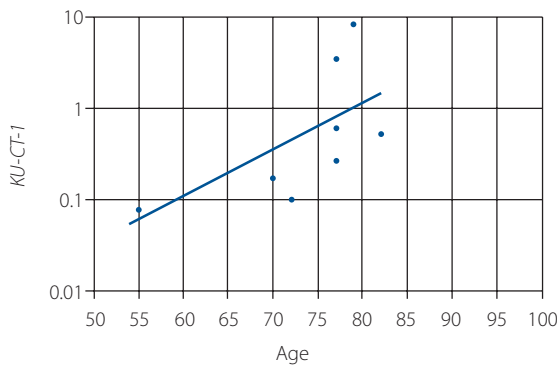
### Cancer/testis gene expression in gastric adenocarcinoma

Analyses of relative CTG expression in adenocarcinoma tissue versus healthy tissue (taken 5 cm from the tumour's edge)

was carried out using the Wilcoxon signed-rank test and demonstrated statistically significant differences for 11 genes: *TTK*, *OIP5*, *FBXO39*, *PLU1*, *LDHC*, *SPAG9*, *HORMAD1*, *IL13RA2*, *KIF2C*, *IGSF11* and *ATAD2* (Tab. II). A correlation between CTG overexpression and patient age at which the gastric adenocarcinoma developed was also shown. The Spearman's rank correlation coefficient showed that AE for the genes *PLAC1*, *SPAG9*, *PRSS55* and *KIF2C* (Fig. 1) in tumour tissue increases with age. Additionally, increased relative expression of the gene *KU-CT-1* was observed in the tumour tissue (Fig. 2). Analyses of potential correlations between CTG expression and patient sex were then performed. Positive correlations were observed between adenocarcinoma occurrence in women and relative expression of the genes *OIP5* ( $p = 0.050$ ) and *HAGE* ( $p = 0.025$ ) (Tab. III). Additionally, patients were divided into 2 groups according to the histological type of adenocarcinoma: tubular and non-tubular. Overexpression of the genes *CAGE1*, *TTK*, *SPA17*, *SPAG9*, *MAGEB6* and *HORMAD1* was typical for tubular adenocarcinomas. When only AE was considered, significant results were obtained for the gene *RGS22* in non-tubular adenocarcinomas ( $p = 0.036$ ) (Tab. III). Due to the small number of patients with G2 and G3 malignancies, the decision was made to combine them into a single group for analysis. The AE of *RGS22* was, however, significantly higher in the G2–G3 group,



**Figure 1.** Positive correlation between absolute expressions (AE) of (A) *PLAC1* ( $R_s = 0.55$ ;  $p = 0.029$ ), (B) *SPAG9* ( $R_s = 0.53$ ;  $p = 0.036$ ), (C) *KIF2C* ( $R_s = 0.54$ ;  $p = 0.029$ ); (d) *PRSS55* ( $R_s = 0.60$ ;  $p = 0.013$ ) in adenocarcinoma tissue and patients' age. The Spearman rank correlation coefficient was used to analyze the correlation between age and cancer/testis gene (CTG) expression level



**Figure 2.** Positive correlation between relative expression (RE) of *KU-CT-1* ( $R_s = 0.76$ ;  $p = 0.006$ ) in adenocarcinoma tissue and patients' age. The Spearman rank correlation coefficient was used to analyze the correlation between age and cancer/testis gene (CTG) expression level

while the AE of *MAGEB6* was higher in the G1 group (Tab. III). In addition, the AE of *PLU1* and RE of *SSX4* and *CTAGE1* were typical for patients with adenocarcinoma without gastritis (Tab. III). Interestingly, the same patients were characterized by the absence of intestinal metaplasia, suggesting that the absence of intestinal metaplasia corresponds to increased expression of the same CTGs. Absolute overexpression of the gene *HORMAD2* (Tab. III) was typical for patients with gastric ulcers. No statistically significant differences in CTG expression in patients with adenocarcinoma were found based on the location from which the sample was collected or the occurrence of endothelial neoplasia.

#### **Expression of cancer/testis genes in gastric adenocarcinoma and adenoma**

We compared the expression of all the previously selected CTGs in gastric adenocarcinoma cases versus gastric adenoma. We noticed a much higher statistically significant RE in adenocarcinomas for the genes *CAGE1*, *FBXO39* and *PLU1* (Tab. IV).

#### **Expression of cancer/testis genes in gastric adenocarcinoma and gastric lymphoma**

In further statistical analysis, we compared the expression of all the previously selected CTGs in gastric adenocarcinomas with the expression of the same genes in gastric lymphomas. We noticed a much higher statistically significant RE in adenocarcinomas for *SPAG4* (Tab. V).

#### **Expression of cancer/testis genes in gastric adenoma and gastric lymphoma**

Finally, we compared the expression levels of all the previously selected CTGs in gastric adenomas with the expression of the same genes in gastric lymphomas. Again, a much higher statistically significant RE was observed in lymphomas for *SPAG4* (Tab. VI).

## **Discussion**

The search for genetic markers that could potentially serve as characteristic biomarkers for specific cancer types has been ongoing for decades [30]; this was also one of the most important objectives of our current research. The salient observation of our study is the identification of new genes correlated with gastric neoplasms. We believe that the above-mentioned changes in gene expression can be considered as potential prognostic biomarkers. Moreover, our findings demonstrate that CTGs may be involved in development of gastric neoplasms. All these suggestions must be confirmed in larger numbers of patients.

First, we verified whether overexpression of the selected CTGs is limited to cancer tissues. If the antigens coded by these CTGs are also capable of invoking an immune response against cancer cells, it would place these markers in a group of very important molecules. We analysed the expression of CTGs in cancerous tissue, as well as healthy tissue, in patients with gastric adenocarcinoma. We examined the relationships between CTG expression in patients with gastric adenocarcinoma and the clinical course of their disease. Finally, we assessed differences in the expression of selected CTGs between different types of gastric neoplasm: adenocarcinoma and adenoma, lymphoma and adenoma, and adenocarcinoma and lymphoma.

Our results indicate a statistically significant overexpression of *TTK* ( $p = 0.003$ ) in gastric adenocarcinoma compared to healthy tissue from the same patient. However, this increased expression was not characteristic of all tissues (a difference greater than 2 was observed in 9 of the 16 adenocarcinoma samples). Interestingly, we previously obtained very similar results for *TTK* ( $p < 0.001$ ) in a similar study in patients with colorectal cancer [31]. High microsatellite instability is believed to induce mutations in many genes, including *TTK*, thereby causing cancer. Additionally, common frameshift mutations in the *TTK* gene have been confirmed in gastric and colorectal cancers [19, 32]. Moreover, these frameshift mutations correlate with increased expression of programmed cell death ligand 1 (PD-L1) in gastric cancers [33]. A very low level of *TTK* expression in normal, healthy gastric mucosa is confirmed not only by our study but also by Mills et al. [34]. In contrast, results obtained by Wang et al. [35] indicate that high expression of the *TTK* gene in gastric adenocarcinoma correlates with a better patient survival rate. These findings would explain why the results obtained here, where the RE of *TTK* was characteristic of tubular adenocarcinoma ( $p = 0.047$ ), gave a much more favourable 5-year survival chance from disease diagnosis than seen for mucinous adenocarcinoma [36].

Interestingly, for tubular adenocarcinoma, a characteristic RE of *HORMAD1* has also been shown. Aung et al. [37] confirmed that the expression of *HORMAD1* in gastric cancer

**Table III.** Analysis of cancer/testis genes (CTGs) expression in relation to clinicopathological features

CTGs	Clinical feature		RE			AE		
			Median	IQR	p value	Median	IQR	p value
<i>OIP5</i>	Gender	F	2.844841	6.275311	<b>0.05</b>	0.000852	0.002245	0.49
		M	1.377583	0.796944		0.000687	0.001115	
<i>HAGE</i>		F	6.136988	11.77483	<b>0.025</b>	0.000082	0.001166	0.63
		M	0.869812	1.165361		0.000207	0.000143	
<i>CAGE1</i>	Histological type of adenocarcinoma	T	1.675143	2.930174	<b>0.047</b>	0.000026	0.000048	0.16
		NT	0.412256	0.455462		0.000003	0	
<i>TTK</i>		T	2.695605	6.463068	<b>0.047</b>	0.00323	0.003909	0.61
		NT	1.136899	0.842932		0.001927	0.000984	
<i>RGS22</i>		T	0.735397	1.218641	0.19	0.000005	0.000011	<b>0.036</b>
		NT	1.497796	4.473127		0.000023	0.000168	
<i>SPA17</i>		T	2.088386	2.120276	<b>0.015</b>	0.001396	0.006806	0.78
		NT	0.666488	0.253483		0.001641	0.003005	
<i>SPAG9</i>		T	2.201953	2.624137	<b>0.047</b>	0.009472	0.015327	0.13
		NT	0.658078	0.978798		0.002955	0.003761	
<i>MAGEB6</i>		T	9.616402	18.46288	<b>0.02</b>	0.000052	0.000138	0.46
		NT	0.748223	0.522369		0.000014	0.000025	
<i>HORMAD1</i>		T	7.177859	7.207974	<b>0.05</b>	0.000159	0.000497	0.19
		NT	1.969562	5.07031		0.000029	0.000125	
<i>RGS22</i>	Grading	G1	0.3778127	0.668473	0.18	0.000001	0.000005	<b>0.025</b>
		G2–G3	3.5319258	6.8011378		0.000028	0.000054	
<i>MAGEB6</i>		G1	5.6743783	15.689210	0.3	0.000072	0.000103	<b>0.025</b>
		G2–G3	0.5364804	5.6297952		0.000013	0.000014	
<i>SSX4</i>	Gastritis	GP	2.632287	17.51189	<b>0.025</b>	0	0.000002	0.53
		GN	0.594935	0.530141		0	0.000001	
<i>PLU1</i>		GP	2.021293	1.402366	0.53	0.011223	0.006718	<b>0.036</b>
		GN	1.190566	1.631837		0.004511	0.003733	
<i>CTAGE1</i>		GP	4.482278	230.5981	<b>0.048</b>	0.000059	0.000065	0.21
		GN	0.273115	0.799865		0.000004	0.000023	
<i>CTAGE1</i>	Intestinal metaplasia	IMP	4.482278	230.5981	<b>0.048</b>	0.000059	0.000065	0.21
		IMA	0.273115	0.799865		0.000004	0.000023	
<i>SSX4</i>		IMP	2.632287	17.51189	<b>0.025</b>	0	0.000002	0.53
		IMA	0.594935	0.530141		0	0.000001	
<i>PLU1</i>		IMP	2.021293	1.402366	0.53	0.011223	0.006718	<b>0.036</b>
		IMA	1.190566	1.631837		0.004511	0.003733	
<i>HORMAD2</i>	Peptic ulcer disease	P	1.043124	300.7225	0.93	0.000005	0.000008	<b>0.034</b>
		N	1.520991	2.436342		0.000017	0.000017	

Differences with p value ≤ 0.05 (Mann-Whitney U test) are in bold; RE — relative expressions; AE — absolute expression; IQR — interquartile range; F — female; M — male; T — tubular; NT — non-tubular; GP — gastritis positive; GN — gastritis negative; IMP — present intestinal metaplasia; IMA — absent intestinal metaplasia; P — positive peptic ulcer disease; N — negative peptic ulcer disease

**Table IV.** Comparison between cancer/testis gene (CTG) expressions in adenocarcinoma and adenoma

CTGs		RE			AE		
		Median	IQR	p value	Median	IQR	p value
<i>CAGE1</i>	ADMA	0.468405	0.694321	<b>0.027</b>	0.000007	0.000026	0.61
	ADCA	1.593094	2.347909		0.000016	0.000048	
<i>FBXO39</i>	ADMA	0.799568	1.369141	<b>0.012</b>	0.000126	0.000116	0.16
	ADCA	3.123984	5.828531		0.000249	0.000655	
<i>PLU1</i>	ADMA	0.873484	0.81754	<b>0.033</b>	0.010759	0.012407	0.82
	ADCA	1.926644	1.492435		0.010307	0.009317	

Differences with p value  $\leq 0.05$  (Mann-Whitney U test) are in bold; RE — relative expressions; AE — absolute expression; IQR — interquartile range; ADMA — adenoma; ADCA — adenocarcinoma

**Table V.** Comparison between cancer/testis gene (CTG) expressions in adenocarcinoma and lymphoma. Differences with p value  $\leq 0.05$  (Mann-Whitney U test) are in bold.

CTGs		RE			AE		
		Median	IQR	p value	Median	IQR	p value
<i>SPAG4</i>	ADCA	1.432069	0.997371	0.07	0.000082	0.000204	<b>0.047</b>
	LYMP	0.529842	0.401008		0.000001	0.000018	

Differences with p value  $\leq 0.05$  (Mann-Whitney U test) are in bold; RE — relative expressions; AE — absolute expression; IQR — interquartile range; ADMA — adenoma; ADCA — adenocarcinoma

**Table VI.** Comparison between cancer/testis gene (CTG) expressions in adenoma and lymphoma

CTGs		RE			AE		
		Median	IQR	p value	Median	IQR	p value
<i>SPAG4</i>	ADMA	1.02886	2.371437	0.29	0.000155	0.000916	<b>0.033</b>
	LYMP	0.529842	0.401008		0.000001	0.000018	

Differences with p value  $\leq 0.05$  (Mann-Whitney U test) are in bold; RE — relative expressions; AE — absolute expression; IQR — interquartile range; ADMA — adenoma; LYMP — lymphoma

is much higher than in 14 other non-cancerous tissues (including stomach tissue). These findings are in accordance with our results, which show that the RE of *HORMAD1* was higher in the adenocarcinoma tissue than in the healthy stomach tissue in 14/16 adenocarcinoma patients.

Expression of the *OIP5* gene has been confirmed in many stomach cancer cell lines, including: SNU1, SNU16, SNU216, SNU638 and AGS [20]. Studies of other groups have indicated that the *OIP5* is expressed in the gastric adenocarcinoma [38] and colorectal cancer [31]. Similar observations were made in our study. The RE of the *OIP5* gene was significantly increased in 7/16 patients with gastric adenocarcinoma ( $p = 0.002$ ). Interestingly, the RE correlated with gender and was higher in women ( $p = 0.050$ ). In another study, similar results were observed for patients with acute myeloid leukemia (AML), in a group where women constituted 41% of the examined individuals [25].

The RE of *FBXO39* in gastric adenocarcinoma was also found to be statistically significant when compared to healthy tissues from the same patients ( $p = 0.039$ ). Moreover, the RE of *FBXO39* was significantly higher in adenocarcinoma than in benign changes (adenoma) ( $p = 0.012$ ).

Equally high results for *FBXO39* were obtained in an analysis of colon cancer [31]. Interestingly, anti-*FBXO39* antibodies were not detected in the serum of patients with gastric cancer (antibodies were detected in 1/24 of patients), potentially making this CTG highly useful in research aimed at developing immune vaccines to stimulate immunogenicity against *FBXO39* [26]. On the other hand, according to Zheng et al. [39], knockout (gene silencing) of *FBXO39* promotes apoptosis and inhibits proliferation of cancer cells in the U-2OS cell line. Moreover, *FBXO39* predicts poor prognosis and correlates with tumour progression in cervical squamous cell carcinoma [40].



We found no statistically significant correlations between the RE of *PLU1* and either patient age at diagnosis or patient gender. Similarly, Wang et al. [21] found no connection between these parameters and the presence of the PLU1 protein in gastric cancer. Moreover, it has been found that the overexpression of *PLU1* is required in gastric cancer for proliferation and metastasis [21]. The results obtained by Wang et al. [21] correlate with our observations, in which the RE of *PLU1* was significantly higher in gastric adenocarcinoma than in benign changes (adenoma) ( $p = 0.033$ ).

Our results indicated a positive correlation between the RE of *KU-CT-1* and the age of the patient at gastric adenocarcinoma diagnosis ( $p = 0.006$ ). In our previous research, we also demonstrated a correlation between the AE of *KU-CT-1* and the age of the patient at diagnosis of colorectal cancer [31]. Nevertheless, the results of our work do not indicate a clear overexpression of *KU-CT-1* in gastric adenocarcinoma compared to healthy tissue. Similar results were obtained in a study by Okada et al. [27], where no expression of *KU-CT-1* was detected in any of the gastric or colorectal cancer tissue examined from the patients.

Another gene observed in our study to have increased expression in gastric adenocarcinoma is *LDHC*. Moreover, expression of *LDHC* has previously been demonstrated in breast, lung, ovarian, colorectal, cervical, thyroid, kidney and prostate cancers, as well as melanoma [41].

Additionally, we observed overexpression of the *SPAG9* gene in gastric adenocarcinoma samples ( $p = 0.010$ ) and, according to the results obtained by Miao et al. [22], these findings may correlate with poor prognosis or even disease relapse following recovery. Moreover, increased expression of the *SPAG9* gene has also been observed in ovarian cancer [28], colorectal cancer [29], hepatocellular carcinoma [42], lung cancer [43], AML [44], breast cancer [45] and cervical cancer [46].

It is worth noting that detection of the protein product *IL13RA2* using immunohistochemical methods may serve as an independent prognostic factor for gastric cancer detection following surgical resection [47]. Overexpression of *IL13RA2*, confirmed by us in adenocarcinoma tissue, is characteristic of many tumours, including glioblastoma multiforme, astrocytoma, and colorectal and pancreatic cancers [48–51].

The expression of *KIF2C* is associated with lymphatic invasion, lymph node metastases and poor survival in patients with gastric cancer [23]. Overexpression of *KIF2C* (understood as expression levels twice as high) in our study was confirmed in 10/16 patients with adenocarcinoma. Interestingly, the AZ521 duodenal adenocarcinoma cell line (which does not demonstrate *KIF2C* expression), demonstrated a high proliferation rate ( $p < 0.001$ ) and migration capacity ( $p < 0.001$ ) compared to sham-transfected cells when transfected with the *KIF2C* gene [23].

*IGSF11* expression has not been observed in many types of diffuse gastric cancer. On the other hand, it is believed that *IGSF11* may be a diagnostic marker for early-stage gastric cancer of the intestinal type [52, 53]. In our study, 14/16 adenocarcinoma samples were of the diffuse type. We observed higher RE of the *IGSF11* gene in lymphoma than in benign changes (adenoma).

Another gene analysed was *ATAD2*, whose function is associated with proliferation, invasion and cellular migration [54]. Numerous literature reports confirm the expression of *ATAD2* in gastric [24, 55], colorectal, breast, lung and uterine cancers [55]. Moreover, some subtypes of gastric cancer with drug resistance (GCIY, GPM1, MKN28) are characterized by high expression levels of *ATAD2*, thus expression of this gene is considered to be one cause of resistance to this drug [56].

## Conclusions

Our results suggest that the overexpression of *ATAD2*, *FBXO39*, *HORMAD1*, *IGSF11*, *IL13RA2*, *KIF2C*, *LDHC*, *OIP5*, *PLU1*, *SPAG9* and *TTK* may be specific for gastric adenocarcinoma. Moreover, we found that the overexpression of *HORMAD1* and *TTK* were positively correlated with tubular gastric adenocarcinoma. Additionally, we observed positive correlations between the RE of *KU-CT-1* and patient age in adenocarcinoma. Our study had some limitations. A study with a small number of patients may not have sufficient statistical power to detect significant differences between the healthy and study group. However, preliminary results show which genes should be confirmed in larger numbers of patients.

## Article information and declarations

### Data availability statement

Available upon request.

### Ethics statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee at the Pomeranian Medical University and the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

All patients gave written informed consent to participate in the study.

### Author contributions

Michał Czerewaty — conceptualization, writing — original draft preparation, writing — review & editing, methodology, investigation, data curation, formal analysis.

Maciej Tarnowski — supervision, investigation, validation.

Krzysztof Safranow — software, formal analysis.

Elżbieta Urańska — resources.

Bernardeta Chajnowska — resources.

Andrzej Pawlik — supervision.

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## Conflicts of interest

The authors declare no conflict of interest.

## Supplementary material

None.

## Michał Czerewaty

Department of Physiology  
Pomeranian Medical University  
ul. Powstancow Wlkp. 72  
70-111 Szczecin, Poland  
tel.: +48 91 466 16 11  
e-mail: [michal.czerewaty@wp.pl](mailto:michal.czerewaty@wp.pl)

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