Endokrynologia Polska
DOI: 10.5603/EP.a2016.0045
Tom/Volume 67; Numer/Number 4/2016
ISSN 0423–104X

Transcriptional activity of TGFβ1 and its receptor genes in thyroid gland

Aktywność transkrypcyjna genów TGFβ1 i ich receptorów w gruczole tarczowym

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Abstract

Introduction: Determination of gene-candidates' profile expression responsible for fibrosis, immunosuppression, angiogenesis, and neoplasia processes in the pathogenesis of thyroid gland disease.

Material and methods: Sixty-three patients underwent thyroidectomy: 27 with non-toxic nodular goitre (NG), 22 with toxic nodular goitre (TNG), six with papillary cancer (PTC), and eight with Graves' disease (GD). In thyroid tissues, transcriptional activity of TGFβ1 and its receptors TGFβRI, TGFβRII, and TGFβRIII genes were assessed using RT-qPCR (Reverse Transcriptase Quantitative Polymerase Chain Reaction). Molecular analysis was performed in tissues derived from GD and from the tumour centre (PTC, NG, TNG) and from peripheral parts of the removed lobe without histopathological lesions (tissue control). Control tissue for analysis performed in GD was an unchanged tissue derived from peripheral parts of the removed lobe of patients surgically treated for a single benign tumour.

Results/Conclusions: Strict regulation observed among transcriptional activity of TGFβ1 and their receptor TGFβRI-III genes in control tissues is disturbed in all pathological tissues – it is completely disturbed in PTC and GD, and partially in NG and TNG. Additionally, higher transcriptional activity of TGFβ1 gene in PTC in comparison with benign tissues (NG, GD) and lower expression of mRNA TGFβRII (than in TNG, GD) and mRNA TGFβRIII than in all studied benign tissues (NG, TNG, GD) suggests a pathogenetic importance of this cytokine and its receptors in PTC development. In GD tissue, higher transcriptional activity of TGFβRII and TGFβRIII genes as compared to other pathological tissues was observed, indicating a participation of the receptors in the pathomechanism of autoimmune thyroid disease (AITD). TGFβ1 blood concentrations do not reflect pathological processes taking place in thyroid gland. **(Endokrynol Pol 2016; 67 (4): 375–382)**

Key words: *TGFbeta1; transforming growth factor beta 1; thyroid; nodular goitre; papillary cancer; Graves' disease*

Streszczenie

Wstęp: Wyznaczenie profilu ekspresji genów-kandydatów odpowiedzialnych za procesy włóknienia, immunosupresji, angiogenezy, nowotworzenia w patogenezie chorób gruczołu tarczowego.

Materiał i metody: W grupie badanej było 63 chorych poddanych tyreoidektomii: 27 z wolem guzkowym nietoksycznym (NG), 22 z wolem guzkowym toksycznym (TNG), 6 z rakiem brodawkowatym (PTC), 8 z chorobą Gravesa-Basedowa (GD). W tkankach tarczycy oceniono ilościowo aktywność transkrypcyjną genów *TGFβ1* i jego receptorów TGFβRI, TGFβRII, TGFβRIII metodą RT-qPCR (ilościową reakcją łańcuchową polimerazy z udziałem odwrotnej transkryptazy). Analizę molekularną wykonano w tkankach pochodzących od GD i z centrum zmiany guzowatej (PTC, NG, TNG) oraz z obwodowych części usuniętego płata w których nie stwierdzono zmian histopatologicznych (tkanka kontrolna). Tkankę kontrolną dla analizy wykonanej u chorych z GD stanowiła niezmieniona tkanka tarczycy pochodząca z obwodowych części usuniętego płata chorych operowanych z powodu pojedynczego łagodnego guza.

Wyniki/Wnioski: Obserwowana ścisła regulacja pomiędzy aktywnością transkrypcyjną genów TGFβ1 i jego receptorów TGFβRI-III w tkankach kontrolnych ulega zaburzeniu we wszystkich tkankach patologicznych – całkowitemu w PTC i GD, częściowemu w NG i TGN. Dodatkowo, większa aktywność transkrypcyjna TGFβ1 w PTC w porównaniu do tkanek łagodnych (NG, GD) oraz mniejsza ekspresja mRNA TGFβRII (niż w TNG, GD) i mRNA TGFβRIII w porównaniu z łagodnymi tkankami (NG, TNG, GD) sugeruje patogenetyczne znaczenie tej cytokiny i jej receptorów w rozwoju PTC. W tkance GD, zwraca uwagę większa aktywność transkrypcyjna genów TGFβRII i TGFβRIII w porównaniu do innych tkanek patologicznych wskazując na udział tych receptorów w patomechanizmie autoimmunologicznej choroby tarczycy (AITD). Stężenia TGFβ1 we krwi nie odzwierciedlają procesów patologicznych zachodzących w gruczole tarczowym. **(Endokrynol Pol 2016; 67 (4): 375–382)**

Słowa kluczowe: *TGF beta 1; transformujący czynnik wzrostu beta 1; tarczyca; wole guzkowe; rak brodawkowaty; choroba Gravesa-Basedowa*

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Introduction

TGFβ1 is a multifunctional cytokine. However, there are three fundamental directions of its activities: I. it regulates cell proliferation, growth, differentiation, and cells movement; II. has immunomodulatory effects; and III. has profibrogenic effects. TGFβ1 action can be local and systemic [1, 2]. It is an endogenous factor controlling apoptosis in normal and pathological tissues and thereby is a factor controlling the balance between replication and cell death [1, 3]. Lack of TGFβ1-dependent growth control may result in oncogenesis. In normal conditions, TGFβ1 is a potent inhibitor of the growth of many cell types, including neoplastic [1, 4]. In the early stages of cancer development, its cells respond to antimitotic effect of TGFβ1 [1, 5]. However, at the entry of tumour cells into the phase of uncontrollable growth, most of them lose sensitivity to the inhibitory effect of TGFβ1. What is more, these cancer cells begin to secrete TGFβ1 themselves [1, 4]. The TGFβ1-dependent immunosuppressive activity and stimulating angiogenesis [4–7] creates a microenvironment favourable to tumour growth and its metastasis [1, 5]. TGFβ1 induces the death of the surrounding healthy cells and thus eliminates their effect designed to inhibit tumour growth [1, 3]. The results of clinical and experimental studies indicate that the molecular reasons for the lack of cell response to TGFβ1 during malignant transformation is caused by mutation in the TGFβRII receptor [8]. TGFβ1 was immunosuppressive in patients with Graves' disease. It inhibited the proliferation of peripheral blood mononuclear cells and of peripheral and thyroid-derived T-cell lines and clones in response to non-specific stimuli. It also suppressed the recognition of thyroid epithelial cells by thyroid autoantigen specific T-cell clones. TGFβ1 may exert a variety of down-regulatory influences in Graves' disease. It may be of importance for the suppression of autoaggression in persons predisposed to autoimmunity; it may be quantitatively overrun by immunostimulatory influences in the acute phase of the disease; and it may be important for the induction of remission in patients with Graves' disease [9, 10]. Triiodothyronine, through binding to its nuclear receptors (TRs), is able to antagonise transcriptional activation by TGFβ/SMAD. This antagonism involves reduced phosphorylation of SMADs. T3 reduces occupancy of SMAD-binding elements in response to TGFβ, reducing histone acetylation and inhibiting transcription [11].

The principle of the study was the determination of gene-candidates' profile expression responsible for immunosuppression, angiogenesis, neoplasia, and fibrosis processes in the pathogenesis of thyroid gland disease. The objective of the study was to investigate the transcriptional activity of TGFβ1 and its receptor (TGFβRI, TGFβRII, TGFβRIII) genes in diseased thyroid tissue in conjunction with the assessment of TGFβ1 concentration in the blood.

Material and methods

The research was approved by the Ethical Committee of the Medical University of Silesia and was carried out in 2002–2007. Studies were performed altogether on 63 patients (48 females, 15 males, mean age: 42.3 years): in eight patients undergoing a near total thyroidectomy due to hyperthyreosis in the course of Graves' disease (GD) and in 55 patients undergoing subtotal or total thyroidectomy due to tuberous changes. In the latter group, patients were divided into groups in dependence on histopathological diagnosis of change in thyroid gland (neoplastic change, benign change) and on hormonal status: the group with papillary cancer (PTC) consisted of six patients, with non-toxic nodular goitre (NG) — 27 patients, and with toxic nodular goitre (TNG) — 22 patients. After thyroidectomy each thyroid specimen was immediately frozen in liquid nitrogen and stored at -80^{0}C until it was used for RNA extraction. Paraffin-embedded thyroid specimens were histologically evaluated. Molecular analysis was performed in thyroid tissue derived from patients with Graves' disease (group GD) and in tissue derived from tumour centre (groups PTC, NG, TNG) and from peripheral parts of the removed lobe without histopathological lesions (tissue control — C). Control tissue (C) for analysis performed in GD was an unchanged thyroid tissue derived from peripheral parts of removed lobe of patients surgically treated for a single benign tumour. In study groups, blood was collected at four points during the day (at 7 am, 1 pm, 7 pm, and 1 am) to determine the presence of a possible circadian rhythm of TGFβ1 and the calculation of its Mean Daily Concentrations (MDC) in the blood. Obtained sera were stored at -80°C until testing. In all those examined, the current endocrinological status was defined on the basis of thyroid gland ultrasound (USG), fine-needle aspiration biopsy (FNAB), serum levels of TSH (thyrotropin), fT4 (free thyroxin), eventually fT3 (free triiodothyronine), TSH receptor antibodies (TRAb), and anti-thyroid peroxidase antibodies (TPOAb). The control group (H) for estimation of TGF$\beta 1_{MDC}$ consisted of 20 healthy volunteers age- and sex-matched to diseased groups. The creation of the control group of healthy people composed from women and men was preceded in our previous study by a comparative analysis of the point concentrations of TGFβ1 in the blood of women (n = 31) and the concentrations of TGFβ1 in the blood of men (n = 28) [10].

Table I. *Primer/probe sequences and size of amplicons generated by real-time RT-qPCR assay*
Tabela I. *Zaprojektowane zestawy starterów i sond hybrydyzacyjnych wykorzystywane w reakcjach RT-qPCR*

Primer/Probe	Oligo sequence	Location mRNA	Size of amplimer (bp)	Gene	Source of sequence
TGFβ1F	5′-TgAACCggCCTTTCCTgCTTCTCATg-3′	1602-1627			
TGFβ1R	5′-gCggAAgTCAATgTACAgCTgCCgC-3′	1729-1753	152 pz	TGFβ1	[13]
TGFβ1S*	5′-FAM-CCgCTggAgAgggCCCAgCATCTgCAAAgC-TAMRA-3′	1634-1663			
TGFβRIF	5′-ACTggCAgCTgTCATTgCTggACCAg-3′	451-476			
TGFβRIR	5′-CCTgAgCCAgAACCTgACgTTgTCATATCA-3′	622-651	201 pz	TGFβ1 receptor I (TGFβRI)	[14]
TGFβRIS*	5′-FAM-TgCCACAACCgCACTgTCATTCACCATCgA-TAMRA-3′	518-547			
TGFβRIIF	5′-ggCTCAACCACCAgggCATCCAgAT-3	1846-1870			
TGFβRIIR	5′-CTCCCCgAgAgCCTgTCCAgATgCT-3′	1960-1984	139 pz	TGFβ1 receptor II (TGFβRII)	[15]
TGFβRIIS*	5′-FAM ACCACgACCCAgAggCCCgTCTCACAgCCC-TAMRA-3	1900-1929			
TGFβRIIIF	5′-ACCgTgATgggCATTgCgTTTgCA-3′	2698-2721			
TGFβRIIIR	5′-gTgCTCTgCgTgCTgCCgATgCTgT-3′	2846-2870	173 pz	TGFβ1 receptor III (TGFβRIII)	[16]
TGFβRIIIS*	5′-FAM AggAAggCAgCAAgTCCCCACCTCCCCgCC-TAMRA-3′	2790-2819			

*probe labelled fluorescent dye FAM (6-carboxyfluoresceine) -5′ end and TAMRA (6-carboxytetramethylrhodamine) -3′ end

Analysis in blood

Serum levels of TGFβ1 were measured by an enzyme-linked immunosorbent assay (ELISA) method using Quantikine Immunoassay (R&D Systems, USA) kits. Tests were performed in the Department of Pathophysiology and Endocrinology with a Universal Microplate Spectrophotometer – μQUANT (BIOTEK INC). The sensitivity of the method was < 7 pg/mL, intra-assay error and inter-assay error were, respectively, 4.9% and 10.3%. TRAb and TPOAb were estimated, respectively, by a radioimmune assay (RIA) method and the ELISA method. Serum levels of TSH and free thyroid hormones were assayed immunoenzymatically (MEIA) by routine laboratory techniques.

Molecular analysis in tissue

The expression of mRNA TGFβ1 system subunits (TGFβ1 and its receptors TGFβRI, TGFβRII, TGFβRIII) in thyroid tissue specimens were evaluated by Reverse Transcriptase Quantitative Polymerase Chain Reaction (RT-qPCR) with GAPDH as an indirect marker of tissue integrity.

RNA extraction from tissue specimens

Total RNA was isolated from the specimens applying commercially available kits (Total RNA Prep Plus A&A Biotechnology; Poland) according to manufacturer's instruction based on the modification of the method of Chomczynski and Sacchi [12]. Quality of extracts was checked electrophoretically using 0.8% agarose gel stained with ethidium bromide. The results were analysed and registered using gel documentation system 1D Bas-Sys (Biotech-Fisher). In the case of RNA extracts contaminated by genomic DNA, the latter was removed by DNAase I (MBI Fermentas) according to the manufacturer's instruction. The concentration of total RNA was determined by spectrophotometric measurement in 5-μL capillaries using a Gene Quant II RNA/DNA Calculator (Pharmacia Biotech) making an assumption that the result of 1 OD, measured in a 10-mm light path cuvette, is equivalent to the concentration of 40 μg/cm^3 RNA extract.

Design of specific primers and probes used in the RT-qPCR reaction

Based on the data previously published in the Internet database GenBank (http://www.ncbi.nlm.nih.gov/irx/genbank) describing mRNA sequences for studied genes, we designed specific primers and probes for mRNA of these genes using the computer program Primer Express ™ Version 1.0 ABI PRISM (Table I).

Both primers as well as the probe were synthesized in the Oligo IBB PAN (Poland). The TaqMan probe consists of an oligonucleotide with a 5′-reporter dye and a downstream, 3′-quencher dye. The fluorescent reporter dye, such as FAM (6-carboxy-fluorescein), is covalently linked to the 5′ end of the oligonucleotide. Each of the reporters is quenched by TAMRA (6-carboxy-tetramethyl-rhodamine), typically located at the 3′ end. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence.

Table II. *The optimum concentrations of primers and probes generated by real-time RT-qPCR assay*

Tabela II. *Optymalne stężenia starterów i sond hybrydyzacyjnych wykorzystywane w reakcjach RT-qPCR*

Gene	Sense primer	Antisense primer	Probe
TGFβ1	0.2 μM	0.9 μM	0.3 μM
TGFβ1RI	0.2 μM	0.4 μM	0.3 μM
TGFβ1RII	0.4 μM	0.9 μM	0.3 μM
TGFβ1RIII	0.4 μM	0.9 μM	0.3 μM
GAPDH	0.3 μM	0.3 μM	0.2 μM

Number of mRNA copies calculation by use of RT-qPCR

Extracted total RNA was a matrix in one-step RT-qPCR reaction, performed by thermostable enzyme *Tth*. One-step quantitative RT-PCR using *Tth* DNA Polymerase depending on the type of ion present in the reaction mixture, manganese and magnesium, acts as either reverse transcriptase or DNA polymerase, respectively. The 10-μL reaction mixture contained five units Tth DNA Polymerase (Epicentre, USA), 1x MasterAmp 10X PCR Enhancer (Epicentre, USA), 200 μM each of dATP, dTTP, dGTP, dCTP, 3 mM $MgCl_2$, 0.5 μM $MnSO_4$, 1 × TaqMan Buffer A (Applied Biosystems, USA), and under 100 ng total RNA. The optimum concentrations of probes and primers were determined — see Table II.

ABI PRISM™ 7700 (TaqMan) sequence detector purchased from Applied Biosystems Co. was used to perform RT-qPCR reaction. RT-qPCR assay was performed in triplicate for each sample. Cycling conditions were as follows: one step at 60°C for 30 minutes, one step at 95°C for 5 minutes, 50 cycles at 95°C for 30 seconds, and 60°C for 1 minute and one step at 72°C for 10 minutes. The hybridisation probe was labelled with a reporter fluorescent dye (FAM) at the 5′ end and a quencher fluorescent dye (TAMRA) at the 3′ end. When this probe was placed in the PCR, the DNA polymerase that catalyses the PCR also cuts off any probe that specifically binds to template. The nucleolytic degradation of the hybridisation probe releases the quenching of FAM fluorescence emission. The higher the starting copy number of the mRNA target, the earlier the significant increase in fluorescence is observed.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control in all individuals RT-qPCR for all samples. Gene expression of GAPDH was analysed using specific sequence primers and probe (Table III).

On the basis the standard curve of each analysis was drawn and then an ABI PRISM™ 7700 sequence detector calculated the number of mRNA copies of the examined gene in analysed samples. RT-PCR products were run on 6% polyacrylamide gels and visualised with silver salts. To further confirm the product identity, they were sequenced with a BigDye Terminator V2.0 Cycle Sequencing Kit (Applied Biosystems, USA) using an automated sequencer (ABI PRISM™ 310 Genetic Analyzer, Applied Biosystems, USA).

Statistical analysis

Results were subjected to routine statistical analysis using the program Statistica, with significance level set at $p < 0.05$. For each quantitative parameter, basic statistical characteristics were made - mean, SD (standard deviation), SEM (standard error of the mean), minimal and maximal value, median, quartiles 25% and 75%, and 95% confidence interval. Chronobiological parameter estimation was performed using the cosinor method [Model: M+A*cos((Pi/12)*T+fi)]. Homogeneity of variance was checked by Fischer test. *U* Mann-Whitney's non-parametric rank sum test was applied where appropriate. For the assessment of intergroup correlations, a Spearman Rank Correlation test was used.

Results

Performing quantitative analysis of mRNA TGFβ1 and its receptors TGFβRI-III in thyroid tissue obtained from patients with papillary cancer (PTC), non-toxic nodular goitre (NG), toxic nodular goitre (TG), and Graves'

Table III. *Primer/probe sequences and size of amplicons generated by real-time RT-qPCR assay*

Tabela III. *Zaprojektowane zestawy starterów i sond hybrydyzacyjnych wykorzystywane w reakcjach RT-qPCR*

Primer/Probe	Oligo sequence	Location mRNA	Size of amplimer (bp)	Gene	Source of sequence
GAPDHF	5′-gAAggTgAAggTCggAgTC-3′	1457-1474			
GAPDHR	5′-gAAgATggTgATgggATTC-3′	3393-3412	226	GAPDH	[17]
GAPDHS*	5′-CAAgCTTCCCgTTCTCAgCC-3′	3364-3383			

* probe labelled fluorescent dye FAM (6-carboxyfluoresceine) -5′ end i TAMRA (6-carboxytetramethylrhodamine) -3′ end

Table IV. *Correlation coefficients among transcriptional activity of TGFβ1 and its receptor (TGFβRI, TGFβII, TGFβIII) genes in diseased thyroid tissue derived from patients with papillary cancer (PTC), non-toxic (NG), and toxic nodular goitre (TNG), Graves' disease (GD), and in control tissue (C)*

Tabela IV. *Analiza korelacji pomiędzy aktywnością transkrypcyjną genów TGFβ1 i jego receptorów (TGFβRI, TGFβII, TGFβIII) w tkankach tarczycy pochodzącej od pacjentów z rakiem brodawkowatym tarczycy (PTC), wolem guzkowym (NG), wolem guzkowym toksycznym (TNG), chorobą Gravesa-Basedowa (GD) oraz w tkance kontrolnej (C)*

mRNA	C		PTC		NG		TNG		GD	
	R	p	R	p	R	p	R	p	R	p
TGFβ1 & TGFβRI	0.6597	0.0008	–0.8061	0.0528	0.1820	0.3636	0.4095	0.0584	–0.1687	0.6897
TGFβ & TGFβRII	–0.5278	0.0116	0.0896	0.8660	–0.4121	0.0327	0.3481	0.1124	0.1078	0.7995
TGFβ1 & TGFβRIII	0.4813	0.0233	0.2687	0.6067	0.3827	0.0488	0.2744	0.2166	0.2755	0.5091
TGFβRI & TGFβRII	0.6425	0.0013	0.3529	0.4926	0.4856	0.0102	0.2950	0.1827	–0.2395	0.5678
TGFβRI & TGFβRIII	0.6096	0.0026	–0.2353	0.6536	0.1592	0.4276	0.3613	0.0985	–0.2635	0.5284
TGFβRII & TGFβRIII	0.9240	0.0000	0.2941	0.5715	0.3121	0.1130	0.8832	0.0000	0.6429	0.0856

R — Spearman's correlation coefficient; p — significance level

disease (GD) has shown no difference in expression of the TGFβ1 system subunits between diseased and control tissues in all studied groups of patients (Table IV). However, strict regulation observed among transcriptional activity of TGFβ1 and their receptors TGFβRI-III genes in control tissues is disturbed in all pathological tissues — it is completely disturbed in PTC and GD, and partially in NG and TNG (Table IV). Additionally, higher transcriptional activity of TGFβ1 gene in PTC tissue in comparison with benign tissue (NG, GD) and lower expression of mRNA TGFβRII (than in TNG, GD) and mRNA TGFβRIII than in all studied benign tissues (NG, TNG, GD) suggest pathogenetic importance of this cytokine and its receptors in PTC development. In GD tissue, higher transcriptional activity of TGFβRII and TGFβRIII genes as compared to other pathological tissues was observed indicating participation of the receptors in the pathomechanism of autoimmune thyroid disease (AITD) (Table V).

Substantially, we did not observe differences in serum TGFβ1 concentrations between the studied groups; only in TNG patients was the cytokine level slightly decreased as compared to healthy controls (Table VI). TGFβ1 blood concentrations rather do not reflect the pathological processes taking place in thyroid gland, although in patients with TNG we noted a correlation between TGFβ1 concentration in blood and mRNA TGFβRII expression in thyroid (Spearman's correlation coefficient (R) = –0.4257; p = 0.0482), which probably indicates the participation of circulating TGFβ1 in the phenomenon of the receptor downregulation. No circadian rhythms of TGFβ1 in all studied groups were found. No significant correlation was found between fT4, fT3, and measured TGFβ1 system subunits expression. We also did not find any correlation between the TGFβ1 system subunits expression and parameters of autoimmune thyroid disease, like TRAb or TPOAb.

Discussion

This study is the first report concerning the simultaneous quantitative analysis (RT-qPCR) of transcriptional activity of TGFβ1 and its receptor (TGFβRI, TGFβRII, TGFβRIII) genes in thyroid tissue. The tissues derived from surgically treated patients with papillary cancer, nodular goitre (non-toxic and toxic), and Graves' disease. In recent years, occasionally TGFβ1 mRNA expression in thyroid tissue was estimated by RT-PCR [18]. Brace et al. (2014) demonstrated, using RT-qPCR, that expression of TGFβ1 but not of TGFβ2 is increased in papillary cancer tissue compared to benign nodule tissue [19]. Most reports describing TGFβ1 in thyroid gland diseases are based on assay of this factor in blood [10] or on qualitative or semi-quantitative but not quantitative analysis of TGFβ1 expression in thyroid tissue [20–23]. Immunohistochemical analyses of resection thyroid showed that the expressions of TGFβ1 in thyroid cancers (papillary, follicular) were higher than in other benign thyroid lesions and normal thyroid tissues. In the study nodular goitre and Graves' disease tissues were also analysed [23]. The other immunohistochemical studies examining the role of TGFβ in human thyroid tissues revealed increased cytoplasmic presence of TGFβ at the periphery of poorly circumscribed PTC. These tumours were associated with increased invasiveness and metastasis with an increased propensity towards epithelial to mesenchymal transition [20, 21, 22]. In PTC cell lines and animal models, TGFβ signalling has been shown to regulate cellular epithelial to mesenchymal transition [19, 20, 22, 25]. In thyroid PTC

Table V. *Quantitative analysis of mRNA TGFβ1 and expression of its receptors (copies/μg RNA) in thyroid tissue of patients with papillary cancer (PTC), non-toxic (NG) and toxic nodular goitre (TNG), and Graves' disease (GD) in comparison to control tissue (C)*

Tabela V. *Ocena ilościowa mRNA TGFβ1 i jego receptorów (l.kopii/μg RNA) w tkance raka brodawkowatego tarczycy (PTC), wola guzkowego (NG), wola guzkowego toksycznego (TNG), tarczycy w chorobie Gravesa-Basedowa (GD) w porównaniu z tkanką kontrolną (C)*

Groups	mRNA TGFβ1		mRNA TGFβ1RI		mRNA TGFβ1RII		mRNA TGFβ1RIII	
	Control tissue (C)	Diseased tissue	Control tissue (C)	Diseased tissue	Control tissue (C)	Diseased tissue	Control tissue (C)	Diseased tissue
PTC	1319349 ± 407839	21509244 ± 12322536	1189096 ± 399369	1172294 ± 465656	543804 ± 464847	93887 ± 41046	164979 ± 99836	645343 ± 585243
NG	3272641 ± 1224427	6297342 ± 3862063	1832109 ± 660609	16877259 ± 14731165	12874886 ± 6648709	11925802 n± 6314538	4999568 ± 2402442	6838578 ± 3751423
TNG	30517625 ± 15005768	6148716 ± 1786329	260164523 ± 138680049	98849035 ± 51478973	1624160098 ± 1073408222	242077706 ± 139319839	794626176 ± 534113964	1047041949 ± 1014411742
GD	3623509 ± 1815087	1847330 ± 1615824	31742449 ± 20463816	30729219 ± 16356403	1152319801 ± 445757158	5774852153 ± 3128663504	9612563802 ± 5028771659	23356636481 ± 14120918743
Comparison between control tissue (C) and diseased tissue:								
PTC *vs.* C	p = 0.078		p = 0.749		p = 0.999		p = 0.522	
NG *vs.* C	p = 0.938		p = 0.736		p = 0.911		p = 0.789	
TNG *vs.* C	p = 0.725		p = 0.907		p = 0.664		p = 0.897	
GD *vs.* C	p = 0.599		p = 0.834		p = 0.529		p = 0.999	
Comparison between groups (diseased tissue):								
PTC *vs.* NG	p = 0.015		p = 0.641		p = 0.0557		p = 0.0357	
PTC *vs.* TNG	p = 0.179		p = 0.467		p = 0.0118		p = 0.029	
PTC *vs.* GD	p = 0,001		p = 0,121		p = 0,0019		p = 0,003	
NG *vs.* TNG	p = 0.041		p = 0.122		p = 0.0704		p = 0.0953	
TNG *vs.* GD	p = 0.075		p = 0.399		p = 0.0017		p = 0.0216	
NG vs. GD	p = 0.556		p = 0.084		p = 0.0001		p = 0.0006	

± SEM — standard error of the mean; p — significance level

Table VI. *Mean Daily Concentrations (MDC) of TGFβ1 in blood of patients with papillary cancer (PTC), non-toxic (NG) and toxic nodular goitre (TNG), Graves' disease (GD), and in healthy controls (H)*

Tabela VI. *Porównanie średnich dobowych stężeń (MDC) dla TGFβ1 we krwi chorych z rakiem brodawkowatym tarczycy (PTC), wolem guzkowym (NG), wolem guzkowym toksycznym (TNG), chorobą Gravesa-Basedowa (GD) i w grupie kontrolnej zdrowych (H)*

Groups	TGFβ1 [ng/mL] MDC ±SD; ±SEM
PTC	38.34 ± 7.8; ± 3.2
NG	34.39 ± 6.8; ± 1.3
TNG	31.69 ± 6.0; ± 1.28
GD	40.08 ± 11.7; ± 4.12
H	38.2 ± 10.9; ± 1.16
Comparison between groups	
PTC *vs.* H	p = 0.734
NG *vs.* H	p = 0.096
TNG *vs.* H	p = 0.004
GD *vs.* H	p = 0.624
PTC *vs.* NG	p = 0.305
PTC *vs.* NTG	p = 0.050
PTC *vs.* GD	p = 0.606
NG *vs.* TNG	p = 0.228
TNG *vs.* GD	p = 0.067
NG *vs.* GD	p = 0.223

cell lines, TGFβ1 treatment was associated with the development more aggressive papillary cancer [24]. Overexpression of TGFβ1 in thyroid tissue is associated with the occurrence of thyroid cancer, which can be used as a candidate for the diagnosis and prognosis of thyroid cancer [23].

In the tissues of thyroid cancers zero or reduced TGFβRII expression has been found compared to benign tumours and normal tissues [1, 26]. In the differentiated and undifferentiated tissues of the thyroid cancers, resistance to TGFβ1 coexisted with a reduction in mRNA and protein TGFβRII expression [1, 27]. TGFβ1 mRNA expression in PTC cells was higher compared to the surrounding tissues, while the TGFβRII was lower. An inverse correlation between TGFβRII and tumour size was found, and there was no such correlation with respect to TGFβ1, which suggests that primarily TGFβRII plays a role in the pathogenesis of PTC [1, 28]. Metastatic thyroid cancer can also be characterised by a decreased sensitivity to the action of TGFβ1 [29]. In human PTC, higher expression levels of TGFβ1 were closely related with lymph node metastasis, whereas Smad3 expression increased significantly with advanced tumour stages. These findings suggest that the activation of TGFβ/Smad3 pathways in cancer cells influences tumour growth [30]. The ability to inhibit tumour growth via TGFβRII, and the discovery of factors inducing the expression of this receptor in tumour cells, may be relevant to the treatment of malignant disease [1].

The application of molecular analysis of gene expression to clinical tissue samples represents one of the most exciting new areas in "translational" thyroid cancer research. Current data suggest that molecular diagnostic assays may improve the sensitivity and accuracy of FNAB of thyroid nodules and lesions, metastases, and detection of recurrent disease in peripheral blood samples [31]. Specified mRNA's TGFβ1 system expression and interactions among the system subunits identified in neoplastic tumour different than in other tissues may be considered in the context of improving PTC diagnostics. On the one hand, thyroid nodules and lesions being one of the commonest clinical situations rarely prove to be malignant, and on the other hand the diagnostic accuracy of FNAB is limited. FNAB is a highly sensitive method in the differential diagnosis of thyroid nodules and lesions; however, a large percentage of thyroid FNABs give a considerable proportion of non-diagnostic results, so there is a need to find new tools for more precise preoperative assessment [32–37]. Patients would benefit from improved preoperative diagnosis, which could reduce the number or extent of surgeries, long-term health costs, and postsurgical complications.

Molecular analysis of gene expression based on RT-qPCR represents new exciting areas of pathophysiology because it creates the opportunity to explore the pathomechanisms occurring in the thyroid gland.

Conclusions

1. Strict regulation observed among transcriptional activity of TGFβ1 and their receptor TGFβRI-III genes in control tissues is disturbed in all pathological tissues — it is completely disturbed in papillary cancer and Graves' disease, and partially in non-toxic and toxic nodular goitre.
2. In papillary cancer tissue, higher transcriptional activity of TGFβ1 gene and lower transcriptional activity of TGFβRII and TGFβRIII genes in comparison with benign tissues suggests pathogenetic importance of this cytokine and its receptors in cancer development.
3. In Graves' disease tissue, higher transcriptional activity of TGFβRII and TGFβRIII genes in comparison with other pathological tissues indicates a partici-

pation of the receptors in the pathomechanism of autoimmune thyroid disease (AITD).

4. TGFβ1 blood concentrations rather do not reflect pathological processes taking place in thyroid gland, although in patients with toxic nodular goitre we noted a correlation between TGFβ1 concentration in blood and mRNA TGFβRII expression in thyroid, which probably indicates the participation of circulating TGFβ1 in the phenomenon of the receptor downregulation.

Acknowledgments

Funding: This work was supported by the State Committee for Scientific Research (Polish — Komitet Badań Naukowych) grant no. 3P05B05322 and 3P05B03123 and Medical University of Silesia grant no. NN1-028/05, NN2-197/05, and NN5-154/04. The funders had no role in the study design, data collection and analysis, the decision to publish, or preparation of the manuscript. Competing interests: The authors have declared that no competing interests exist.

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