TFF3 and *TIMP3* – the candidate marker genes for differentiation diagnosis of follicular cell-derived thyroid tumors

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Background:

Thyroid cancer is a serious epidemiological problem of endocrine diseases. Thyroid carcinoma develops as a result of malignant transformation of nodular goiter (NG), which at an early stage can lead to the development of follicular adenoma (FA). The progression of FA can be associated with the transformation of this benign neoplastic lesion into: papillary thyroid carcinoma (PTC), characterized by slow growth and mild outcome, and thyroid follicular cancer (FTC), the more aggressive form of cancer. The final differentiation of thyroid lesions (FA, PTC, FTC) is usually carried out post-operatively. Therefore, it appears advisable to look for markers enabling the proper preoperative diagnosis.

The search for new differentiating biomarkers in follicular-cell derived thyroid tumors (FCDT), especially for the FNAB with underdetermined cytology, is an important scientific task. Disturbed expression of tumor suppressor genes plays important role in thyroid carcinogenesis.

In this study we focused on epigenetic mechanism influencing on *TIMP3* (*Tissue Inhibitor Of Metalloproteinases 3*) and *TFF3* (*trefoil factor 3*) expression in follicular cell-derived thyroid tumors.

The aim of study:

Evaluation gene expression and promoter methylation of *TFF3* and *TIMP3* as candidate biomarkers in differentiating diagnosis of follicular cell-derived thyroid tumors.

Material:

- Thyroid neoplasms (N) and matching macroscopically unchanged tissues (Control) from 86 patients with preoperative FNAB diagnosis: PTC/ *„follicular neoplasm"*.
- Patients aged 16 to 76 years (average 49 years).
- 70 women (81.4%) and 16 men (18.6%).

Table 1. Histopathological classification of patients

Histopathological classification	n	%	Women/ Men
nodular goiter (NG)	33	38.4%	28 /5
follicular adenoma (FA)	9	10.5%	7 /2
carcinoma papillare (PTC)	35	40.6%	29 /6
follicular carcinoma (FTC)	9	10.5%	6 /3

Methods:

- RNA and DNA isolation (Universal RNA Purification Kit – Eurx; QIAamp DNA Mini kit (QIAGEN®), qualitative and quantitative spectrophotometric analysis of RNA and DNA (BioPhotometer, Eppendorf).
- mRNA expression level (RQ) using Taq Man Low Density Arrays, TaqMan Array Micro Fluidic Card in 7900 HT Fast Real-Time PCR System (Applied Biosystems, USA). Relative gene expression level $\mathbf{RQ = 2^{-\Delta\Delta CT}}$
- DNA bisulfite conversion (EpiMark® Bisulfite Conversion Kit), followed by promoter methylation level evaluation in methylation specific PCR with methylated and unmethylated primers, Methylation Index (MI) calculation. $IM = \frac{M\left[\frac{ng}{\mu l}\right]}{U + M\left[\frac{ng}{\mu l}\right]}$
- Statistical analysis (Statistica for Windows 10.0).

Results:

TIMP3 and *TFF3* promoter methylation

***TIMP3* gene**

- presence of both „U" and „M" alleles in all samples (n=86, 100%) derived from macroscopically unchanged tissue (C) and thyroid tissue - neoplasms (N)
- Highest ***TIMP3*** mean MI was detected in FAs (0.871) and FTCs (0.878)
- ***TIMP3*** MI value was significantly higher in follicular lesions (FTC/FA), than in NGs and PTCs (p=0.049, Kruskal – Wallis test).

***TFF3* gene**

- presence of both „U" and „M" alleles in 47.7% of controls (C) and 54.7%of neoplasms (N); presence of only „M" allele in 52.3% of C and 45.3% of N
- Highest ***TFF3*** mean MI was detected in FAs (0.916) and NGs (0.913)
- ***TFF3*** MI value revealed the opposite correlation to ***TIMP3*** – low MI in FTC/FA.
- In PTC ***TFF3*** MI correlates with RQ level (p=0.01).

TFF3 and *TIMP3* expression

***TIMP3* gene :** highest RQ value among patients with NG (1.823), PTC (1.498), lowest in FTC+FA (1.738), (Kruskal-Wallis test; p>0.05).

***TFF3* gene:** highest RQ value among patients with FTC+FA (10.770), PTC (6.949), lowest in NG (4.360), (Kruskal-Wallis test; p>0.05).

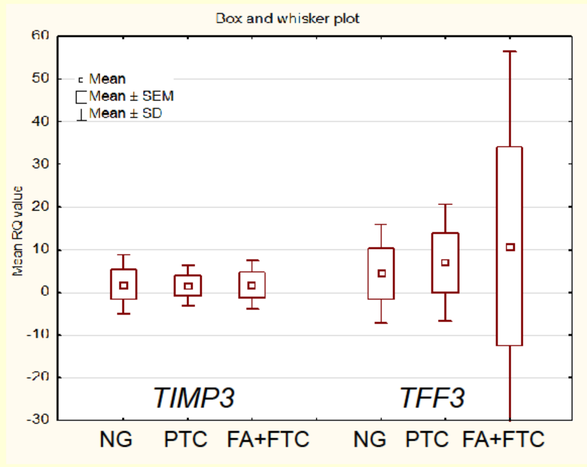

Figure 1. Box and whisker plot showing mean RQ values in histopathological types of thyroid tumors (NG *vs* FA+FTC *vs* PTC).

Correlation of expression and methylation of *TIMP3* and *TFF3*

(Spearman's rank correlation coefficient)

- ***TIMP3*** MI correlates with RQ (p=0.029)
- ***TIMP3*** MI and RQ are higher in woman vs. men (p=0.016).
- In PTC ***TFF3*** MI correlates with RQ level (p=0.01).
- In women positive correlation between MIs and RQs for ***TIMP3*** and ***TFF3*** were found (p=0.0004, p=0.00 respectively).
- ***TFF3*** and ***TIMP3*** RQ values in groups regarding pTNM groups reversely correlate with MI for both genes (p>0).
- Significant correlation between methylation levels in ***TIMP3*** and ***TFF3*** (p=0.00009), as well as between expression levels in both genes (p=0.000000).

Conclusions:

The increased *TIMP3* MI values in FA/FTC, *TFF3* in PTC and correlation between MIs with RQs suggest that can be regarded as promising biomarkers for distinguishing follicular cell-derived thyroid tumors. Our research indicates that simultaneous analysis of methylation profile and expression level of *TIMP3* and *TFF3* may be diagnostically useful. Further studies are needed.

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