Vitamin-D neutralizing CYP24A1 gene expression in thyroid fine-needle aspiration biopsy samples

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Abstract

Objectives: We previously published the result of 24-hydroxylase (*CYP24A1)* gene expression in one hundred, solely papillary thyroid carcinoma (PTC) compared to its own tumor free control from the same patient. We report an increase in *CYP24A1* gene transcription in more than half of analyzed PTCs. Elevated CYP24A1 protein expression was also observed in the cancerous tissue section compared to peritumoral normal thyroid tissue. In the present study we aimed to examine *CYP24A1* gene transcription in thyroid fine-needle aspiration biopsy (FNAB) specimens and follow up the patients for two years.

Methods: The gene expression analyses of forty-two thyroid FNABs were carried out by Taqman probe-based quantitative real-time RT-PCR. The somatic mutation states of *BRAF, NRAS, HRAS, KRAS* oncogenes as well as *ELE1/RET* and *CCDC6/RET* rearrangements were also tested. Genomic DNA and total RNA were isolated from each sample using Roche High Pure kits.

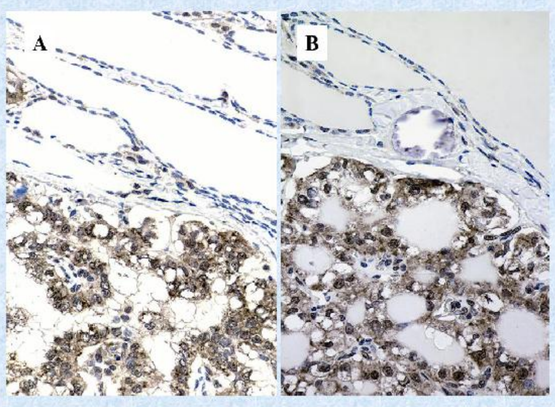
Results: Eight males and 34 females participated in the study. The mean age was 51.43 years. Cytology results of 28 FNABs were benign and 14 were malignant. Within the malignant specimens 13 papillary and 1 follicular type carcinoma were recognized. Altogether, 6 *BRAF* (rs113488022) mutations, 1 *ELE1/RET* translocation were detected in the malignant FNAB samples and one benign biopsy carried *HRAS* (rs28933406) mutation. *CYP24A1* gene expressions were noticed only in five FNAB samples diagnosed with PTC. We could not determine *CYP24A1* specific mRNA in the benign samples. During the follow up period we identified malignant transformation in three cases from the 28 initially cytological benign FNABs. In all of these three cases PTC were certified.

Conclusion: It is well established, that *CYP24A1* gene activity is elevated in various cancers including thyroid carcinoma might be to protect tumor tissue from the anti-proliferative and pro-apoptotic effects of 1,25-vitamin D3. Our results show that changes of *CYP24A1* gene expression have no predictive value in precancerous states of thyroid and it could not help to complete the diagnosis of FNAB cytology.

Introduction

Nowadays, it is well known that key players of vitamin-D metabolism show altered expression in various types of thyroid cancers.

- Work of Clinckspoor I. et al. (J Histochem Cytochem 2012;60) indicated enhanced vitamin-D receptor (*VDR*), *CYP27B1* and *CYP24A1* (respectively activating and catabolizing vitamin-D) expression in differentiated thyroid cancers compared with normal thyroid. However, papillary carcinoma (PTC) with lymph node metastasis was characterized by lower *VDR* and *CYP24A1* transcription than non-metastasized PTC.
- Zou M. et al. (Clin Endocrinol 2014;81) reported up-regulated *CYP24A1* expression in PTC compared to benign multinodular goitre. The expression was further increased in advanced tumor stages. There was a strong correlation between *CYP24A1* overexpression and *BRAFV600E* mutation.
- We previously published the result of vitamin-D neutralizing 24-hydroxylase (*CYP24A1)* gene expression in one hundred, solely papillary thyroid carcinoma (PTC) compared to its own tumor free control from the same patient (Balla B. et al. J Endocrinol Invest 2014). We report an increase in *CYP24A1* gene transcription in more than half of analyzed PTCs. We also found association between higher *CYP24A1* expression rate and the occurrence of point mutations in oncogenic tumor markers (*BRAF, HRAS, CCDC6/RET*) as well as tumor malignity in a multiparametric method.
- We observed elevated CYP24A1 protein expression in the cancerous tissue section compared to peritumoral normal thyroid tissue.



Immunohistochemical detection of CYP24A1. Representative cases demonstrate virtually negative peritumoral follicules (upper half) and strongly positive neoplastic tissue with granular cytoplasmic and nuclear staining (lower half) CYP24A1 (brown) and nuclei are counterstained with hematoxylin (blue).

- **Although, vitamin-D signaling and the expression of *CYP24A1* are widely investigated in histologically confirmed malignant thyroid conditions. We aimed to examine *CYP24A1* gene transcription in thyroid fine-needle aspiration biopsy specimens as a prediction marker and follow up the patients for two years.**

Materials & Methods

Study population:
Ultrasound guided thyroid FNAB sampling was carried out in forty-two Hungarian patients. The FNA biopsy sample was dispensed into 1x phosphate buffered saline (PBS) solution and stored at -80°C until nucleic acid extraction. The study was approved by the Regional Committee of Science and Research Ethics, Semmelweis University (SOTE-TUKEB 1160-0/2010- 1018EKU).

Somatic oncogenic mutation and rearrangement analysis:
Genomic DNA was isolated using Roche High Pure PCR template Preparation Kit according to the manufacturer's protocol. DNA was tested for BRAF codon 600, HRAS codon 61, KRAS codons 12, 13 and NRAS codon 61 using real-time PCR and fluorescence melting curve analysis (Roche Light Cycler 2.0 Instrument). ELE1/RET, CCDC6/RET rearrangements were detected on RNA by RT-PCR ABI Prism 7500 with primers designed to flank the respective fusion point.

Gene expression analysis:
Total RNA was isolated from each sample with Roche High Pure Total RNA Isolation kit according to the manufacturer's recommendations. 500 ng of total RNA was reverse-transcribed to cDNA. The expression of the selected *CYP24A1* genes (*ID: Hs00167999_m1, Applied Biosystems*) was analyzed by Taqman probe-based quantitative real-time PCR. *GAPDH* (*ID: Hs99999905_m1*) was used as endogenous control.

Results

Basic characteristics of patients

Patients n = 42	**Male** n = 8 (19%)	**Female** n = 34 (81%)
Age (Mean ± SD) 51.43 ± 14.59 years	50.13 ± 9.99 years	51.74 ± 14.59 years

Aspiration cytology sample results

FNAB samples	**BRAF** (rs113488022)	**HRAS** (rs28933406)	**KRAS** (rs121913535)	**NRAS** (rs79057879)	**ELE1/RET**	**CCDC6/RET**	**CYP24A1 expression**
Benign n = 28	0	**1**	0	0	0	0	**0**
Malignant n = 14	**6**	0	0	0	**1**	0	**5**

Within the malignant specimens 13 papillary and 1 follicular type carcinoma were recognized.
***CYP24A1* gene expressions were noticed only in five FNAB samples diagnosed with PTC. The detected avarege expression level of *CYP24A1* was very low (*GAPDH* normalized relative quantity, RQ = 0.032 AU). We could not determine *CYP24A1* specific mRNA in the benign samples.**

Patients follow-up

During the two-year follow-up period we identified malignant transformation in three cases from the 28 initially cytological benign FNABs. In all of these three cases PTC were certified.

Summary & Discussion

- **The tumor cell growth-inhibiting role of the active metabolite of vitamin-D has been extensively studied in different malignancies. It is well established, that the neutralizing *CYP24A1* gene activity is elevated in various cancers including thyroid carcinoma might be to protect tumor tissue from the anti-proliferative and pro-apoptotic effects of 1,25-vitamin-D3.**
- **In this study we could determine very weak *CYP24A1* expression in thyroid FNAB samples, solely in patients with cytologically malignant result.**
- **Our findings show that changes of *CYP24A1* gene expression have no predictive value in precancerous states of thyroid and it could not help to complete the diagnosis of FNAB cytology.**

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