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Original research

Vitamin D receptor expression is linked to potential markers of human thyroid papillary carcinoma

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ABSTRACT

Genes regulated cell–cell and cell-matrix adhesion and degradation of the extracellular matrix (ECM) have been screened as potential markers of malignant thyroid nodules. The mRNA expression levels of two of them, the ECM protein-1 (ECM1) and the type II transmembrane serine protease-4 (TMPRSS4), were shown to be an independent predictor of an existing thyroid carcinoma. The vitamin D receptor (VDR) is expressed in epithelial cells of the normal thyroid gland, as well as in malignant dividing cells, which respond to the active metabolite of vitamin D by decreased proliferative activity *in vitro*. We evaluated the relationship between mRNA gene expressions of TMPRSS4, ECM1 and VDR in 21 papillary thyroid carcinoma samples and compared it to 21 normal thyroid tissues from the same patients. Gene expression was considered as up- or down-regulated if it varied by more or less than 2-fold in the cancer tissue relative to the normal thyroid tissue (Ca/N) from the same patient. We found an overall significant adjusted correlation between the mRNA expression ratio (ExR) of VDR and that of ECM1 in Ca/N thyroid tissue ($R = 0.648$, $P < 0.001$). There was a high ExR of VDR between Ca/N thyroid tissue from the same patient (3.06 ± 2.9), which also exhibited a high Ca/N ExR of ECM1 and/or of TMPRSS4 (>2, $P = 0.05$).The finding that increased VDR expression in human thyroid cancer cells is often linked to increased ECM1 and/or TPMRSS4 expression warrants further investigation into the potential role of vitamin D analogs in thyroid carcinoma.

1. Introduction

Differentiated thyroid carcinoma (DTC), which includes the papillary and follicular carcinomas, is the most common endocrine cancer. DTC patients have a good prognosis, but once the cancer cells metastasize, the prognosis is significantly worsened. The extracellular matrix protein-1 (ECM1) is the 85 kDa glycoprotein that is associated with maintenance of the extracellular matrix, bone formation and lymphangiogenesis [1]. The ECM1 gene is located on chromosome 1q21 [2] and expressed around blood vessels, promoting blood vessel formation, stimulating proliferation of malignant epithelial cells and leading to tumor progression [3]. Up-regulation of ECM1 has been detected in numerous malignant epithelial tumors, such as invasive breast ductal carcinoma, esophageal squamous carcinoma, and gastric and colorectal cancer [4].

The type II transmembrane serine protease-4 (TMPRSS4) belongs to the family of cell surface serine proteases that are involved in regulating cell–cell and cell-matrix adhesion, motility and homeostasis. The TMPRSS4 gene is located on chromosome 11. q23.3 [5], which is expressed at the cell surface and modulates invasion, metastasis, migration and adhesion, as well as epithelial-mesenchymal transition in cancer cells [6,7]. Up-regulation of TMPRSS4 has been reported in several types of epithelial cancer, including pancreatic, prostatic, colon and gastric, as well as in DTC [8,9]. The high mRNA expression level of the ECM1 and the

Abbreviations: DTC, differentiated thyroid carcinoma; 1,25D, 1,25-dihydroxyvitamin D3; cDNA, complementary deoxyribonucleic acid; Ca/N, cancer tissue relative to the normal thyroid tissue; ECM, extracellular matrix; ECM1, extracellular matrix protein 1; ER-α, estrogen receptor α; ER-β, estrogen receptor β; ExR, expression ratio; hTBP, human TATA-binding protein; mRNA, messenger ribonucleic acid; 1-OH-ase, 25-hydroxyvitamin D3 1-alpha-hydroxylase; PTC, papillary thyroid carcinoma; RT-PCR, quantitative real-time polymerase chain reaction; TMPRSS4, transmembrane serine protease; TTF2, thyroid transcription factor; VDR, vitamin D receptor.

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Table 1
Demographic and clinicopathological characteristics of thyroid cancer patients ($n = 21$).

Characteristic	Mean (±SD)	Range/percent
Age (years ± SD)	38.3 ± 16.6	15–71
Gender		
Male	6	29
Female	15	71
Tumor size (cm ± SD)	2.0 ± 1.5	0.5–6.0
Stage		
I	14	67
II	0	0
III	3	14
IV	4	19
Pathology, *n*		
PTC CV	7	33
PTC FV	14	67
Multicentricity, *n*		
Negative	8	38
Positive	13	62
Extrathyroidal extension, *n*		
Negative	13	62
Positive	8	38
Vascular invasion, *n*		
Negative	18	86
Positive	3	14
Lymph node metastasis, *n*		
Negative	8	38
Positive	13	62
Distant metastasis, *n*		
Negative	20	95
Positive	1	5
Thyroiditis, *n*		
Negative	12	57
Positive	9	43

PTC CV, papillary thyroid carcinoma classical variant; PTC FV, papillary thyroid. carcinoma follicular variant.

TMPRSS4 genes was identified as an independent predictor of malignant thyroid neoplasms [10].

Several epidemiological studies have shown an inverse association between levels of vitamin D and the risk of developing cancer [11]. By binding to the specific vitamin D receptor (VDR), the active form of vitamin D, 1,25-dihydroxyvitamin D3 (1,25D), has reportedly exerted anti-tumor activity, that includes the inhibition of cell proliferation and angiogenesis and the promotion of cell differentiation and apoptosis in various cancers, both *in vitro* and *in vivo* [12–16]. The VDR is expressed in epithelial cells of the normal thyroid gland. It is also present in various malignant dividing cells, which respond to 1,25D by decreased proliferative activity *in vitro* [17].

The objective of the present study was to evaluate the relationship between VDR and the genes that regulate cell–cell and cell-matrix adhesion and degradation of the extracellular matrix, the TMPRSS4 gene and the ECM1, in patients with papillary thyroid carcinoma (PTC), which is the most common endocrine malignancy.

Table 2
Comparison of mRNA gene expression[a] of ECM1, TMPRSS4, VDR, and TTF2 in malignant and normal thyroid tissues from the same patients.

Gene	Malignant tissue		Normal tissue		P-value
	Median	IQR	Median	IQR	
ECM1	0.78	4.26	0.25	0.35	0.01
TMPRSS4	0.71	2.82	0.01	0.02	0.001
VDR	0.21	0.40	0.08	0.17	0.001
TTF2	6.03	9.9	16.76	17.08	0.001

IQR, interquartile range; ECM1, extracellular matrix protein-1; TMPRSS4, type II transmembrane serine protease-4; $P < 0.05$ is considered statistically significant by Wilcoxon signed rank test.

[a] mRNA gene expression, the relative difference in gene expression of the gene of interest and that of the internal reference gene is represented by $2^{(-\Delta ct)}$.

2. Materials and methods

2.1. Study population

Fresh surgical specimens from 21 PTC patients who had undergone thyroidectomy in the Department of Otolaryngology at the Tel Aviv Sourasky Medical Center were evaluated. All cases had two samples collected after micro-dissection as follows: thyroid cancer tissue and the corresponding normal thyroid tissue, located more than 2 cm away from the tumor margins on the same lobe or from opposite lobe. Seven cases of PTC were of the classical variant, and the remaining 14 cases were of the follicular variant. The study was approved by the Human Subject Committee of the Tel Aviv Sourasky Medical Center.

2.2. Preparation of total RNA, RT-PCR and RNA quantification

Gene expression of EMC1, TMPRSS4, VDR, thyroid transcription factor 2 (TTF2), 25-hydroxyvitamin D3 1-alpha-hydroxylase (1-OH-ase), estrogen receptor α (ER-α), and estrogen receptor β (ER-β) was measured by quantitative real-time PCR (RT-PCR). Total RNA from thyroid tissue was extracted using the Trizole reagent (Gibco Life Technologies) according to the manufacturer's instructions. Extracted RNA (1 μg) was then reverse transcribed using the Applied Biosystems High-Capacity cDNA Transcription kit. mRNA expression was quantified with an ABI ONE STEP RT-PCR system using specific primer probe sets for ER-α, ER-β, VDR, 1-OH-ase, TMPRSS4, ECM-1, and TTF2. We used the Taqman Universal FAST Master MIX and Assay: on demand Gene Expression Assay Mix for ERalpha-HS00174860_m1, ERbeta-HS00230957_m1, VDR-HS00172113_m1, TMPRSS4-HS00212669_m1, ECM-1-HS00189435_m1, TTF2-HS00916085_s1, and 1-OH-ase was ordered according to the following sequence:

Forward = CACCCGACACGGAGACCTT; Reverse = TCAA-CAGCGTGGACACAAACA;

Probe = TCCGCGCTGTGGGCT. hTBP was used as the internal reference gene. The relative difference in gene expression of the gene of interest and that of the internal reference gene is represented by $2^{(-\Delta ct)}$. Similarly to the study of Kebebew et al. [10], gene expression was considered as being either up- or down-regulated in cancer tissue if it varied by more or less than 2-fold in the cancer tissue relative to the normal thyroid tissue from the same patient.

2.3. Statistical analysis

Statistical analysis was performed using the SPSS version 19.0. Correlation analyses of the gene expressions were performed by the non-parametric Wilcoxon signed rank for dependent groups, the non-parametric Wilcoxon test for independent groups and the non-parametric Spearman's rho. Correction for the α-value for multiple comparisons by False Discovery Rate controlling procedures was carried out. All statistical tests were two-sided. A P value < 0.05 was considered statistically significant.