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Expression of E-Cadherin/Beta-Catenin in Epithelial Carcinomas of the Thyroid Gland

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Abstract

BACKGROUND: The aberrant activation of Wnt signalling pathway may be a common denominator for the development of thyroid tumorigenesis. It was announced that the loss of E-cadherin rather than β-catenin mutation represents a crucial event in determining the degree of differentiation of thyroid carcinomas.

AIM: The aim of the study was to evaluate the expression of E-cadherin and β-catenin in the thyroid cancer tissue and to correlate these data with some histological and clinical parameters of the tumours.

MATERIAL AND METHODS: We investigated 112 patients, having thyroid tumours – papillary, follicular, anaplastic and oncocytic carcinomas immunohistochemically with antibodies against E-cadherin and β-catenin. Survival analyses were done.

RESULTS: E-cadherin expression was focally retained in the tumour cell membranes and the tumour cell cytoplasm of the papillary, follicular and oncocytic thyroid cancers, weather in anaplastic cancers it was almost lost ($p = 0.0042$, and $p = 0.019$, respectively, Fisher's Exact Test). The expression of β-catenin in tumour cytoplasm and membrane in papillary cancers was higher as compared to that in the other tumours ($p = 0.111$, and $p = 0.0104$, respectively).

CONCLUSION: Not surprisingly, the presence of aberrant expression of E-cadherin and β-catenin in thyroid cancer has been associated with better patients' prognosis and better differentiated tumour histology.

Introduction

Thyroid cancer is one of the most frequent endocrine tumours at present and appears to be a particularly interesting neoplastic process in which some authors cited as major prognostic markers: age, sex, tumour size, hormonal regulation, iodine exchange, genetic polymorphisms, in anti-tumor immunity violations. This tumour is usually slow in growth, and fatal cases are rare, except for anaplastic carcinomas which have a rapid progression and dissemination [1]. Papillary carcinoma is the most common thyroid neoplasm representing about 80% of all thyroid malignancies [2], while follicular and anaplastic cancers are quite rare. The oncocytic thyroid tumour, originating from C-cells in the thyroid gland is usually benign and might have malignant behaviour.

Different factors - cellular and molecular play a role in development and progression of thyroid cancer. A dominant role of Wnt/β-catenin signalling in the proliferation of normal and neoplastic thyrocytes was reported [3]. The aberrant activation of the Wnt-signaling pathway may be a common denominator for the development of tumours and strongly involved in thyroid tumorigenesis [4]. β-Catenin was originally described as an element of the E-cadherin/catenin complex. It is, however, an element of the Wnt signalling pathway and β-catenin may be tumorigenic [5]. β-Catenin is a multifunctional protein that plays an important role in signal transduction. In normal resting cells, it is mainly localised to the adherent junctions, while free cytosolic-β-catenin is recruited to a "destruction" complex that includes Axin, the tumour suppressor adenomatous polyposis coli (APC) and glycogen synthase kinase 3 (GSK3β). When the pathway is activated β-catenin is stabilised, that results in its nuclear accumulation and interaction with T-cell factor/lymphoid enhancer factor (TCF/LEF) or other transcription factors, and activation of genes required for cell proliferation (c-Myc and cyclin D1) [3]. Mutations in genes coding the proteins participating in

the regulation of β-catenin turnover (GSK3β, APC, AXIN) cause disturbance of the process of β-catenin protein degradation and its increase in the cellular cytoplasm [6].

Cadherin's act as Ca^{2+}- dependent adhesion molecules in the cell-cell adherence junction, a specialised region of the plasma membrane that is connected with cytoskeletal actin filaments. Cadherin molecules are integral membrane glycoproteins having a single transmembrane domain. The extracellular domain of E-cadherin is composed of five cadherin domains (EC1 to EC5) [7]. The cytoplasmic undercoat proteins for cadherins have been named catenins. β-Catenin interacts with cadherins through its cytoplasmic domain, which exhibits the strongest degree of homology between different members of the cadherin family. α-Catenin connects the E-cadherin and β-catenin complex to actin filaments. The interaction between cadherins and cytoskeletal proteins through catenins confer stability on the cell-cell adherent junctions. These observations suggested the possibility that suppression of E-cadherin activity triggers the release of cancer cell from primary cancer nests [5, 7].

The expression of E-cadherin and β-catenin in thyroid cancer was investigated mainly in immunohistochemical panels for differential diagnosis between different thyroid malignancies [8, 9]. Other investigators correlated the expression of both proteins with some clinical and histological parameters and tumour progression [9]. It was announced that the loss of E-cadherin rather than β-catenin mutation represents a crucial event in determining the degree of differentiation of thyroid carcinomas [8, 10].

In this study, we used immunohistochemistry with anti-E-cadherin and anti-β-catenin antibodies to determine their various expressions in different epithelial thyroid malignancies and to determine their correlation with some parameters of tumour progression and overall survival data.

Materials and Methods

Patients

For this retrospective study, we investigated 112 patients (22 men and 90 women) mean age 57.9 years (from 30 to 78 years). These thyroid cancer patients had been surgically treated for a period of 17 years (from 1998 to 2015) in the University Hospital of Stara Zagora, Bulgaria. The patients were followed up until 01st January 2016 year. The overall survival data were used only for papillary thyroid cancers since the group was largest. Tumors were divided into four groups: papillary thyroid cancer (PTC) (n = 69) and follicular variant of papillary thyroid cancer (FVPTC) (n = 10), follicular thyroid cancer (FTC) (n = 13), anaplastic thyroid cancer (ATC) (n = 10) and oncocytic carcinoma (OC) (n = 10). Deeper invasion (higher pT staging) and advanced stages (stage III or IV) were a more frequent event in ATC and OTC than in PTC and FTC ($p < 0.0001$, and $p = 0.010$, respectively). Lack of tumour capsule was more often detected in specimens from ATC and OTC than from PTC and FTC ($p = 0.008$) (Table 1). Tumor staging was defined as 45.5% (n = 51) for the Ist stage, 14.3% (n = 16) for the IInd stage, 16.1% (n = 18) for the IIIrd stage and 2.7% (n = 3) for the IVth stage.

Immunohistochemistry

Biopsy specimens were treated for routine histology (staining with hematoxylin and eosin) and for immunohistochemistry. Samples were fixed in 10% formalin, included in paraffin and the sections were 5mm thick. For antigen retrieval, the deparaffination was done in hot xylene, at 56°C. Blocking of endogenous peroxidase was performed with 1.2% hydrogen peroxide solution in methanol for 30 minutes. The specificity of the immune response is determined by substituting primary antibody with normal mouse serum. The duration of incubation with primary antibody and normal mouse serum was overnight at room temperature. The sections were counterstained with Mayer's hematoxylin for better visualisation of the DAB reaction product and then mounted with entering an for light microscopy. The antibodies used were the following: monoclonal mouse anti-human E-cadherin (M3612) both from DAKO A/S, Denmark, and anti-beta-catenin-Mhb (C19220) from Transduction Laboratories, USA.

Evaluation of E-cadherin/β-catenin expression

It was evaluated semi quantitatively as negative – (-); low – (-/+); and high – (++/+++). E-cadherin immune reaction was evaluated in tumour cytoplasm and membranes, and β-catenin – in tumour cytoplasm, membranes and nuclei.

Statistical analysis

Statistical analyses were performed using SPSS software package (SPSS, Inc., Chicago, IL, USA) and STATVIEW. The descriptive statistical tests, including the mean, standard deviation, and median, were calculated according to the standard methods. The associations E-cadherin/β-catenin expression and clinicopathological parameters were assessed by χ^2 Test. $P < 0.05$ was considered to be statistically significant. Survival plots were drawn by the Kaplan–Meier test and survival periods were compared by log-rank test.

Results

Immunohistochemical study

Expression of E-cadherin. We observe high expression in the cytoplasm and cell membrane in PTC and FTC and lower expression in the cytoplasm in OC. Patients with ATC showed low expression.

Level of expression of E-cadherin and β-catenin

In PTC, OC and FTC the positive expression of E-cadherin in tumor membrane is more frequent (87.5%, 71.4% and 75% respectively) compared with ATC, where it is 25% (p = 0.0042). In membranes of PTC tumor expression of E-cadherin, more often is higher (+ + / + + +) (68.75%) compared with that in the OC, FTC and ATC (28.6%, 25% and 12.5%, respectively) (p = 0.0065).

Expression of E-cadherin in tumor cytoplasm in a PTC is often high (+ + / + + +) (59.4%) compared with that in the OC, FTC and ATC (14.3%, 25% and 12.5%, respectively) (p = 0.019). A tendency of higher expression of β-catenin in the cytoplasm in tumor PTC (66.7%) compared with FTC (33%) and absence of expression in ATC (0%, p = 0.111).

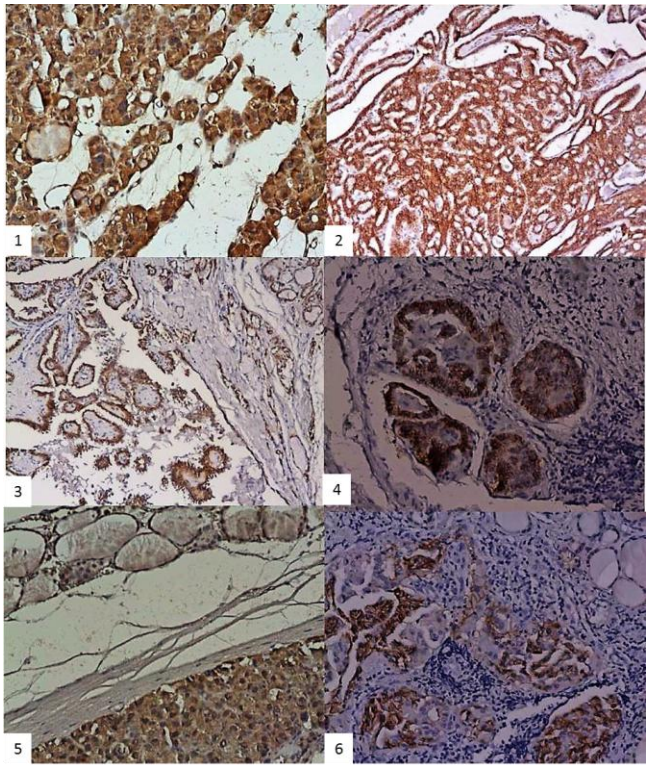

Figure 1: Expression of β-catenin. the β-catenin immune response in tumour cytoplasm and membrane. 1) β-catenin positive expression in FTC X 200. 2) β-catenin positive expression in PTC X 100. Expression of E-cadherin. E-cadherin immune response in tumour cytoplasm and membrane. 3) E-cadherin positive expression in OC X 200. 4) E-cadherin positive expression in PTC X 200. 5) E-cadherin positive expression in FTC X 200. 6) E-cadherin positive expression in PTC X 200

Observed was statistically more frequent high expression of β-catenin in tumour membrane in PTC (83.3%), while in other tumours, the expression of β-catenin in tumour membranes is weak indicative (p = 0.0104). All tumors showed low expression of β-catenin in tumor nucleus ($\chi 2$ = 1.298, p = 0.730) (Figure 1).

Comparing the level of expression with clinical data

Comparing the expression of E-cadherin in tumour cytoplasm and the development of metastases, it was obtained that in 100% of the tumours with an expression of E-cadherin there were no metastases, ($\chi 2$ = 3.6, p = 0.058).

Comparing the expression of E-cadherin in tumour membrane with tumour stage in patients with PTC turns out that 80.8% of tumours in stage I and II express E-cadherin in tumour membrane, while 50% of tumours in stage III and IV express E-cadherin ($\chi 2$ = 3.41, p = 0.065) (Table 1).

Table 1: Clinical data and histological and pathological characteristics of the tumour specimens according to the thyroid tumour type

Characteristics	PTC N (%)	FVPTC N (%)	FTC N (%)	ATC N (%)	OTC N (%)	p-value
Age (mean±SD)	54.17±14.48	53.00±12.08	56.67±9.59	59.40±12.05	56.62±8.52	0.718*
Gender						
males	9 (13.0)	2 (20.0)	5 (38.5)	3 (30.0)	3 (30.0)	0.316**
females	60(87.0)	8 (80.0)	8 (61.5)	7 (70.0)	7 (70.0)	
pT classification						
T1-T2	51 (91.1)	8 (80.0)	8 (72.7)	4 (40.0)	10 (100)	<0.000
T3-T4	5 (8.9)	2 (20.0)	3 (27.3)	6 (60.0)	0 (0)	1**
Lymph node metastases						
no	54 (96.4)	10 (100)	13 (100)	9 (90)	8 (80.0)	0.214**
yes	2 (3.6)	0 (0)	0 (0)	1 (10)	2 (20.0)	
Distant metastases						
no	69 (100)	10 (100)	13 (100)	10 (100)	9 (90.0)	0.065**
yes	0 (0)	0 (0)	0 (0)	0 (0)	1 (10.0)	
pTNM staging						
I stage	35 (62.5)	8 (72.7)	8 (72.7)	3 (30)	6 (60.0)	
II stage	14 (25.0)	2 (18.2)	0 (0)	1 (10)	1 (10.0)	0.005**
III stage	7 (12.5)	0 (0)	2 (18.2)	6 (60)	2 (20.0)	
IV stage	0 (0)	0 (0)	1 (9.1)	0 (0)	1 (10.0)	
Capsule invasion						
Non capsular	9 (18.0)	9 (18.0)	5 (45.5)	7 (70.0)	5 (50.0)	0.008**
Capsular	51 (82.0)	51 (82.0)	6 (54.5)	3 (30.0)	5 (50.0)	

* - ANOVA test; ** - $\chi 2$ test

There was no significant difference in β-catenin expression between other clinical data, except for high expression in 73% of tumours in stage I and II compared with low expression of the catenin in the tumor in stage III and IV ($\chi 2$ = 3.59, p = 0.046).

Overall survival in the group of patients with PTC

Complete clinical and survival data were available from the Oncological archives for 56 of the patients. The patients were followed-up until 01st January 2016 year. At the end of the follow-up period there were 42 patients alive, as 30 of them with PTC (3/37), 6 with FTC (6/7), 3 with ATC (3/6) and 3 with OC (3/6). The medial survival period for all patients was 104.41 months, ranging from 1.64 to 197.07

months. Patients with PTC had a median survival of 104.27 months (12.76 – 182.28 months), those with FTC –median of 123.38 months (18.15-197.08 months); with ATC – median of 71.85 months (1.64-163.86 months), and patients with OTC – median survival of 116.00 months (7.93 – 192.86 months). Due to the very small number of patients with some types of thyroid cancer, we performed survival analyses only in the group of patients with PTC. Samples with the presence of expression of E-cadherin tumour membrane show a tendency for longer overall survival than those with absent expression (p = 0.072) (Figure 2).

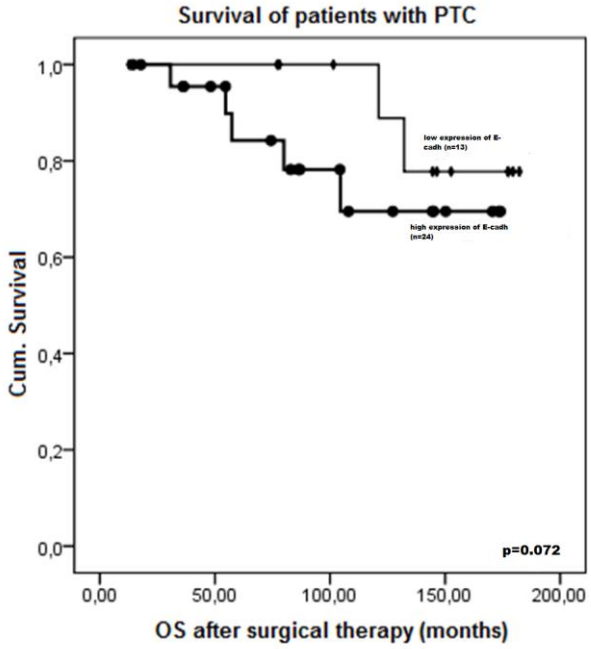

Figure 2: Kaplan-Mayer survival plot for overall survival (OS) after surgical therapy of patients with PTC according to the expression of E-cadherin (Log-rank test)

Discussion

Beta-catenin is involved in the regulation of gene expression as a mediator of the Wnt-signaling pathway. The expression and intracellular localisation of beta-catenin are altered in many types of cancers. Beta-catenin acts as an integral component of adherence junctions in addition to being a component of the Wnt-signaling. Beta-catenin links to epithelial cadherin, a major cell-cell adhesion molecule, with two other catenin members (α and γ) at pericellular membrane [11]. In our study, we found high expression of β-catenin in tumour membrane in PTC while in other tumours, the expression of β-catenin in tumour membranes is weak indicative.

E-cadherin is normally expressed in the thyroid gland and represents the main cell-cell adhesion molecule of the thyroid follicular cells. This cadherin is necessary for normal epithelial function, and it is responsible for calcium assisted cell adhesion. It can prevent the invasion of malignant tumours, and its synthesis in malignant cells is reduced. Epithelial cadherin levels in malignant tumours are variable and reduced [9]. E-cadherin has a significant function in intercellular adhesion of epithelial cells, the establishment of epithelial polarisation, glandular differentiation and stratification. Down-regulation of E-cadherin expression has been observed in some carcinomas and is usually associated with advanced stage and progression. The antibody is useful for the identification of E-cadherin-positive cells in normal and neoplastic tissues. In our study, we found that highly differentiated epithelial carcinomas (PTC, FTC, OC) of the thyroid gland express in greater cell adhesion marker E-cadherin compared with non-differentiated anaplastic carcinomas. The low expression of E-cadherin in PTC confirms the results of Soares at al. 1997 and Brabant et al. 1993 [6, 18] who observed a reduction of this cadherin in a PTC compared with normal tissue. In our investigation, there was a tendency for statistical significance in E-cadherin expression between highly differentiated thyroid cancers (high expression) and anaplastic cancers (low expression). Also in our study, we found that tumours expressing E-cadherin in tumour cell cytoplasm develop metastases with very small probability. Erdem at al. 2011 [9] found the same results, generally, the presence of E-cadherin in tumour epithelium correlated with the absence of metastases and local invasion. Similar results were reported from Rocha et al. 2003 [10] and other author's [12-14] who observed that in patients with over 30% E-cadherin staining, local recurrence and distant metastasis were rarely seen.

In our investigation, there was a tendency for statistical significance of E-cadherin expression in tumour membrane with tumour stage in patients with PTC. We found that in stage I and II expression of E-cadherin are high, while in stage III and IV the expression is less. The E-cadherin/catenin and its associated complexes participate in tumour development as suppressors or stimulators of growth, differentiation and invasion. Consequently, tumors which express E-cadherin can serve as a quality mark for their staging. This cadherin is also involved in adhesion between epithelial cells, and it seems to have a protective role in cancer since its loss is associated with tumour progression and metastases formation in a series of different cancers [12, 15, 16]. It has been proposed that reduced aberrant expression of E-cadherin is critical for the pathogenesis and biological behaviour of certain thyroid cancers [17-19]. It was possible that E-cadherin expression was lost as an epiphenomenon of tumour dedifferentiation, rather than being a true pathogenic factor [20-24]. The role of adhesion factors in thyroid malignancies may be superior in

comparison with cell proliferation. Not surprisingly, the presence of aberrant expression of E-cadherin and β-catenin in thyroid cancer has been associated with better patients' prognosis and better differentiated tumour histology.

References

1. Takano T. Fetal cell carcinogenesis of the thyroid. Endocrine journal. 2004;51(6)509-515. https://doi.org/10.1507/endocrj.51.509 PMid:15644567

2. Ito Y, Miyauci A. Prognostic factors and therapeutic strategies for diferentiated carcinomas of the thiroid. Endocr J. 2009;56177-192.

3. Ishigaki K, Namba H, Nakashima M, Nakayama T, Mitsutake N, et al. Abberant localization of beta-catenin correlates with overexpression of its target gene in human papillary thyroid cancer. J Clin Endocrinol Metab. 2002;87:3433-3440. PMid:12107263

4. Van Aken E, De Wever O, da Rocha ASC, Mareel M. Defective E-cadherin/catenin complexes in human cancer. Virchows Arch. 2001;439:725-751. https://doi.org/10.1007/s004280100516 PMid:11787845

5. Brabletz T, Jung A, Kirchner T. β-Catenin and the morphogenesis of colorectal canvcer. Virchows Arch. 2002;441:1-11. https://doi.org/10.1007/s00428-002-0642-9 PMid:12111194

6. Barabant G at al. E-cadherin a differentiation marker in thyroid malignancies. Cancer Res. 1993;135:575-581.

7. Hirohashi S, Kanai Y. Cell adhesion system and human cancer morphogenesis. Cancer Sci. 2003;94(7):575-581. https://doi.org/10.1111/j.1349-7006.2003.tb01485.x PMid:12841864

8. Wiseman SM, Masoudi H, Niblock P, Turbin D, Rajput A, Hay J, Filipenko D, Huntsman D, Gilks B. Derangement of the E-cadherin/catenin complex is involved in transformation of differentiated to anaplastic thyroid carcinoma. Am J Surgery. 2006;191:581-587. https://doi.org/10.1016/j.amjsurg.2006.02.005 PMid:16647341

9. Erdem H, Gundogdu C, Sipal S. Correlation of E-cadherin, VEGF, COX-2 expression to prognostic parameters in papillary thyroid carcinoma. Exp Mol Pathol. 2011;90:312-317. https://doi.org/10.1016/j.yexmp.2011.01.008 PMid:21335003

10. Rocha AS, Soares P, Fonseca E, et al. E-cadherin loss rather than beta-catenin alterations is acommon feature of poorly differentiated thyroid carcinomas. Histopathology. 2003;42:580-587. https://doi.org/10.1046/j.1365-2559.2003.01642.x PMid:12786894

11. Garcia-Rostan G, Robert L, David L, Giovanni T. β-catenin Dysregulation in thyroid neoplasms down-regulation, aberrant nuclear expression, and CTNNB1 Exon 3 mutations are markers for aggressive tumor phenotypes and poor prognosis. American journal of pathology. 2001;158(3)987-996. https://doi.org/10.1016/S0002-9440(10)64045-X

12. Yap AS. The morphogenetic role of cadherin cell adhesion molecules in human cancer: a thematic review. Cancer Invest. 1998;16(4):252-261. https://doi.org/10.3109/07357909809039774 PMid:9589034

13. Rocha AS, Smit DI, Thibodeau SN . Frequent mutation and nuclear localization of E-cadherin in anaplastic thyroid carcinoma. Cancer Res. 2000;26:146-147.

14. El Demellawy D, Nasr A, Alowami S. Application of CD56, P63 and CK19 immunohistochemistry in the diagnosis of papillary carcinoma of the thyroid. Diagn Pathol. 2008;3:5. https://doi.org/10.1186/1746-1596-3-5 PMid:18254952 PMCid:PMC2267445

15. Kapran Y, Ozbey N, Molvalilar S, Snecer E, Disdalogru F, Ozarmagan S. Immunohistochemical detection of E-cadherin alpha- and beta-catenins in papillary thyroid carcinoma. J Endocrinol Invest. 2002;25:578-585. https://doi.org/10.1007/BF03345079 PMid:12150330

16. Guarino V, Castellone MD, Avila E, Rosa MM. Thyroid cancer and inflammation. Molecular and cellular Endocrinology. 2010;321:94-102. https://doi.org/10.1016/j.mce.2009.10.003 PMid:19835928

17. Gilbert-Sirieix M, Makoukji J, Kimura S, Talbot M, Caillou B, Massaad C, Massaad-Massade L. Wnt/β-catenin signaling pathway is a direct enhancer of thyroid transcription factor-1 in human papillary thyroid carcinoma cells. Plos one. 2011;6(7): e22280. https://doi.org/10.1371/journal.pone.0022280 PMid:21814573 PMCid:PMC3141030

18. P. Soares, G. Berx, F.V Roy, M. Sobrinho-Simões. E-cadherin gene alteration are rare events in thyroid tumors. Int J Cancer. 1997;70:32-38. https://doi.org/10.1002/(SICI)1097-0215(19970106)70:1<32::AID-IJC5>3.0.CO;2-7

19. Batistatou A, Konstantinos C, Yukihiro N, Constantine V, Setsuo H, Niki JA, Chrissoula DS. Differential Expression of Dysadherin in papillary thyroid carcinoma and microcarcinoma: correlation with E-cadherin. Endocr Pathol. 2008;19:197-202. https://doi.org/10.1007/s12022-008-9035-1 PMid:18677652

20. Fluimara A, Russo G, Salomone E et al. In situ evidence of neoplastic cells phagocytes by macrophages in papillary thyroid cancer. Clin Endocrinol Metab. 1997;82:1615-1620.

21. Mantovani A, Schippa T, Porta C, Allavena P, Sica A. Role of tumor-associated macrophages in tumor progression and invasion. Cancer Metast Rev. 2006;25:315-322. https://doi.org/10.1007/s10555-006-9001-7 PMid:16967326

22. Ryder M, Ghossein RA, Ricarte-Filho JCM, Knauf JA, Fagin JA. Increased density of tumor -associated macrophages is associated with decreased survival in advanced thyroid cancer. Endocr Relat Cancer. 2008;15(4):1069-1074. https://doi.org/10.1677/ERC-08-0036 PMid:18719091 PMCid:PMC2648614

23. Underwood JCE. Lymphoreticular infiltration in human tumors. Br J Cancer.1974;30:538-548. https://doi.org/10.1038/bjc.1974.233 PMid:4614858 PMCid:PMC2009334

24. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenviroments. Cancer Research. 2006;66:605-612. https://doi.org/10.1158/0008-5472.CAN-05-4005 PMid:16423985