



E-CADHERIN/ β -CATENIN AND CD68 IMMUNOEXPRESSION IN THYROID CANCER

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ABSTRACT

Thyroid tumors are relatively common, especially in women. These cancers appeared to be a particularly interesting neoplastic process, despite numerous data on the importance of cellular and molecular factors.

The **aim** of the present study was to investigate CD68 distribution and E-cadherin/ β -catenin expression in thyroid cancer and the impact of this expression to tumor differentiation and eventually progression.

Materials and methods: We investigated thyroid tumor tissue collected from 57 patients (12 males and 45 females), having thyroid tumors - papillary, follicular, anaplastic and oncocyctic carcinomas. Immunohistochemistry was done with anti-E-cadherin antibody; anti- β -catenin antibody; and anti-CD68 antibody.

Results: The expression of E-cadherin in PTC/FTC in tumor cytoplasm and membranes was stronger as compared to that in ATC. All tumors that showed E-cadherin expression in tumor cytoplasm didn't develop metastases ($\chi^2 = 3.6$, $p = 0.058$). Most of the tumors expressing E-cadherin in their cytoplasm had low CD68 infiltration in the invasive margin, ($\chi^2 = 3.81$, $p = 0.051$). The most PTC/FTC showed β -catenin expression in tumor cells, while ATC cases had no such an expression.

Conclusion: Not surprisingly, the presence of aberrant expression of E-cadherin and/or β -catenin in thyroid cancer has been associated with more differentiated tumor histology. For the first time we correlated E-cadherin/ β -catenin expression with TAMs infiltration in thyroid cancers and found their impact on tumor differentiation and progression.

Key words: thyroid carcinoma, CD68, E-cadherin, β -catenin

INTRODUCTION

Thyroid cancer is the most frequent endocrine neoplasia, and its incidence is rapidly increasing (1). Thyroid carcinomas are usually slow in growth, and fatal cases are rare, except for anaplastic carcinomas which show a relentless and deadly clinical course with rapid progression and dissemination (2). Papillary carcinoma is the most common thyroid cancer representing about 80% of all thyroid malignancies (3). The frequency of thyroid neoplasms varies widely according to

geographical dissemination and its traceability at different periods. Different factors - cellular and molecular play a role in development and progression of thyroid cancer. A dominant role of Wnt/ β -catenin signaling in the proliferation of normal and neoplastic thyrocytes was reported (4). The aberrant activation of the Wnt-signaling pathway may be a common denominator for the development of tumors and strongly involved in thyroid tumorigenesis (5). E-cadherin is necessary for normal epithelial function and it is responsible for calcium assisted cell adhesion. It can prevent the invasion of malignant tumors and its synthesis in malignant cells is reduced. Epithelial cadherin levels in malignant tumors are variable and generally reduced (6). It was

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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 (7, 8). E-cadherin has a significant function in intercellular adhesion of epithelial cells, the establishment of epithelial polarization, glandular differentiation and stratification. Down-regulation of E-cadherin expression has been observed in a number of 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. 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 tumor progression and metastases formation in a series of different cancers (9). E-cadherin is normally expressed in the thyroid gland and represents the main cell-cell adhesion molecule of the thyroid follicular cells. It has been proposed that reduced aberrant expression of E-cadherin is critical for the pathogenesis and biological behavior of certain thyroid cancers (10, 11).

Beta-catenin is involved in the regulation of gene expression as a mediator of the Wnt-signaling pathway. The expression and intracellular localization of beta-catenin is altered in many types of cancers. Beta-catenin acts as an integral component of adherens 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. The interaction between E-cadherin and β -catenin is lost in normal epithelial cells, the regular polarity disappears and cells do not form the follicular structure or they transform into papillary phenotype (5).

On the other side in many human tumors (breast cancer, thyroid cancer etc.) a high frequency of infiltrating tumor associated macrophages (TAMs) has been associated with tumor development and poor prognosis (12, 13). Macrophages may be found in a variety of malignancies and are commonly believed to represent the host's immune response to the tumor (14). It was shown that in poorly differentiated thyroid cancers TAMs support tumor progression (13). Macrophages were identified by immunohistochemistry with specific antibody anti-CD68. Understanding

the functions of TAMs on tumor progression is complex due to the pleiotropic action of macrophages on tumor progression (15).

In this study we observed the tissue expression of proteins from Wnt/ β -catenin signaling pathway, namely E-cadherin and β -catenin and of TAMs, in papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), anaplastic thyroid cancer (ATC) and oncocyctic carcinoma (OC). Our findings suggest that in well differentiated thyroid cancers the immune expression of E-cadherin and β -catenin was better preserved as compared to poorly differentiated ones. The low infiltration of tumors with TAMs inversely correlated with higher E-cadherin expression suggesting the dual role of TAMs in highly and poorly differentiated thyroid cancers in the context of tumor suppression and tumor promotion.

MATERIALS AND METHODS

For this retrospective study we investigated 57 patients (12 men and 45 women) mean age 57,9 years (from 30 to 78 years). These thyroid cancer patients had been surgically treated in a period of 15 years (from 1998 to 2012) in the University Hospital of Stara Zagora, Bulgaria. Tumors were divided into three groups of which the first group were united papillary and follicular carcinomas as follows: papillary thyroid cancer (PTC) (n=32) / follicular thyroid cancer (FTC) (n=8), anaplastic thyroid cancer (ATC) (n = 9) and oncocyctic carcinoma (OC) (n = 8).

The antibodies used were the following: monoclonal mouse anti-human CD68 (N1576) and monoclonal mouse anti-human E-cadherin (M3612) both from DAKO A/S, Denmark, and anti-beta-catenin-Mhb (C19220) from Transduction Laboratories, USA .

Biopsy specimens were treated for routine histology (staining with hematoxylin and eosin) and for light microscopical immunohistochemistry. Samples were fixed in 10% formalin, included in paraffin and the sections were 5 μ m 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 visualization of the DAB reaction product and then mounted with entelan for light microscopy.

Cell counting. TAMs were counted in tumor nests and at the invasive margin of tumors, at 5 randomly chosen fields of vision, at magnification of 400 (0.74 mm^2).

Evaluation of E-cadherin/ β -catenin expression. It was evaluated semiquantitatively as missing – (-); weak – (+); moderate – (++) ; strong – (+++). For the statistical analysis we assessed the immune reaction as weak to missing (-/+) and expressed (+++/+++). E-cadherin immune reaction was evaluated in tumor cytoplasm and membranes, and β -catenin – in tumor 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 and CD68 expression and clinicopathological parameters were assessed by χ^2 Test. $P < 0.05$ was considered to be statistically significant.

RESULTS

When comparing the expression of E-cadherin in PTC/FTC with that in ATC we found that 82.5% of PTC/FTC have E-cadherin expression in the tumor cytoplasm, while 62.5% from ATC didn't express E-cadherin in tumor cells, ($\chi^2 = 7.2$, $p = 0.007$). In 82.9% from PTC/FTC E-cadherin had membrane location in tumor cells, while in 75% from ATC didn't have the epithelial cadherin in their cell membranes ($\chi^2 = 11.5$, $p = 0.01$), (**Fig. 1**).

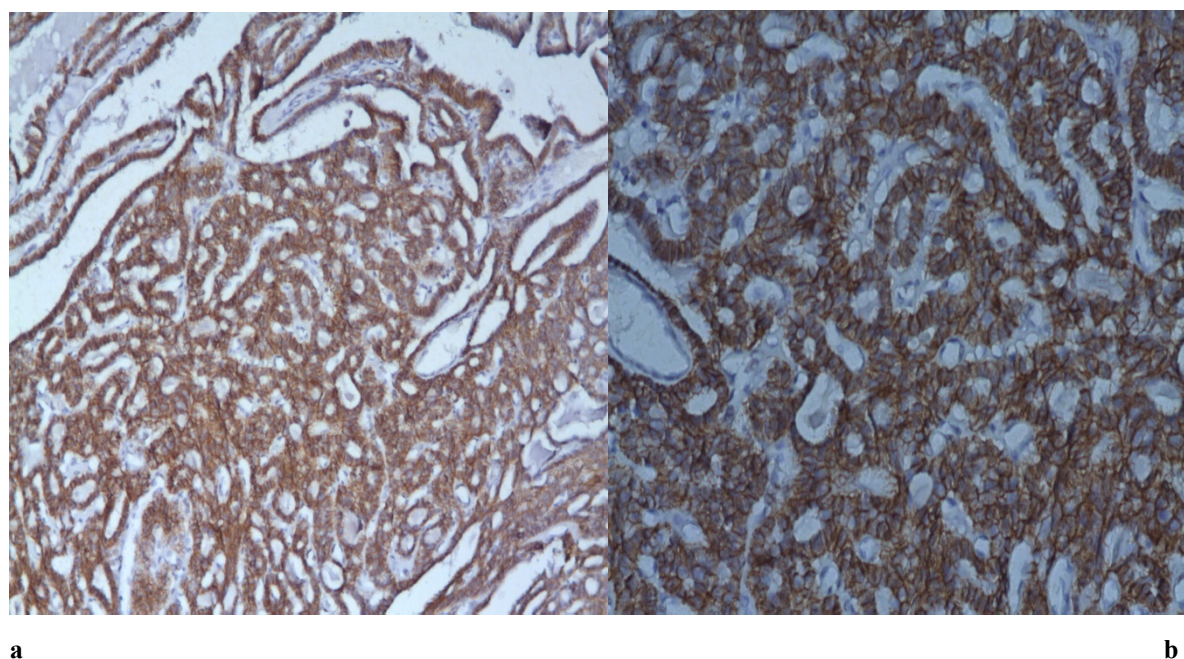
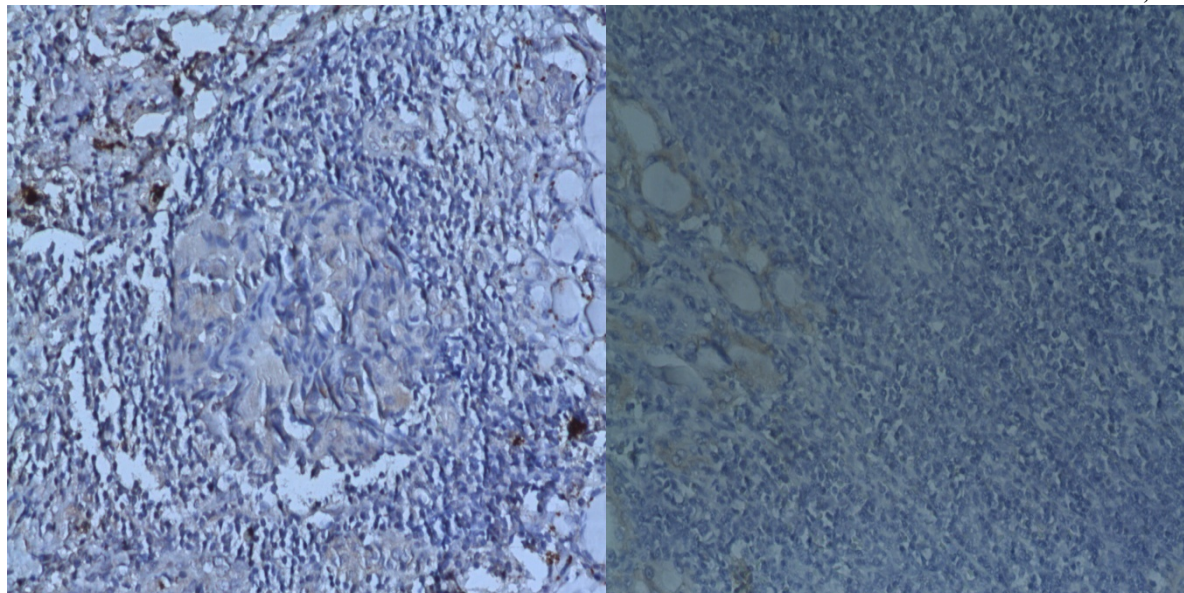


Fig. 1. Papillary thyroid carcinoma: high immunohistochemical expression of E-cadherin in tumor membranes.(Magnification a x 100; b x 200).

Comparing the expression of E-cadherin between all cancers with the infiltration with CD68 we obtained that 58.9% of tumors expressing E-cadherin in tumor cytoplasm had low infiltration with CD68 in the invasive margin, ($\chi^2 = 3.81$, $p = 0.051$). In 61.5% of tumors expressing E-cadherin in tumor cytoplasm we observed low infiltration with

CD68 in the tumor stroma, ($\chi^2 = 6.63$, $p = 0.01$).

Comparing the expression of E-cadherin in tumor cytoplasm and the development of metastases, it was obtained that in 100% of the tumors with expression of E-cadherin there were no metastases, ($\chi^2 = 3.6$, $p = 0.058$), (**Fig. 2a, b**)



a **b**
Fig. 2. a - Papillary thyroid carcinoma - low immunohistochemical expression of CD68 in tumor stroma. (Magnification x 200); **b** - Anaplastic thyroid tumor - negative expression of E-cadherin. (Magnification x 200).

There was a tendency when comparing the expression of β -catenin in tumor cell cytoplasm and membrane, where the larger percentage of PTC/FTC were manifested with high β -catenin expression (55.6%) there, while

ATC cases had no such an expression ($\chi^2 = 3.611$, $p = 0.105$, Fisher's Exact Test).

There was no difference in levels of β -catenin expression in tumor nuclei in the different types of thyroid cancers (**Fig. 3**).

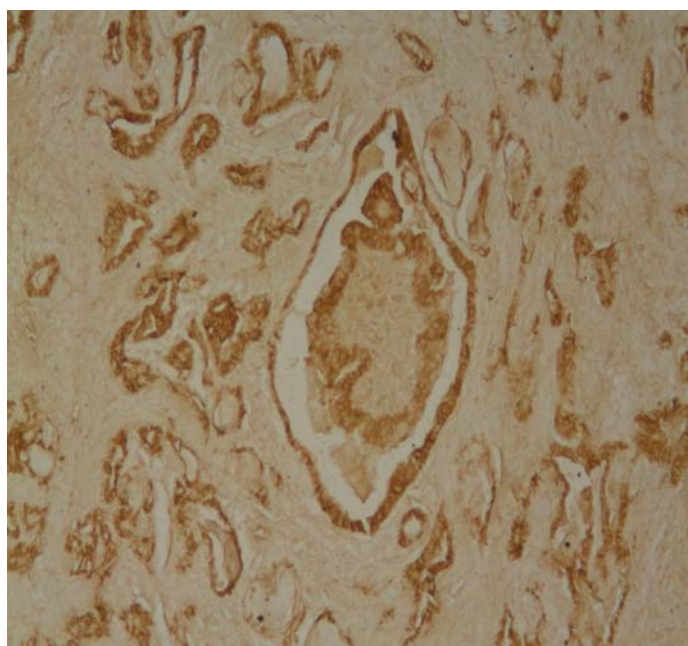


Fig. 3. Papillary carcinoma - positive expression of β -catenin in tumor membranes. (Magnification x100).

DISCUSSION

E-cadherin is normally expressed in thyroid follicles and represents the main cell-cell adhesion molecule of the thyroid gland. It has been proposed that reduced aberrant

expression of this marker is critical for the pathogenesis and biological behavior of certain thyroid carcinomas (16).

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. Batistatou et al. have found that in papillary thyroid carcinoma the expression of E-cadherin was low in 40% of cases and high in 20%. 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). Rocha et al (16) concluded that was the loss of E-cadherin rather than β -catenin mutation that represents the crucial event in determining the degree of differentiation of thyroid carcinomas. Consequently, the expression of E-cadherin can serve as a marker for higher tumor differentiation. Concerning the expression of β -catenin in the tumor cell membrane and cytoplasm we detected a tendency of higher expression of the antibody in PTC/FTC as compared to ATC. Like others (8) we have found that the expression of E-cadherin and β -catenin was decreased in PTC and FTC and lost in ATC. The loss of E-cadherin expression was lower than the loss of β -catenin expression by anaplastic tumors. These observations suggest that β -catenin rather than E-cadherin may have a more important role in anaplastic transformation of differentiated thyroid carcinoma. As it was previously supposed (5), β -catenin was liberated from E-cadherin/ β -catenin complex and the last may activate Wnt-signaling pathway, resulting in unlimited cell proliferation.

With regard to clinical relevance, we like others (5) did not find any correlation between β -catenin staining pattern in thyroid neoplasms and various clinicopathological parameters.

In our study we found that tumors expressing E-cadherin in tumor cell cytoplasm develop metastases with very small probability. Erdem et al. (2011) (6) found the same results, generally, the presence of E-cadherin in tumor epithelium correlated with absence of metastases and of local invasion. Rocha et al. (2003) (7) reported that in patients with over 30% E-cadherin staining local recurrence and distant metastasis were rarely seen.

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/or β -catenin in thyroid cancer has been

associated with better patients' prognosis and more well differentiated tumor histology.

TAMs are classified in two different phenotypes M1/Th1 associated and M2/Th2 associated according to immune cells and cytokine profile of the tumor microenvironment (13). Comparing the expression of E-cadherin between all cancers and infiltration with CD68 we obtained that in tumors highly expressing E-cadherin in tumor cytoplasm there was low infiltration with CD68 in the invasive margin and in tumor stroma, which means that Wnt-signaling pathway was involved in the regulation of the accumulation of TAMs. It was known that TAMs through IL-1 β , promote Wnt signaling in tumor cells, supporting tumor growth (17). Expression of oncoproteins involved in thyroid cell transformation may directly stimulate the recruitment of TAMs through increased expression of macrophage chemoattractants. The correlation between TAMs and E-cadherin expression in thyroid cancers suggest that the macrophages may actively participate in the biology of tumor process (15, 17).

In our study for the first time we correlated E-cadherin/ β -catenin expression with TAMs infiltration in thyroid cancers and found their impact on tumor differentiation and progression. Further investigations are necessary to elucidate that connection.

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