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ROLE OF p53 AND CD68 IN DEVELOPMENT OF THYROID CANCER

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ABSTRACT

Thyroid carcinoma is the most frequently diagnosed endocrine malignancy with a prevalence in Bulgaria in 2010 accounting for 1.6%. This tumor is usually slow in growth, and fatal cases are rare, except for anaplastic carcinomas which have a rapid progression and dissemination. Different factors - cellular and molecular play a role in development and progression of thyroid cancer. p53 protein is one of those factors, which function is to control the cell cycle and protect the organism from the development of cancers.

The **aim** of this study was to evaluate the expression of p53 protein and tumor associated macrophages CD68 in the thyroid cancer tissue and to correlate these data with some clinicopathological parameters of the tumors.

Materials and methods: For this retrospective study we investigated 52 patients (10 men and 42 women) mean age 55,8 years (from 22 to 79 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.

We investigated the patients having thyroid tumors papillary, follicular, anaplastic and oncocytic carcinomas imunohistochemically with antibodies against p53 and CD68.

Results: We found that 56,3% of p53 positive cells in tumors have low infiltration with CD68, while 13,9% of negative p53 have significantly higher infiltration with CD68. (x2=5,56; p= 0.018).

We also observed that 62,5% of p53 positive cells in tumor border was lower, although not significantly, in tumors with expression of CD68, while 19,4% of negative p53 have higher infiltration. (x2=1,92; p= 0.165).

In **conclusion**, we suggest that p53 protein profile analysis by IHC be useful in the differential diagnosis of thyroid lesion, appears with respect to aggression of the tumor, histogenesis, respectively the progression of cancer.

Key words: p53 protein, CD68, thyroid cancer

INTRODUCTION

Thyroid cancer is one of the most frequent endocrine tumors at present (1). This tumor is usually slow in growth, and fatal cases are rare, except for anaplastic carcinomas which have a rapid progression and dissemination (2). Papillary carcinoma is the most common thyroid neoplasm representing about 80% of all thyroid malignancies (3), while follicular and anaplastic cancers are quite rare. The oncocytic thyroid tumor (oncocytoma), 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. p53 protein is one of those factors, which function is to control the cell cycle and protect the organism from the development of cancers. It plays an important role in cell cycle control and apoptosis (10). Defective p53 could allow abnormal cells to proliferate, resulting in cancer. Such mutations are found in all of the major histogenetic groups, including cancers of the colon, stomach, breast, lung, brain, thyroid gland and esophagus (4, 8, 11) . It is estimated that *p53* mutations is the most frequent genetic event in human cancers and accounts for more than 50% of cases

In normal cells, the p53 protein level is low. DNA damage and other stress signals may trigger the increase of p53 proteins, which have three major functions: growth arrest, DNA repair and apoptosis (cell death), (13). The growth arrest stops the progression of cell cycle, preventing replication of damaged DNA. During the growth arrest, p53 may activate the transcription of proteins involved in DNA repair. Apoptosis is the "last resort" to avoid proliferation of cells containing abnormal DNA. In cancer cells that bear a mutant p53, this protein is no longer able to control cell proliferation, which results in inefficient DNA repair and the emergence of genetically unstable cells (4, 5).

The cellular concentration of p53 must be tightly regulated. While it can suppress tumors, high level of p53 may accelerate the aging process by excessive apoptosis. The major regulator of p53 is Mdm2, which can trigger the degradation of p53 by the ubiquitin system (6, 12).One of the most striking features of the inactive mutant p53 protein is its increased stability (half-life of several hours compared with 20 min for wild-type p53) and its accumulation in the nucleus of neoplastic cells. Positive immunostaining is usually indicative of abnormalities of the *p53*gene and its product, but it is highly dependent on the type of p53 mutation (7, 11). Tumor-associated macrophages (TAMs) play a tumorigenic role related to advanced staging and poor prognosis in many human cancers including thyroid cancers.CD-68 is widely regarded as a selective marker for human monocytes and macrophages and is commonly used in human pathology studies (6, 9, 10).

MATERIALS AND METHODS

Specimens were obtained from 52 patients who underwent resection of thyroid cancer at the Department of Surgery, University Hospital "Prof. St. Kirkovich", Medical Faculty, Trakia University, Stara Zagora, between 1998 and 2012. The patients comprised 10 males and 42 females, aged 22 to 79years (mean 55,8 years). No patient received anti-cancer treatment prior to surgery. Tumor staging was defined as 60% (n=21) for the Ist stage, 11,4% (n=4) for the IInd stage, 25,7% (n=9) for the IIIrd stage and 2,9% (n=1) for the IVth stage . Tumor grading and staging was performed according to the TNM Classification of Malignant Tumours 7 th Edition, by UICC 2009.

Thirty one patients (59,6%) had the papillary histologic type tumor (PTC), 7 patients (13,5%) had oncocytic type (OTC), 7patients 13,5% had follicular type (FTC) and the other 7 (13,5%) had anaplastic type (ATC). Tumor specimens were fixed in 10% buffered formalin and embedded in paraffin. Histological grading was performed on hematoxyllin and eosin-stained sections according to the protocols.

For immunohistochemical staining, the paraffin blocks were prepared using tumor tissues from the periphery of the tumor adjacent to the normal tissues. Paraffin sections 5 µm thick were dewaxed in two xyllenes (for 30 min each at 56º) and were rehydrated in ethanol. Then the sections were soaked in 10 % sucrose in distilled water, overnight. Later, they were washed in 0.1 M phosphate buffered saline (PBS), pH 7.4, incubated in 1.2 % hydrogen peroxide in methanol for 30 min, and rinsed in 0.1 M PBS, pH 7.4, for 15 min. Then the slides were incubated in a humid chamber until night, at room temperature with antibody Monoclonal Mouse Anti-Human CD68 clone: PG-M1 (DAKO, Denmark) and Monoclonal Mouse Anti-Human p53 protein clone:DO-7(DAKO, Denmark) .After washing three times in PBS, the slides were incubated with DAKO-REALTM En-VisionTM detection system (DAKO) for 60 min, then visualized with diaminobenzidine and counterstained with Mayer's hematoxylin. For negative control, the primary antibody was replaced with PBS.

The SPSS 16.0 program for Windows was used for statistical analysis. The chi-squared test and Fisher's exact test were used to compare the immunohistochemical staining and the clinicopathological parameters. Correlations were tested by Spearmen and Person tests. The accepted level of significance was set at $p<0.05$.

RESULTS

In our study 52 patients were investigated immunohistochemicaly for p53 in tumor cell cytoplasm.Of them, 36 displayed negative expression of p53 and 16 displayed positive expression. When comparing the expression of p53 between CD68+ cells in tumor and CD68+ cells in tumor we found that in 56,3% of p53 positive cells in tumors have low infiltration with CD68, while 13,9% of negative p53 have significantly higher infiltration with CD68.

(x2=5,56; p= 0.018).

We also observed that 62,5% of p53 positive cells in tumor border was lower, although not significantly, in tumors with expression of CD68, while 19,4% of negative p53 have higher infiltration. (x2=1,92; p= 0.165).

After analysis, it is found that in 30.8% of the examined cases there is expression of the p53 protein.. In addition, there was a tendency in gender expression of p53, as registered in 30% of men and 31% of women (χ2 = 6.53; p = 0.088). **(Figure 1** and **Figure 2).**

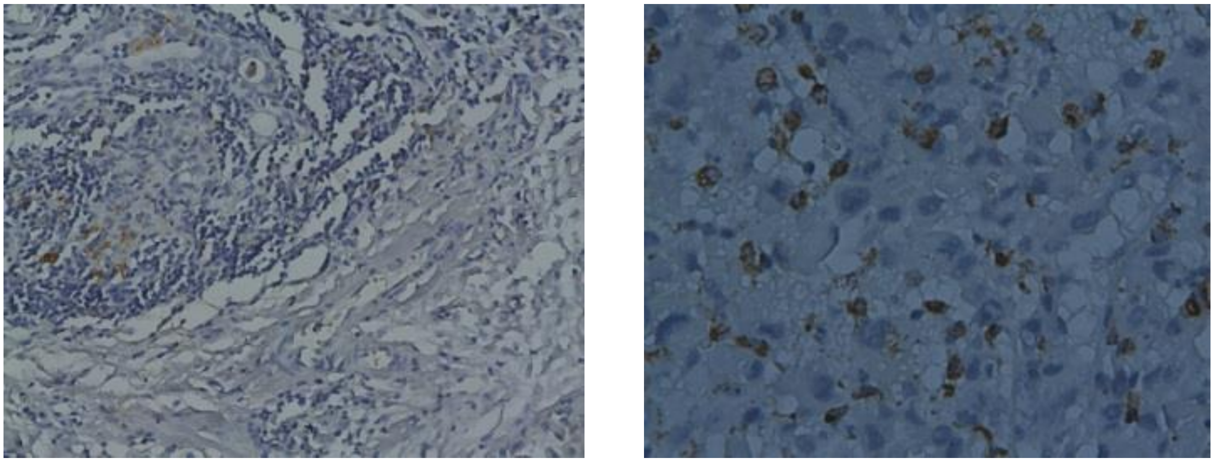

Figure 1. Anaplastic thyroid carcinoma: CD68 expression in tumor border and tumor tissue (Magnification a x 200; b x 400).

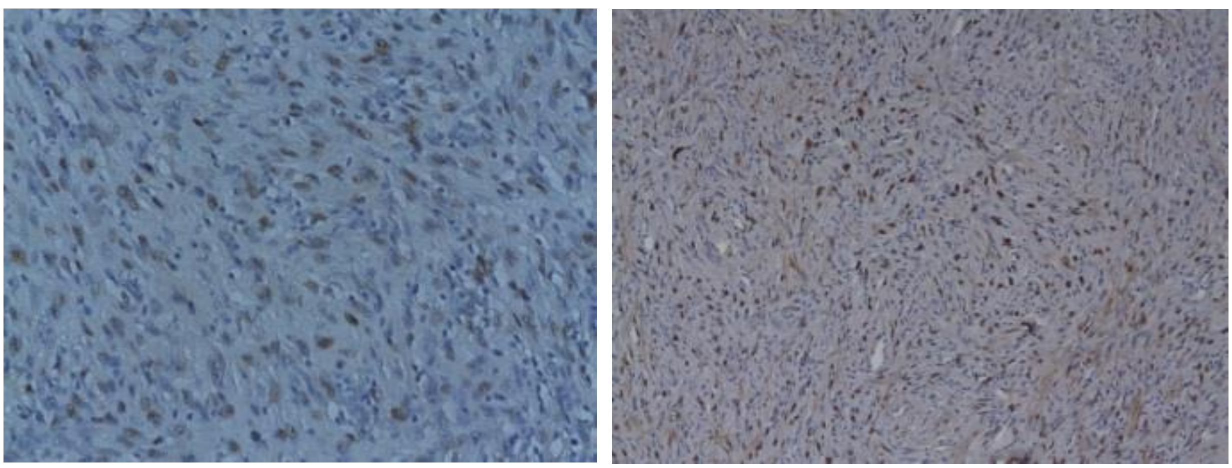

Figure 2. Anaplastic thyroid carcinoma: p53 expression possitive cells in tumor (Magnification a x 400; b x 100).

DISCUSION

The study of p53 has revealed many of the principles underlying human tumorigenesis. These include the critical differences between an oncogene and a tumor suppressor gene, the relationship between environmental exposures and cancer, the mechanisms through which cancer genes stimulate cell birth or inhibit cell death, and the striking networks that control the transcription, translation, and function of key cellular proteins(2,9). We observed that tumors positive for p53 expression frequently presented an low infiltration of CD68, *Marcello et all, 2013*, found the same relationship between these markers, which means that the association of p53 and CD68 may help to explain, the aggressivity of thyroid cancer. In fact, the aberrant expression in non tumor cells provides an immunological window for the use of p53 as a tumor antigen for immunotherapy. (5, 10, 13). The many facets of these studies, coupled with the fact that p53 inactivation is essential for the formation of the majority of human tumors. In conclusion we can say that after an analysis of the literature data and the data obtained from our study, p53 is a quality marker in terms of the aggressiveness of the tumor histogenesis, respectively progression of cancer.

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