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 immunohistochemically 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.
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
p53gene 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 79 years (mean 55.8 years).
No patient received anti-cancer treatment prior to
surgery. Tumor staging was defined as 60%
(n=21) for the I stage, 11.4% (n=4) for the II
stage, 25.7% (n=9) for the III stage and 2.9%
(n=1) for the IV stage. Tumor grading and
staging was performed according to the TNM
Classification of Malignant Tumours 7th

Thirty one patients (59.6%) had the papillary
histologic type tumor (PTC), 7 patients (13.5%)
had oncocytic type (OTC), 7 patients 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 hematoxylin 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 xylenes (for 30 min each at 56°)
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 Spearman 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.

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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. ($\chi^2=1.92; p=0.165$).

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 ($\chi^2=6.53; p=0.088$). (Figure 1 and Figure 2).

**DISCUSSION**

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 al, 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

![Figure 1](image1.png)  
*Figure 1. Anaplastic thyroid carcinoma: CD68 expression in tumor border and tumor tissue (Magnification a x 200; b x 400).*

![Figure 2](image2.png)  
*Figure 2. Anaplastic thyroid carcinoma: p53 expression positive cells in tumor (Magnification a x 400; b x 100).*
the tumor histogenesis, respectively progression of cancer.

REFERENCES