P53 and C-FOS overexpression in patients with thyroid cancer: an immunohistochemical study

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A sequence of genetic events characterized by deletion and expression of several oncogenes may lead progressively to tumorgenesis. The expression of certain oncogenes is believed to be related with thyroid carcinogenesis and tumor progression. We investigated immunohistochemically p53 tumor suppressor gene and c-fos oncogene expression in forty patients with thyroid cancer. Thyroid biopsies from twenty patients with benign thyroid diseases were also examined. The forty patients with thyroid cancer varied histologically; 24 with papillary carcinoma (60%), 12 with follicular carcinoma (30%), 3 with anaplastic carcinoma (7.5%) and one with medullary carcinoma (2.5%). The patients with benign thyroid diseases consisted of 10 with adenomatous goiter (50%), 7 with goiter (35%) and three with Hashimoto thyroiditis (15%). Individual p53 and c-fos expression was more prevalent in thyroid carcinomas compared to benign tumors (p=0.001 and p=0.04, respectively). A marked increase of p53 and c-fos coexpression was found (p=0.02) in patients with thyroid cancer and metastasis to the regional lymph nodes. Furthermore c-fos was overexpressed in only female thyroid cancer patients. In conclusion, p53 and c-fos are significantly overexpressed in thyroid cancer patients, indicating their role in the genetic mechanisms leading to thyroid tumorigenesis. This hypothesis is further supported by the observation that p53/c-fos coexpression was related with more advanced disease status.

Key words: Thyroid cancer, goiter, thyroiditis, adenoma, p53, c-fos.

Thyroid tumors represent an attractive model for the identification of genetic changes involved in tumorigenesis. They show a stepwise progression from hyperplasia to solitary nodule, differentiated and anaplastic carcinoma.

The development and progression of thyroid tumors involve several genetic mechanisms. The mutation of p53 tumor suppression gene located in the short arm of chromosome 17, is frequently reported to be mutated in malignant tumors [15, 23]. This gene encodes, a 53 kDa nuclear phosphoprotein, which plays a role as a check point control for recognising DNA damage, resulting in either a delay in progress through the cell cycle to permit repair process or to initiate apoptosis [16], eliminating this way the abnormal clones of cells that lead to cancer [1]. The half-life of mutated p53 gene is subsequently much longer than wild-type and high levels of mutated p53 protein accumulate in the nucleus. For this reason p53 immunohistochemistry represents a possibility to get inside into the process of neoplastic transformation.

The protooncogene c-fos also plays a central role in cell proliferation and its protein product is an essential component of transcription factor AP-1 [3, 20]. The activity and function of c-fos is also implicated in hormone-dependent transcriptional regulation by fos-steroid receptor fusion proteins [7, 27]. The aim of this study was to investigate the clinical significance of the p53 tumor suppressor gene and c-fos oncogene overexpression in patients with thyroid cancer.

Material and methods

Pathological specimens from 40 patients with thyroid cancer and 20 patients with benign thyroid diseases were

Table 1. Pathological findings in patients with thyroid cancer

Diameter of the tumor	Mean: 3.1 ± 0.32 Median: 2.5	
Tumor location	N=	%
Left lobe	10	25
Right lobe	12	30
Isthmus	3	7.5
Multifocal	15	37.5
Histological type	N=	%
Papillary	24	60
Follicular	12	30
Undifferentiated	3	7.5
Medullary	1	2.5
Stage	N=	%
Stage I	16	40
Stage II	14	35
Stage III	7	17.5
Stage IV	3	7.5

retrieved from the archives of the Department of Pathology, Hippokration Hospital, University of Athens. All patients were diagnosed and treated in the First Department of Propaedeutic Surgery, Hippokration Hospital, Athens Medical School from 1990 to 1995. These patients were divided into two groups with the following characteristics: Group A: forty patients with thyroid cancer and Group B: twenty patients with benign thyroid diseases.

Group A. All thyroid cancer patients were classified according to the recommendation of the World Health Organization (WHO) [12]. The group consisted of 10 males and 30 females and the mean age of the patients was 52 ± 2.5. The majority of the patients underwent total thyroidectomy (n=31, 77.5%) and nine patients were treated by near-total thyroidectomy (22.5%). The findings from the pathology reports are demonstrated in Table 1. In ten patients (25%) the regional lymph nodes were found positive. Adjuvant treatment with I^{131} was administrated to 23 patients (57.5%), 11 of which also received a replacement of T4 thyroid hormone. Two patients received adjuvant radiation therapy.

The 5-year survival rate of thyroid cancer patients was 92.5% while nine patients (22.5%) relapsed during follow-up.

Group B. The group of patients with benign thyroid tumors consisted of one male and 19 females with mean age 46.8 ± 3.37.Ten patients were diagnosed with adenomatous goiter (50%), 7 patients with goiter (35%) and 3 patients with Hashimoto thyroiditis (15%).

Immunohistochemical determination of p53 and c-fos. Immunohistochemical staining was performed on formalin fixed and paraffin embedded sections using the streptavidin-biotin-peroxidase method (Cadenza Tags kit, Shandon Inc., Pitsburg, Pensylvania U.S.A.) as described elsewhere [10] with monoclonal antibodies specific for c-fos (Ab1 – OP17, Oncogene Science) (dilution 1:100) and p53 (YLEM, Rome, Italy) (dilution 1:50). All of the assignments were performed on biopsy specimens obtained before the administration of any therapy.

Statistical analysis. Statistical analysis was performed using the Fisher's exact test. Differences in the mean values were examined for statistical significance by Student's t-test. We were unable to conduct a survival analysis because only 3 patients (7.5%) died during the five year follow up. However, in order to identify possible factors that contribute to the development of recurrence we performed a regression analysis. A p value less than 0.05 denoted a statistically significant difference.

Results

Single oncogene expression.

P53. The percentage of positive specimens expressing the p53 protein was 57.5% in patients with thyroid cancer (Fig. 1a) and 10% in patients with benign thyroid diseases. This data strongly support the notion that p53 expression occurred significantly more often in carcinomas (p=0.001) (Fig. 2). In detail, 12 out of 24 patients with papillary (50%) and 8 out of 12 patients with follicular carcinoma (66.7%) were found positive for p53 expression. All patients with undifferentiated carcinoma were found positive (100%). A marked increase in the expression of p53 protein was found in patients with lymph node metastasis since eight out of 10 patients with lymph node metastasis (80%) showed immunoreactivity for p53, while only 15 out of 30 patients with local disease (50%) expressed the p53 protein. However, this difference was not statistically significant (p=0.14). No significant correlations were found between the expression of p53 protein and several clinical and pathological factors, such as age, sex, tumor size, location and histological type.

C-fos. Immunopositivity of c-fos was detected in 12 patients (30%) with thyroid cancer (group A) (Fig. 1b) and in 1 patient (5%) with benign thyroid disease (group B), indicating significant expression of c-fos in malignant tumors (p=0.04) (Fig. 3). It is of note that no males were included among positive patients. This difference of c-fos expression between the two genders in group A was found statistically significant. (p=0.019). No further significant correlations were identified between the expression of c-fos protein and the clinical and pathological characteristics of our study group.

Oncogene coexpression. Six patients with thyroid cancer (15%) were found immunoreactive for both p53 and c-fos proteins. Coexpression of p53 and c-fos oncoproteins was strongly correlated to the presence of disease involved

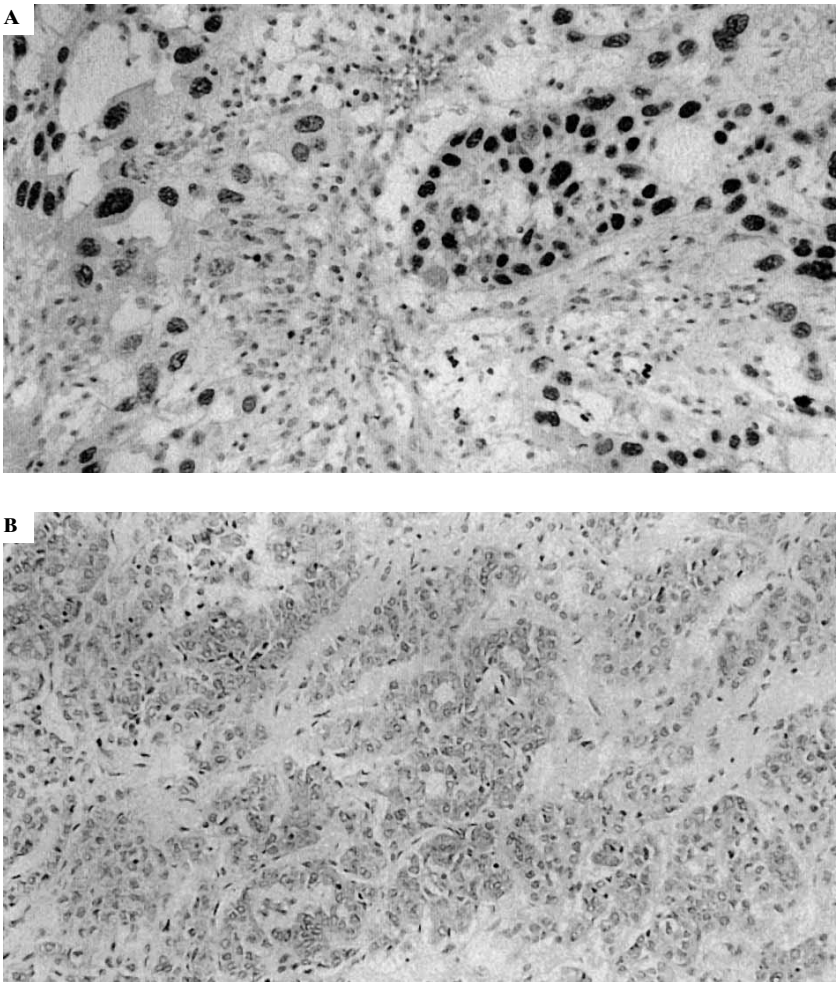

Figure 1. A – p53 expression in thyroid cancer tissue. Darkly staining nuclei indicate a positive reaction (x400), B – a section of thyroid cancer stained for c-fos expression (x400).

lymph nodes. More than half (66.7%) of the patients expressing both proteins, presented disease involved lymph nodes, while only 17.6% of the cancer patients who expressed one or none of the genes had positive lymph nodes (p=0.02) (Fig. 4).

Disease outcome. Several variables known to significantly influence the survival of patients with thyroid cancer, such as age, sex, stage, histological type, tumor size, and lymph node status were included in the regression analysis. None of the above variables, including the expression of p53 and c-fos proteins, were found to be significantly associated to the development of recurrence.

Discussion

Cellular oncogenes have provided the theoretical target for mutagenic events in carcinogenesis. Their transformation to deregulated oncogenic forms of proto-oncogenes, caused by mutation, potentially establishes a basis for the multistep development towards malignant phenotype. Studies on human cancer suggest that not only activation but also inactivation of another category of genes, the tumor suppressor genes, may lead to tumorigenesis [5, 30, 32]. The development and progression of thyroid tumors may be due to a similar genetic mechanism.

The expression of p53 protein is a late event in thyroid carcinomas. In our study all the patients with undifferentiated thyroid carcinoma were found to overexpress p53. On the contrary p53 overexpression was detected in low frequency in patients with benign thyroid disease (goiter, adenoma, Hashimoto thyroiditis). These findings are consistent with studies in the literature reporting that p53 immunoreactivity is more frequent in thyroid carcinomas than in adenomas and especially in poorly differentiated and anaplastic carcinomas [6, 8, 9, 31]. There was also found a very high proportion of differentiated thyroid tumors with overexpression of p53, (57.5%). This percentage is much higher than found in the previous studies and might be explained by the fact that the incidence of differentiated thyroid cancer has increased and gene rearrangements have been detected in thyroid cancer patients after the Chernobyl nuclear accident happened at 1986 in Belarus and in regions affected by the radioactive clouds [3, 4, 25, 26, 29]. The relationship between radiation exposure and differentiated thyroid carcinoma is well recognized. Many investigators have reported an increased incidence of p53 mutation in thyroid cancer patients from these areas [17, 19, 20, 24].

Fos family genes encode for four cellular proteins c-fos, FosB, Fra-1 and Fra-2. When activated by external stimulus c-fos interacts with one of three cellular members of the jun family proteins to form AP-1 (Activating Protein 1) complex which then binds to located promoters/enhancers of target genes [3, 20]. The variety of stimuli inducing fos synthesis and activity, as well as the detection of the specific binding areas of AP-1 in promoters of numerous genes with diverse function suggest that the biological role of fos is complex. Indeed the fos family members have been implicated in most fundamental processes occurring in mammnalian cells such as cell cycle control [11], apoptosis [18], cell differentiation [14], oncogenic transformation and tumor progression [21, 22]. Additionally the c-fos activity found

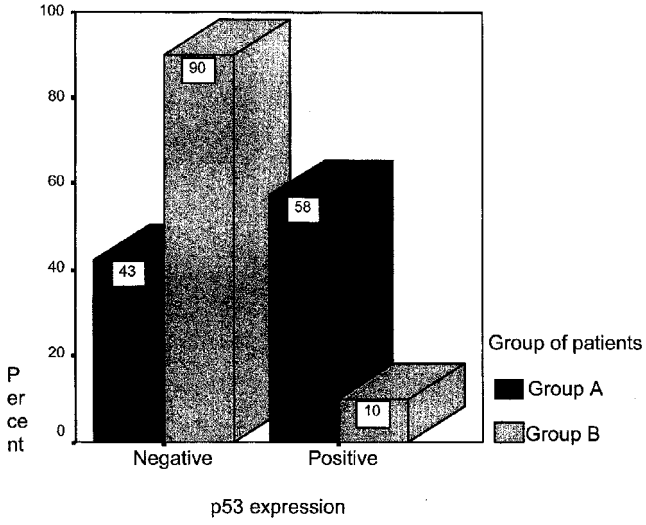

Figure 2. P53 expression in patients with thyroid cancer (group A) and benign thyroid diseases (group B). P53 overexpression is more frequent in patients with thyroid cancer.

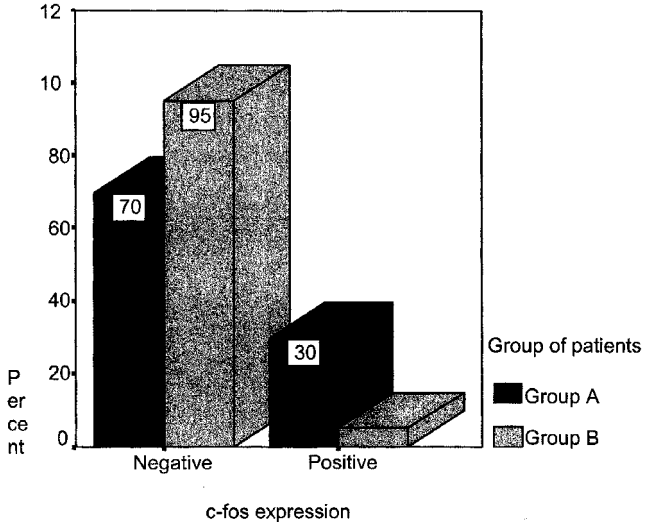

Figure 3. C-fos expression in patients with thyroid disorders. C-fos oncogenes is more prevalent in thyroid cancer (group A) rather in benign thyroid diseases (group B).

to be implicated in hormone-dependent trancriptional regulation by Fos-steroid receptor fusion proteins [7, 27]. In our study c-fos overexpression was detected in patients with thyroid carcinoma rather than in patients with benign thyroid tumors. Further more c-fos was expressed only in female patients with thyroid cancer. This preference of c-fos expression in malignant thyroid tumors is controversial with previous studies [13, 28, 32]. Keeping in mind that the expression and the function of c-fos is not clear and depends on the external or internal stimuli and since female sex carries a prolonged survival compared to that of males in thyroid malignancies, the expression of c-fos gene only in

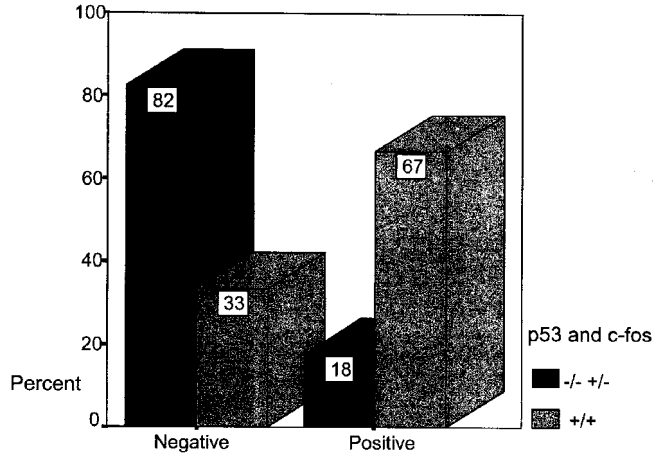

Figure 4. Coexpression of p53 and c-fos oncogene in relation with disease involved lymph nodes in patients with thyroid cancer. The majority of the patients with disease-involved lymph nodes found to overexpress both oncogenes.

females may imply a different, hormone-dependent mechanism of tumorigenesis in thyroid cancer.

We have shown that patients with thyroid cancer who had lymph nodes metastasis (n=66.7%) overexpressed the p53/c-fos genes. P53 is a tumor suppressor gene and its mutation seems to deregulate its protein function. Additionally c-fos seems to be involved in the tumor cells' invasiveness since c-fos-estrogen receptor chimera induces the expression of several matrix proteinases [2, 21], whose activation is associated with invasive behaviour of the tumors. Thus, it may be suggested that the tumors expressing both p53 and c-fos gain a more aggressive phenotype.

In conclusion we demonstrated that c-fos and p53 show significantly increased expression in patients with thyroid cancer. Furthermore all thyroid cancer patients who expressed c-fos gene were females indicating that this oncogene may account for the different clinical behavior of thyroid cancer in female patients. The combined study of p53/c-fos provides the clinician with important information, which may prove useful in selecting a high-risk patient group who will be candidate for a more aggressive treatment protocol. Environmental, sex-hormone dependent and growth factors seem to be implicated in thyroid tumorigenesis and more studies focusing on the multiple gene expression and their inducers are needed in order to unmask the molecular mechanisms of thyroid tumorigenesis.

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