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Original Contribution

EXPRESSION OF PROLIFERATIVE ANTIGENS IN HUMAN THYROID DISEASES

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ABSTRACT

The aim of the present study was to examine the local expression of proliferative markers PCNA, EGFR and Ki67 in patients with different thyroid diseases. Thirty-eight biopsy materials from the thyroid gland were examined. The biotin-streptavidin-peroxidase system with primary monoclonal antibodies to PCNA, EGFR and Ki67 was applied on cryostat sections of these biopsy specimens. The intensity of the reaction was analyzed semiquantitatively. In multinodular goiter, Hashimoto's thyroiditis and thyroid adenoma EGFR expression was detected in numerous follicules in well-defined zones. In patients with carcinoma EGFR showed diffuse and strong accumulation. In benign thyroid diseases PCNA was expressed in large areas, but in carcinoma the marker was found only on single cells. Ki67 showed diffuse reactivity with different intensity in Hashimoto's thyroiditis and in carcinoma. High level of Ki67 was detected in single cells lining the follicules in patients with adenoma and multinodular goiter. A correlation between PCNA and EGFR expressions in benign thyroid lesions was registered. Ki67 expression in the absence of PCNA suggested malignant proliferating potential. On the basis of this study PCNA, EGFR and Ki67 could offer a possible diagnostic and prognostic marker profile in benign thyroid diseases.

Key words: Thyroid gland, Proliferation, EGFR, PCNA, Ki67

INTRODUCTION

An increased incidence of thyroid diseases has been registered in recent years in our country. The reason for this could have been due to better diagnosis but could have also been due to the real growth in the number of affected patients. Multinodular goiter occurs at about 4% of the adult population (1) Thyroid carcinoma constitutes approximately 2% of all registered neoplasms (2). Thyrotoxicosis, which includes conditions such as toxic adenoma and toxic Hashimoto's thyroiditis, is found in 0,25% of the population (3). Different mechanisms leading up to glandular dysfunction, and therefore thyroid diseases, have been proposed. Pathophysiological changes are often associated with hyperplasia of thyroid epithelium. Detection of proliferative activity is important for monitoring the clinical course, treatment and prognosis of these conditions. Therefore the introduction of new diagnostic methods for early detection of proliferating cells is of utmost importance. The expression of PCNA, Ki67 and EGFR has been well studied in thyroid carcinoma only, but not in benign conditions.

The epidermal growth factor receptor (EGFR) has been extensively investigated over the years. It serves as a model receptor that mediates some cellular phenomena like proliferation and differentiation. The ligand, epidermal growth factor (EGF), induces dimerization of the receptor leading to the activation of its intrinsic protein, tyrosine kinase and its subsequent activity (4). Phosphorylation creates binding sites for interaction with enzymes such as phospholipase CY1 and phosphatidylinositol-3-kinase PI3K. These interactions are crucial and critical for the induction of signaling events regulated by EGFR (5). EGFR immunoreactivity can be detected in both normal and abnormal thyroid tissues and is mainly confined to the basolateral surface of polarized epithelial cells in normal thyroid tissues and toxic diffuse goiter. In neoplastic thyroid gland, however, it is over-expressed

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on the cells' apical surface, as well as on the pericellular and cytoplasmic regions (6). In the thyroid, EGFR promotes growth different from that caused by thyrotropic disease, follicular adenoma and carcinoma but it is absent in papillary carcinoma (7).

PCNA (proliferating cell nuclear antigen), on the other hand, is a cell-cycle-related protein with maximal concentration in S- and G2- phases of the cell cycle. The protein functions as a co-factor for DNA-polymerase-δ. It is an essential element of the DNA-replication and DNA-repair machinery (8). PCNA expression is regulated at transcriptional and posttranscriptional levels. For example, growth factors, such as platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF), may stabilize PCNA mRNA and consequently affect PCNA expression (9). PCNA interacts with various proteins involved in cell cycle control, that is, with p21, Gadd45, cyclins and replication factor C (10). In disease conditions, PCNA expression has been investigated in different tumors and inflammatory conditions and it has been found in Grave's thyroid glands (11). The immunocytochemical staining rarely shows PCNA expression in anaplastic thyroid carcinoma cell lines (12).

Unlike any of the other proteins, Ki67 is a nuclear protein present in proliferating cells only. It is present in two isoforms in human cells and is generated by alternative splicing of an mRNA. It is evolutionarily conserved in most species. Though it is well characterized on the molecular level, its full biological roles are still relatively unknown (13). However, it is a DNA-binding protein affecting chromatin structure and function; it is present in cells during the G1-, S-, G2-, and M- phases of the cell cycle but never found in the G0-phase. In this respect its expression could provide information only about whether a cell is in cycle or not, but may not define the phase and the length of the cell cycle.

Ki67 is a commonly applied marker for the assessment of proliferative activity and is used to calculate the so-called "proliferative index" (dividing/non-dividing cells). In this wise Ki67 has been shown to be a predictor of malignancy in pituitary adenomas, pancreatic endocrine tumors, parathyroid carcinomas and adrenocortical carcinomas (14). Unlike some other cell-cycle-associated molecules, such as PCNA, Ki67 is never expressed during DNA repair processes (15). With all these in view it could be reasonable to hypothesize the roles of these proteins in some cell-growth-related disturbances.

Therefore, the aim of the present study is to examine the proliferative potential in different thyroid diseases by assessment of the local expression of proliferative antigens PCNA, EGFR and Ki67.

MATERIALS AND METHODS

Biopsy samples from 38 thyroid glands were obtained after surgical operations. All patients with hyperthyroidism were rendered euthyroid prior to surgery.

Cryostat sections (4-5μm in thickness) from patients with multinodular goiter (n=16), thyroid adenoma (n=12), Hashimoto's thyroiditis (n=5) and thyroid carcinoma (n=5) were made. The sections were then fixed in 80% ice-cold acetone. Endogenous peroxidase was blocked with a 5% solution of H_2O_2. Nonspecific staining was inhibited with 5% normal serum. To determine the expressions of EGFR, Ki67 and PCNA monoclonal antibodies to human EGFR, Ki67 and PCNA (DAKO) were used as primary antibodies. The immunohistochemical study was then performed with the Biotin-Streptavidin-Peroxidase labeling system (DAKO) and visualized using aminoethylcarbazole as substrate reagent. The red-brownish granular accumulations in different cell zones were indicative of a positive reaction. The nuclei were counterstained with Mayer hematoxylin.

A semiquantitative detection scale was introduced relating to the types of reactivity observed: in single cells (+); in single follicules (2+); in large zones (3+) and diffuse reactivity (4+).

RESULTS

Samples obtained from multinodular goiter, thyroid adenomas and Hashimoto's thyroiditis revealed EGFR expression in numerous follicules located in well-defined zones as follows: EGFR was positive in 100 % of nodular goiter and Hashimoto's thyroiditis, but only in 83% of adenomas (**Figure 1a**). The positive reaction was found mostly in cells lining the follicules. Larger reddish-brown zones characterized the staining reactions in tissues with Hashimoto's thyroiditis; this was different from other benign thyroid lesions. A strong and diffuse accumulation of EGFR was detected in all carcinoma cases (**Figure 1b**).

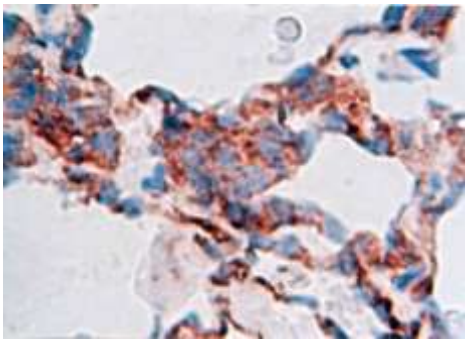

Figure 1a. Expression of EGFR in thyroid adenoma (x 400)

Figure 1b. Expression of EGFR in thyroid carcinoma (x 200)

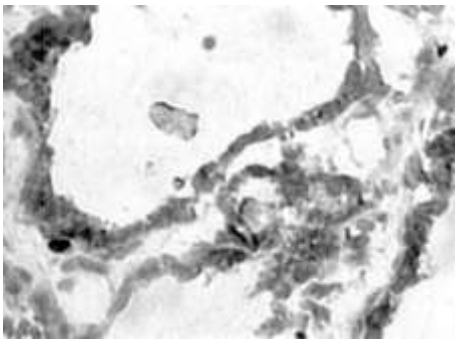

Figure 2a. Expression of PCNA in thyroid adenoma (x 400)

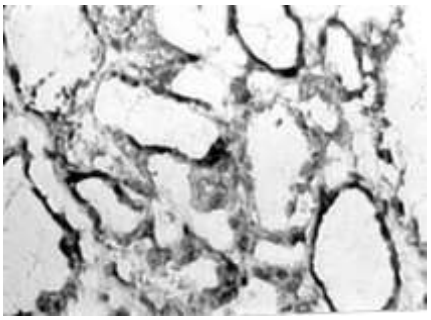

Figure 2b. Expression of PCNA in multinodular goiter (x 400)

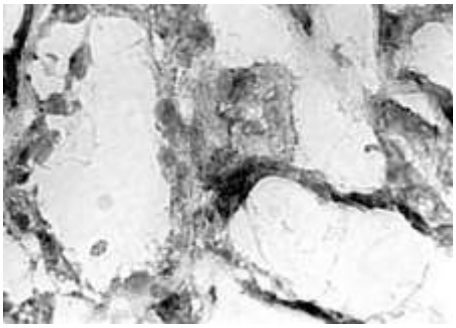

Figure 3a. Expression of Ki67 in multinodular goiter (x 400)

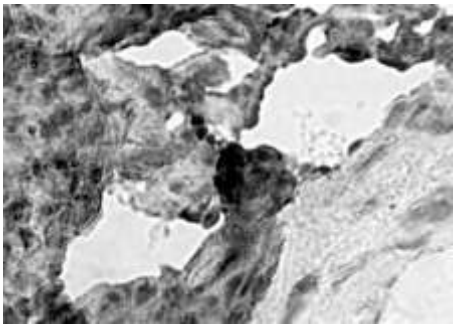

Figure 3b. Expression of Ki67 in Hashimoto's thyroiditis (x 400)

In benign thyroid diseases PCNA was expressed in large areas, but in carcinomas this marker was found only in single cells. PCNA was positive in all examined benign diseases. High levels were found in adenomas and in multinodular goiter (**Figure 2a, Figure 2b**). Single positive thyrocytes characterized the staining reaction in 30% of carcinomas.

The indirect immunohistochemical technique revealed a perinuclear expression of Ki67 in single cells in patients with nodular goiter and toxic adenoma (**Figure 3a**). 15% of multinodular goiters stained negative. All carcinomas and Hashimoto's thyroiditis cases were positive for Ki67 (**Figure 3b**). In these diseases Ki67 showed diffuse reactivity with varying intensities. In Hashimoto's thyroiditis Ki67 was expressed in zones with lymphocytic infiltration.

DISCUSSION

In this study we examined the local expression of proliferative markers EGFR, PCNA and Ki67 in different pathological conditions of the thyroid gland. Proliferative activity with different intensities and localizations is already known (16). In non-malignant thyroid lesions a correlation between PCNA and EGFR expression was found. These pathological processes could

probably be associated with normal functioning DNA-repair system and could be controlled by growth factors outside the thyroid gland. It is possible that PCNA expression is controlled by EGF binding to its receptor, EGFR. The latter action leads to cell proliferation. The studies of Lin suggested that EGFR could function as a transcription factor and could enhance gene expression by binding to DNA in the nucleus (17). Growth promoted by EGFR is different from thyrotropin-induced growth. This could explain why some thyroid hyperplastic lesions do not associate with glandular hyperfunction. The larger EGFR positive areas in patients with Hashimoto's thyroiditis might indicate a permanent stimulation of large glandular areas by growth factors. This could lead to a fast depletion of compensatory thyroid potential.

PCNA has a relatively long half-life, so that the antigen could still be detected long after protein production. In M-phase PCNA staining could also be seen in the cytoplasm due to a break of nuclear membrane or aberrant cytoplasmic synthesis (18). Diffuse non-polarized location of EGFR was detected in carcinomas and in some cases of adenomas. It suggests a loss of the normal epithelial cell polarity. In adenomas this could be interpreted as an early sign of de-differentiation and development of neoplasia. Probably the role of EGF and EGFR in growth control differs between papillary carcinoma and follicular adenomas/carcinomas of the thyroid (7).

The proliferative activity estimated for Ki67 was higher in malignant than in benign tumors. Ki67 expression in the absence of PCNA suggests malignant proliferating potential. The proliferative activity in Hashimoto's thyroiditis registered in areas with lymphocytic infiltration confirms the involvement of immune mechanisms in the development of these lesions and submits for discussion a consideration for a more radical surgical approach.

On the basis of the results obtained so far the expression profiles of PCNA, EGFR and Ki67 are being proposed as a possible diagnostic and prognostic tool in benign thyroid diseases. Assessment of proliferative activity might help correct diagnosis and support the right choice of treatment.

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