Frequency and Distribution of DNA Fragmentation in Hashimoto's Thyroiditis and Development of Papillary Thyroid Carcinoma

Zhong Jiang, MD, Lou Savas, MS, Nilima A. Patwardhan, MD, Joanne Wuu, MS, and Ashraf Khan, MD

Abstract

Downregulation of apoptosis and high expression of bcl-2 play an important role in the development of follicular lymphoma. However, little is known about apoptosis in thyroid disease, particularly with respect to the development of papillary carcinoma from Hashimoto's thyroiditis. To study the early stages of cell death in various types of thyroid disease, surgical specimens from 31 patients including Hashimoto's thyroiditis (HT, n = 7), papillary carcinoma (PC, n = 12), Hashimoto's thyroiditis with papillary carcinoma (HTPC, n = 5), and Graves' disease (GD, n = 7) were examined by an in situ nucleotidyl transferase assay (ISNTA), which detects DNA fragmentation. Control normal thyroid tissue (NT, n = 7) was obtained from surgically resected papillary thyroid carcinomas sampled away from the primary tumor. An immunohistochemical (IHC) method was used to detect bcl-2 expression. Positive ISNTA nuclei in thyroid follicular cells or tumor cells per section were counted in all parenchymal areas, excluding areas of lymphocyte aggregates. The intensity of bcl-2 staining was graded on a scale of 1+ to 3+. The number of ISNTA-positive thyroid follicular cells was a significantly higher in HT compared to GD. In addition, there was significantly lower number of ISNTA positive non-neoplastic thyroid follicular cells in HTPC compared to HT alone. Strong expression of bcl-2 was found in all cases of GD and NT, but much less bcl-2 staining was seen in HT. There was moderate expression of bcl-2 in HTPC and PC. These findings suggest that (1) DNA fragmentation of the thyroid follicular cells plays an important role in the thyroid injury in HT but not in GD, (2) expression of bcl-2 may overcome the apoptosis in GD but not in HT, and (3) downregulation of DNA fragmentation of the follicular cells in Hashimoto's thyroiditis associated with papillary carcinoma may suggest an important mechanism for tumor pathogenesis.

Key Words: Apoptosis; Hashimoto's thyroiditis; papillary carcinoma.

Introduction

Apoptosis is a process by which cell death occurs in a highly specific series of reactions resulting in fragmentation of the DNA [1-4]. Apoptosis may occur as a programmed cell death triggered by multiple factors such as inflammatory cytokines and chemical or physical agents. bcl-2 is a proto-oncogene and inhibits apoptosis in hematopoietic and nonhematopoietic cells [5,6]. Hashimoto's thyroiditis (HT) and Graves' disease (GD) are two autoimmune diseases of the thyroid. The thyroid-specific cytotoxic or stimulating antibodies, antibody-dependent, cell-mediated cytotoxicity, and direct lymphocyte cytotoxicity may play important role in the development of autoimmune thyroid diseases [7-11]. As for cellular immunity, recent
studies have indicated that cytotoxic lymphocytes induce apoptosis in target cells [12–14]. However, little is known about apoptosis in autoimmune thyroid disease.

There is an established relationship between Hashimoto's thyroiditis and the development of thyroid papillary carcinoma. The prevalence of Hashimoto's thyroiditis is found to be significantly higher in individuals with papillary carcinoma than in those with follicular adenomas or adenomatous goiter [15]. This may be caused by either an increase in cell proliferation or a decrease in cell death (apoptosis), or both in cases of Hashimoto's thyroiditis, which progress to papillary carcinoma.

Methodologically, it is difficult to explore apoptosis in situ because apoptotic cells are abruptly phagocytosed in vivo, and their histological changes are difficult to detect. In this study, we used the in situ nucleotidyl transferase assay (ISNTA) [16] to assess fragmentation of the DNA, and immunohistochemistry to detect bcl-2 expression in Hashimoto's thyroiditis with and without associated papillary thyroid carcinoma, Graves' disease, papillary thyroid carcinoma, and normal thyroid tissue. Hashimoto's thyroiditis with and without associated papillary thyroid carcinoma was studied to see if apoptosis of thyroid follicular cells played any role in the development of papillary thyroid carcinoma in patients with Hashimoto's thyroiditis.

**Materials and Methods**

**Cases**

A total of 31 surgical specimens from an equal number of patients including Hashimoto's thyroiditis (HT, n = 7), Graves' disease (GD, n = 7), papillary thyroid carcinoma (PC, n = 12), and Hashimoto's thyroiditis with papillary thyroid carcinoma (HTPC, n = 5) were randomly selected from the surgical pathology files of the University of Massachusetts Medical Center between February 1990 and September 1996. Control normal thyroid tissue (NT, n = 7) was obtained from surgically resected papillary thyroid carcinomas received during the same period. The normal tissue was sampled away from tumor.

**In Situ Nucleotidyl Transferase Assay**

The principal of the ISNTA is the fact that DNA fragmentation, an early event in the apoptotic process, yields free DNA ends with 3'-OH groups that can be enzymatically elongated by terminal deoxynucleotidyl transferase (TdT) and labeled nucleotides that can be subsequently visualized using the ApopTag kit (Oncor, Gaithersburg, MD, USA). Paraffin-embedded sections were cut at 5 μm. After deparaffinization the sections were incubated with hydrogen peroxide to quench endogenous peroxide. Sections were subjected to enzymatic homopolymeric tailing with TdT and digoxigenin-labeled nucleotides for 1 h at 37°C in a humidified atmosphere. The incorporated nucleotides were revealed by incubation with antidigoxigenin antibody conjugated to peroxidase for 30 min at room temperature. The peroxidase label was then visualized by the diaminobenzidine reaction. All sections were counterstained with hematoxylin. For negative control, TdT was replaced by water in the reaction buffer.

**Morphological Evaluation and Data Analysis**

The ISNTA-positive nuclei stained strongly and were easily identified. ISNTA-positive follicular cells were identified carefully by their location. The size of the positive nuclei and their location helped in distinguishing thyroid follicular cells