Autoantibodies to p53 in Sera of Patients with Autoimmune Thyroid Disease

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Abstract. Mutations in the tumor suppressor gene, p53, lead to intracellular accumulation of abnormal p53 protein and are associated with p53 autoantibodies. p53 also accumulates in autoimmune diseases and Hashimoto's thyroiditis, but it is unknown if p53 autoantibodies occur in the latter. We measured p53 autoantibodies in the sera of 93 patients with thyroid disease and 19 patients without thyroid disease. Anti-p53 antibodies were detected in the sera from 4.2% (2/48) of patients with autoimmune thyroid disease and 19 patients without thyroid disease. Anti-p53 antibodies were detected in the sera from 4.2% (2/48) of patients with autoimmune thyroid disease, including one patient with Hashimoto's thyroiditis (3.7%, 1/27) and one with Graves' disease (4.8%, 1/21). A third patient with pseudohypoparathyroidism, but without thyroid disease, was also positive (1/19; 5.2%). None of 19 patients with differentiated thyroid cancer had anti-p53 antibodies. We conclude that anti-p53 antibodies can be detected in the sera from approximately 4% of patients with autoimmune thyroid disease. This finding suggests that increased DNA damage and apoptosis may be associated with autoimmune thyroid disease.

Keywords: p53, thyroid, autoimmunity, tumor suppressor gene

Introduction

The p53 tumor suppressor gene encodes a 53 kDa nuclear phosphoprotein believed to function as part of the “cell cycle checkpoint,” which protects cells against the accumulation of genetic alterations. Abnormalities in the p53 gene are the most common genetic alteration in human cancer [1-6], and are particularly frequent in tumors of the colon, breast, lung, stomach, and brain, as well as leukemia and osteosarcoma [5]. More than 95% of these alterations are single-point somatic mutations in the conserved regions of one of the p53 alleles accompanied by complete loss of the other allele [4-6]. Germline mutations have also been identified and implicated in the Li-Fraumeni inherited cancer syndrome [5].

Benchimol et al showed by radioimmunoassay that p53 protein was overexpressed in transformed cells but undetectable in normal cells [5,7]. In contrast, mutant p53 protein can be readily identified in all cell types because mutations induce a conformational change, which is associated with a prolonged half-life and intracellular accumulation [1,3]. These mutant p53 proteins may also serve as tumor specific antigens and become targets for the host immune system [1,2,4]. The mutant p53 protein, accompanied by abnormal expression of differentiation antigens, may induce clonal expansion of specific cytotoxic T lymphocytes and/or circulating anti-p53 antibodies. Previous studies have identified anti-p53 antibodies in the sera of patients with malignant disease [1], and Lubin et al [3] confirmed by isotyping that circulating anti-p53 antibodies belong to the IgG1, IgG2, IgM, and IgA classes of immunoglobulins.

Antibodies against p53 protein have also been identified in the sera of patients with autoimmune disorders, including systemic lupus erythematosus.
(15/50; 32%), Sjogren's syndrome (2/3; 66%), and systemic sclerosis (1/3; 33%) [7]. Mutant p53 protein also accumulates in inflamed tissues, including gastric mucosa infected with Helicobacter pylori, colonic mucosa with active ulcerative colitis or Crohn's disease, and synovia during active rheumatoid arthritis [9]. Anti-p53 antibodies have also been reported in a single patient with autoimmune hepatitis [1]. Based on these observations, Okayasu et al [9] postulated that p53 over-expression may be indicative of DNA damage and could be linked to apoptosis or DNA repair.

Recent studies suggest that abnormal p53 protein also accumulates in thyroid disorders [9,10]. Okayasu et al [9] showed that p53 protein can be detected by immunohistochemistry in malignant thyroid tumors and lymphocytic thyroiditis. Chetty et al [10] demonstrated mutant p53 in thyroid glands of patients with Hashimoto's thyroiditis and primary thyroid lymphomas, and also showed that the intensity of staining correlated with the severity of thyroiditis.

Because, to our knowledge, no previous study has done so, this investigation was designed to determine if anti-p53 antibodies can be detected in sera from patients with benign and malignant thyroid diseases. The major finding of our study is that anti-p53 antibodies can be detected by ELISA in sera from patients with autoimmune thyroid disorders.

**Materials and Methods**

This study received prior approval from the Institutional Review Board and Human Use Committee, Department of Clinical Investigation, Walter Reed Army Medical Center.

**Collection of samples.** Following informed consent, sera were obtained from 112 patients (70 adults and 42 children) with benign, malignant, or no known thyroid disease. The median age was 35 yr (range 4-85). Of the 112 patients, 19 (17%) had no known thyroid disease; 22 (19.6%) had multinodular goiter with negative antithyroid peroxidase (anti-TPO) antibodies; 27 (24.1%) had Hashimoto's thyroiditis with positive anti-TPO antibodies; 21 (18.8%) had Graves' disease with positive TSH-receptor antibodies; 3 (2.7%) had subacute thyroiditis; 1 (0.8%) had congenital hypothyroidism; and 19 (17%) patients had treated thyroid cancer (15 papillary [PTC] and 4 follicular [FTC]). Hashimoto's thyroiditis was defined as the presence of anti-TPO antibodies in association with an elevated thyroid stimulating hormone (TSH) level and goiter. Graves' disease was defined as the presence of TSH-receptor antibodies associated with a suppressed TSH level and goiter.

**Enzyme-linked immunosorbent assay.** Anti-p53 antibodies were detected by an Enzyme Linked Immuno-Sorbent Assay (ELISA, Inc., #ELAP5301, Westbrook, ME), according to the manufacturer's instructions. In brief, sera were added to microtiter plates coated with either recombinant wild type human p53 protein (to detect specific anti-p53 antibodies) or control protein (to detect non-specific binding). A peroxidase-conjugated goat anti-human IgG secondary antibody was added to bind anti-p53 antibodies and the specific p53/anti-p53/conjugate complexes were then detected by addition of a peroxidase substrate (tetramethylbenzidine, TMB). Anti-p53 antibody titer was quantified by the final colorimetric reaction at 450 nm. Duplicate assays were performed on all equivocal or positive samples.

**Dot-blot analysis.** Dot-blot analysis was performed on all samples that had equivocal or positive ELISA, as well as 3 random samples that were negative by ELISA. Two different anti-p53 antibodies were used. Antibody 1 (Oncogene Research Products, Inc., #OP32, Cambridge, MA) is a monoclonal antibody that reacts with only wild type p53, and antibody 2 (Oncogene Research Products, Inc., #OP09) is a pantropic monoclonal antibody, which reacts with both the mutant and wild type p53. Three different antigenic lysates that contained one of the p53 proteins were used to confirm the specificity of the p53 antibodies. The mutant p53 protein alone was present in recombinant baculovirus lysate (Pharmingen, Inc., #162364, San Diego, CA). The wild type p53 protein was present in recombinant baculovirus lysate (Pharmingen, Inc., #162264). Both mutant and wild type p53 protein were present in Cos 7 cell lysate (Pierce Biochemicals, Inc., #29985, Rockford, IL). One μl of each lysate was applied to strips of nitrocellulose membrane and allowed to dry. Sample buffer without cell lysate was used as the antigen
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![Figure 1: Dot-blot analysis. Five identical nitrocellulose membranes were dot-blotted with cellular lysates containing both wild type (WT) and mutant (M) p53 from Cos 7; only M p53 from recombinant baculovirus; and only WT p53 from recombinant baculovirus. Antibody 1 (Ab 1) and antibody 2 (Ab 2) were used to verify the presence of the WT or the M p53 protein as expected in the cell lysates (lanes 1 and 2). The sample buffer containing no antigenic lysates demonstrated no detectable signal. Sera from patients P13, P23, and A45 were shown to contain antibodies capable of detecting both the WT and M p53 proteins (lanes 3, 4, and 5).]

Three samples were positive for anti-p53 antibodies and confirmed by duplicate ELISA. These were from two children with autoimmune thyroid disease and one adult without any evidence of thyroid disease.

The first patient (P13) is a 17-year-old female with a 7-year history of Hashimoto's thyroiditis, evidenced by goiter and positive anti-TPO antibodies (1:400). At the time the serum was collected, she was clinically euthyroid on thyroid hormone replacement, but her TSH was suppressed at 0.28 mU/L (normal range 0.51 - 4.9 mU/L). Her free T4 was 24.1 pmol/L (normal range 10.3 - 30.6 pmol/L), and her free T3 was 5.7 pmol/L (normal range 1.5 - 6.9 pmol/L). Her thyroid gland was mildly enlarged, with each lobe measuring 3.4 cm in length.

The second patient (P23) is a 12-year-old male with a 6-month history of Graves' disease, evidenced by suppressed TSH, elevated free T3, and positive thyroid-stimulating immunoglobulin (158%, normal < 130%). He had initially been treated with propylthiouracil and propranolol without improvement. At the time the serum was obtained, he was receiving treatment with supersaturated potassium iodide. His TSH was 0.01 mU/L (normal range 0.51 - 4.9 mU/L) and his free T4 was 27.3 pmol/L (normal range 10.3 - 30.6 pmol/L). His thyroid gland was mildly enlarged, with the right lobe measuring 3 cm and the left lobe measuring 2 cm in length.

The third patient (A45) is a 46-year-old postmenopausal female with pseudohypoparathyroidism evidenced by short stature (55 inches), elevated parathyroid hormone (PTH) levels (24-36 pmol/L [intact assay, normal range 3-17.6 pmol/L]), low serum calcium levels (1.62-2.12 mmol/L [normal range 2-2.5 mmol/L]), elevated serum phosphate levels (1.5-1.8 mmol/L [normal range 0.8-1.5 mmol/L]), and basal ganglion calcification on cranial computed tomography. She had maintained normal 1-25 dihydroxy-vitamin D levels (57.6-113 pmol/L [normal range 36-144 pmol/L]), normal 25 hydroxy-vitamin D levels (42-52 nmol/L [normal range 22-130 nmol/L]), and normal renal function. She had no evidence...
of thyroid disease (TSH 1.44 mU/L [normal range 0.5-4.9 mU/L]), free T4 (11.4 pmol/L [normal range 10.3-30.6 pmol/L]), negative anti-TPO and antithyroglobulin antibodies, and a normal thyroid gland to palpation.

Dot-blot analysis was performed on the sera from these three patients, as well as four patients for whom the initial ELISA was equivocal and two patients with negative ELISA (Fig. 1). All three patients with positive ELISA (P13, P23, and A45) were confirmed by dot-blot analysis. All of the negative and equivocal samples were negative by dot-blot (data not shown).

Of note, all of 19 patients with thyroid cancer (15 with PTC and 4 with FTC) were negative for anti-p53 antibodies. Absence of anti-p53 antibodies in these patients is consistent with the fact that all of the patients had well-differentiated thyroid cancer [11-13].

Discussion

Diverse physiological events can induce DNA damage, including inappropriate oncogene activation, cytokine stimulation or withdrawal, hypoxia, and heat shock [5,7,14]. In response to DNA damage, normal proliferating cells respond with either cell cycle arrest or programmed cell death (apoptosis) [14]. The tumor suppressor protein, p53, is implicated in both. p53 can cause growth arrest at the cell cycle checkpoints, of which the best understood is late G1 phase arrest [14]. p53 triggers G1 arrest through specific transcriptional activation of the cyclin-dependent kinase inhibitor, p21 [9]. p53 is also required for cells to undergo apoptosis.

Human autoimmune diseases share the common feature of imbalance between the production and destruction of tissue-specific cells [14]. Under normal conditions, apoptosis is tightly counterbalanced against cell stimulation and proliferation. However, in autoimmune disease, dysregulated apoptosis at critical stages of lymphocyte maturation or differentiation may occur. Although lymphocyte activation usually induces the production of immune mediators and entry into the proliferative phase of the cell cycle, activation can also lead to cell death through apoptosis [14].

Apoptosis and DNA damage have also been identified in autoimmune thyroid disease. Okayasu et al [9] demonstrated that p53 and p21WAF1 proteins were overexpressed in thyroid follicular cells in response to chronic lymphocytic thyroiditis. This observation suggests that lymphocytic thyroiditis may also be associated with enhanced DNA damage. Histologic evidence of increased apoptosis has been shown in chronic lymphocytic thyroiditis, and tissue-specific overexpression of Fas antigen has also been detected [9]. Okayasu and colleagues [9] demonstrated that the number of p53- and p21WAF1-positive cells correlated with the severity of thyroiditis, regardless of the presence or absence of neoplastic lesions. They speculated that although overexpression of p53 and p21WAF1 proteins did not directly link autoimmune thyroiditis to thyroid tumorigenesis, cell cycle disturbances might have an important role in the pathogenesis of both [9].

In this study, anti-p53 antibodies were detected in the sera of 4% of patients with autoimmune thyroid disease (Hashimoto's thyroiditis plus Graves' disease). Patient P13 is a 17-year-old female with a 7-year history of Hashimoto's thyroiditis; patient P23 is a 12-year-old male with recent onset Graves' disease. Patient A45 is a 46-year-old female with pseudohypo-parathyroidism, but without known history of thyroid disease. Based on a careful review of the medical records, none of these three patients had any evidence of malignancy. Soussi has shown that in normal patients, anti-p53 antibodies are very rare and can be detected in less than 0.5% of 1,000 patients analyzed [4]. Our data therefore support previous observations, which have identified the accumulation of mutant p53 in autoimmune thyroid disease [9,10].

Our study found no anti-p53 antibodies in the sera of 19 patients with thyroid cancer. Although the number of thyroid cancer patients is too small to exclude a low p53 mutation rate in differentiated thyroid cancer, the data are consistent with studies that have failed to identify p53 mutations in differentiated thyroid cancer tissue [11-13]. None of our patients had anaplastic thyroid cancer, in which p53 mutations have been more frequently identified [11-13].

In conclusion, our data show that anti-p53 antibodies can be detected in sera from approximately 4% of patients with autoimmune thyroid disease. This finding may be indicative of DNA damage and increased apoptosis associated with autoimmune thyroid disease [9].
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References


