Immunohistochemical detection of p53 and Bcl-2 proteins in Hashimoto's thyroiditis and primary thyroid lymphomas

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Abstract

***Aims*—To investigate whether immunohistochemical staining using p53 and/or bcl-2 distinguishes between florid Hashimoto's thyroiditis and low grade mucosa associated lymphoid tissue (MALT) lymphoma of the thyroid.**

***Methods*—Ten cases of Hashimoto's thyroiditis and eight of primary thyroid lymphoma were stained with monoclonal antibodies directed against p53 and bcl-2.**

***Results*—In Hashimoto's thyroiditis most small lymphoid cells in mantle zones, within the thyroid parenchyma and in lymphoepithelial lesions expressed bcl-2 protein. Very occasional centroblasts in reactive germinal centres were positive for p53, but all other lymphoid cells from cases of Hashimoto's disease were negative for p53. In diffuse, low grade lymphomas bcl-2 protein was uniformly expressed by most tumour cells. However, low grade lymphomas with a follicular pattern did not express bcl-2. The diffuse, low grade lymphomas were negative for p53, while occasional larger cells in the follicular subtype were positive. Both high grade lymphomas were bcl-2 negative but strongly p53 positive.**

***Conclusions*—This study indicates that there is an inverse correlation between p53 and bcl-2 immunostaining in thyroid lymphomas (low grade lymphomas: bcl-2 positive, p53 negative; high grade lymphomas: bcl–2 negative, p53 positive). Furthermore, immunohistochemical staining for bcl-2 and p53 proteins does not distinguish florid Hashimoto's thyroiditis from diffuse, low grade thyroid lymphoma.**

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The role of p53 and bcl-2 genes in the regulation of apoptosis is rapidly being elucidated. This has important ramifications for their role in oncogenesis, especially with regard to lymphomas because these are commonly deranged with resultant aberrent protein expression.

Bcl-2 is the prototype of a family of genes that inhibit apoptosis. Lymphoid and myeloid cells that were "rescued" from cell death did not proliferate, showing that bcl-2 is a true "survival factor", distinct in action from mitogens.[1 2] It is thought that bcl-2 protein acts close to the final, irreversible step in the pathway of apoptosis.[2]

Wild-type p53, on the other hand, is thought to promote apoptosis, whilst mutant p53 has a similar effect on apoptosis as bcl-2—that is, inhibition of programmed cell death.[3] Thus, p53 is a well established activator of one of the pathways leading to mammalian apoptosis and differentially affects bcl-2 protein concentrations.[2]

The p53 protein is abnormally expressed in a large proportion of non-Hodgkin's lymphomas and also has an inverse relation with bcl-2 expression in non-Hodgkin's lymphomas.[4]

The purpose of the current investigation was to explore this relation in Hashimoto's thyroiditis and primary lymphomas (mucosa associated lymphoid tissue (MALT) lymphomas) with the view of ascertaining the following: (1) the immunohistochemical staining pattern of p53 and bcl-2 proteins in Hashimoto's thyroiditis and thyroid lymphomas; (2) whether p53 and bcl-2 have the same relation in thyroid disease as they do in reactive lymph nodes and nodal B cell lymphomas; and (3) whether p53 and/or bcl-2 staining is of help in resolving the vexing morphological problem of distinguishing florid Hashimoto's thyroiditis from diffuse, low grade MALT lymphomas of the thyroid.

Methods

Ten cases of Hashimoto's thyroiditis and eight of primary thyroid lymphoma (six low grade and two high grade) were examined. All eight patients with lymphoma had positive autoimmune profiles typical of Hashimoto's thyroiditis during the course of their disease. Two of the patients with lymphoma also had coexistent Hashimoto's thyroiditis. For cases to be accepted as Hashimoto's thyroiditis, the following criteria had to be met: (1) dense lymphoid aggregates (often replete with germinal centres) together with plasma cells; (2) lymphoepithelial lesions; and (3) oxyphil or Hurthle cell metaplasia of thyroid follicle cells.

The lymphomas were diagnosed on the basis of (1) pervasive, destructive lymphoid infiltrates within the thyroid parenchyma; (2) lymphoepithelial lesions; and (3) immunohistochemical demonstration of unequivocal light chain restriction.

Sections of formalin fixed, paraffin wax embedded tissue were stained with DO7 (mono-

bcl-2 and p53 staining in Hashimoto's thyroiditis and thyroid lymphomas

Disease	*bcl-2*	*p53*
Hashimoto's thyroiditis		
Germinal centres	Negative	Occasional positive cells
Mantle zone lymphocytes	Positive	Negative
Interfollicular lymphocytes	Positive	Negative
Lymphocytes in LEL	Positive	Negative
Thyroid lymphomas		
Low grade, diffuse	4/4 Positive	0/4 Positive
Low grade, follicular	0/2 Positive	Occasional positive cells
High grade	0/2 Positive	2/2 Positive

LEL = lymphoepithelial lesions.

clonal antibody for p53; Dako, Glostrup, Denmark) and bcl-2 124 (Dako), using the Streptavidin horseradish peroxidase technique.[5] Blocking for endogenous peroxidase was carried out before microwave treatment of the sections in 0·01 M sodium citrate for 20 minutes.

Sections were also stained with leucocyte common antigen (LCA), B and T cell antigens, cytokeratins, and κ and λ light chains.

Results

The findings are summarised in the table. Expression of bcl-2 was deemed to be present when cytoplasmic staining was present. For Hashimoto's thyroiditis, the most intense staining was noted in more than 90% of mantle zone lymphocytes. Cells within germinal centres were resolutely negative, save for the very occasional (less than 5%) small, centrocytic lymphocyte that was present within germinal centres. The lymphocytes in lymphoepithelial lesions also exhibited bcl-2 expression.

Two of the low grade lymphomas had a follicular pattern composed of both centrocytes and centroblasts. These follicles were negative for bcl-2 expression. The lymphocytes (more than 90%) around and between the follicular structures were intensely bcl-2 positive. In the diffuse, low grade lymphomas at least 90% of the tumour cells were positive. Occasional larger cells interspersed amongst the small tumour cells were negative. No expression was detected in either of the high grade lymphomas.

For p53, a positive reaction was recorded when nuclear staining was present. In cases of Hashimoto's thyroiditis p53 staining was noted in less than 5% of follicle centre cells within the germinal centres and these were invariably centroblasts. Mantle zone lymphocytes were negative. Lymphocytes in lymphoepithelial lesions were also immunonegative. Thyroid epithelium showed strong cross-reactivity with p53.

Small lymphocytes (centrocyte-like) in the diffuse, low grade lymphomas were uniformly negative. Occasional centroblastic cells showed nuclear labelling. The two cases of low grade lymphoma with a follicular pattern contained occasional centroblastic cells with positive staining. Small cells were again negative. In the high grade lymphomas most tumour cells were positive. One of the cases showed almost 75% tumour cell positivity, whilst the other case had 50–55% tumour cell labelling.

Discussion

With both bcl-2 and mutant p53 inhibiting apoptosis, it might be expected that their respective proteins would be expressed similarly in tumours. However, this study has demonstrated an inverse correlation between p53 and bcl-2 expression—that is, p53 expression was present in most tumour cells in high grade lymphomas, while bcl-2 was present alone in most tumour cells in low grade lymphomas. This is the general staining pattern despite isolated larger cells in low grade lymphomas showing p53 expression. Thus, a similar correlation between p53 and bcl-2 expression was noted in Hashimoto's thyroiditis and thyroid lymphomas as exists in lymph node non-Hodgkin's lymphomas.[4] It has been proposed that inactivation of a tumour suppressor gene such as p53 could result in an oncogenic effect similar to that achieved by overexpression of bcl-2 protein.[4] This inverse correlation is difficult to explain. It could be ascribed to several phenomena: (1) genotypic drift and heterogeneity within high and low grade lymphomas; (2) immunohistochemistry providing a "biological window" at a particular point in the cell cycle of the various tumour cells; and (3) bcl-2 protein expression mainly occurs when cells are not entering the cell cycle rapidly (low grade lymphomas) and p53 is overexpressed when there is rapid cell turnover (high grade lymphomas).

The pattern of bcl-2 staining found in this study is in keeping with other studies in MALT lymphomas,[6] and in other low grade lymphomas.[7] As regards the two low grade thyroid lymphomas with a follicular pattern, the nodules of centrocytes and centroblasts did not show immunohistochemical evidence of bcl-2 protein expression. This pattern of staining is a variance with that encountered in nodal follicular lymphomas, where the majority of cases show a strong positive staining reaction. However, examples of follicular lymphoma, especially those with a high proportion of centroblasts, have previously been shown to be negative.[7] An alternative explanation for the lack of staining is the phenomenon of "follicular colonisation", where the normal follicles are attenuated and occupied by neoplastic centrocyte-like cells. Six MALT lymphomas with colonised follicles were negative for bcl-2 protein expression.[8] This has been ascribed to a high proliferation rate within these colonised follicles. Although both high grade lymphomas in the current study were negative for bcl-2, considerable overlap in staining patterns between high and low grade MALT lymphomas has been demonstrated.[6] Of the high grade lymphomas, 40% show bcl-2 expression in some large cells, with nearly one third of the low grade lymphomas containing a small proportion of bcl-2 negative cells.[6] These small negative cells could be activated T or B lymphocytes, which are generally negative for bcl-2.

Within the histological spectrum of Hashimoto's disease, bcl-2 expression paralleled that of reactive lymph nodes. In the florid cases of Hashimoto's thyroiditis the small, diffusely pervasive lymphoid cells, including those in

lymphoepithelial lesions, were bcl-2 positive. An identical staining reaction was seen in the diffuse lymphoid infiltrate of the low grade lymphomas. Therefore, bcl-2 staining is not a discriminant between florid Hashimoto's disease and early/established diffuse low grade lymphomas of the thyroid.

Cases of Hashimoto's thyroiditis showed occasional p53 positive staining in germinal centres. This could represent activated lymphoid cells, which are known to express p53 depending on their phase in the cell cycle. The lack of staining for p53 protein in low grade lymphomas, and the strong immunopositivity in high grade lymphomas, is consistent with the findings in similar nodal lymphomas.[4 9] The two patients with high grade lymphomas died two and five years after diagnosis and interestingly, it was the patient with the greater percentage of cells exhibiting p53 positivity who died after two years. Patients with high grade nodal B cell lymphomas which are p53 positive also show a decrease in life expectancy during the first few months after diagnosis.[10] Expression of p53 in tumours other than lymphomas has also been correlated with a poor prognosis.[11 12] A possible role of p53 immunostaining in the context of thyroid lymphomas could be in identifying those tumours that will behave more aggressively. Also p53 does not appear to be of value in separating the dense lymphoid infiltrates seen in Hashimoto's thyroiditis from low grade MALT lymphomas of the thyroid.

The results of this immunohistochemical study show that the staining patterns of p53 and bcl-2 in Hashimoto's thyroiditis and primary thyroid lymphomas parallel their lymph node counterparts—that is, reactive lymphadenopathy and nodal low and high grade non-Hodgkin's lymphomas. Furthermore, neither can be used to separate florid Hashimoto's thyroiditis from low grade thyroid lymphoma. A possible role for the immunohistochemical detection of p53 and bcl-2 proteins may be in indicating the evolution of low grade (bcl-2 positive; p53 negative) to high grade (bcl-2 negative; p53 positive) lymphoma. Indeed, within the subset of high grade lymphomas, it appears a greater degree of p53 positivity may help identify those tumours that will behave more aggressively.

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