Do *Bcl-2* and Survivin Help Distinguish Benign From Malignant B-Cell Lymphoid Aggregates in Bone Marrow Biopsies?

Amish M. Gandhi and Jonathan M. Ben-Ezra*

Department of Pathology, School of Medicine of Virginia Commonwealth University, Richmond, Virginia

Bcl-2 and survivin are cellular proteins that are known to be inhibitors of apoptosis and are commonly found in malignant tissues, including lymphomas. In previous studies, it has been shown that staining for *bcl-2* can help distinguish between benign and malignant lymphoid aggregates in bone marrow biopsies. To determine whether staining for survivin expression in lymphoid aggregates can aid investigators in making this clinically important distinction, we stained bone marrow biopsies from 10 patients with benign lymphoid aggregates, and 15 malignant ones derived from B cells (six mantle cell, four follicular cells, two diffuse large cell, two small lymphocytic cell, and one marginal zone lymphoma) with antibodies to CD3, CD20, *bcl-2*, and survivin by an

indirect immunoperoxidase technique. Whereas staining for *bcl-2* was significantly stronger in the malignant lymphoid aggregates ($P=0.001$), both the control and malignant cases were almost uniformly negative for survivin expression. Only three cases (two mantle cell and one small lymphocytic lymphoma) showed very faint expression of survivin. Although *bcl-2* and survivin both act to inhibit apoptosis, their expressions do not parallel each other. Survivin is not significantly expressed in either benign or malignant bone marrow aggregates, and therefore measuring its expression does not help distinguish benign from malignant B-cell bone marrow lymphoid aggregates. J. Clin. Lab. Anal. 18:285–288, 2004. © 2004 Wiley-Liss, Inc.

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INTRODUCTION

Bcl-2 and survivin are members of a class of anti-apoptotic proteins that are commonly expressed in primary tumors. The more-studied member, *bcl-2*, was originally found in follicular lymphomas as the up-regulated protein product of the t(14;18) chromosome translocation. As a cellular protective mechanism, it functions to increase cell survival, rather than to promote further cell growth. It is believed that the physiological role of *bcl-2* is to prevent apoptosis in cells damaged by ultraviolet irradiation, cytokine withdrawal, and cytotoxic drugs (1). Studies have shown *bcl-2* to be a prognostic indicator of poor survival in non-Hodgkin's lymphoma (2) and acute myeloid leukemia. Conversely, in non-hematopoietic malignancies, *bcl-2* acts as a protective factor in some carcinomas, such as breast (3) and colon (4).

Survivin, the more recently identified anti-apoptotic protein, is not found in normal adult tissues (5). It is known to be present only in fetal tissues, and most cancerous tissue. It has been suggested that its expression may be a negative prognostic factor for survival in some cancers (4). In a previous study (6), it was shown that reactivity for the anti-apoptosis protein *bcl-2* can

help distinguish benign from malignant lymphoid aggregates in the bone marrow. In this study, we examined lymphocytic aggregates (both benign and malignant ones derived from B cells in bone marrow biopsies) to determine whether there is a relation between *bcl-2* and survivin expression, and whether determination of survivin expression can help distinguish benign from malignant B-cell lymphoid aggregates.

MATERIALS AND METHODS

Control cases without evidence of lymphoma but with bone marrow lymphoid aggregates, and cases of B-cell lymphoma with bone marrow involvement were identified. Patients with a previous diagnosis of lymphoma whose bone marrow showed a benign lymphoid aggregate were eliminated from the potential control

*Correspondence to: Dr. Jonathan Ben-Ezra, Department of Pathology, Virginia Commonwealth University, P.O. Box 980250, Richmond, VA 23298-0250. E-mail: jbenezra@vcu.edu

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group. In the control group (Table 1) were two patients with treated acute myeloid leukemia (AML), two patients with human immunodeficiency virus (HIV) infection, and one each with breast cancer, thrombocytopenia, anemia, pyelonephritis, viral infection, and plasma cell dyscrasia. The cases of malignant lymphoid aggregates included six mantle cell lymphomas, four follicular lymphomas, two diffuse large-cell B-cell lymphomas, two small lymphocytic lymphomas, and one marginal zone lymphoma (Table 1).

The bone marrow biopsies were fixed in AZF fixative, decalcified in hydrochloric acid for 30 min, and embedded in paraffin. Biopsy samples were stained for CD3, CD20, *bcl-2* (DAKO, Carpinteria, CA), and survivin (Alpha Diagnostic International, San Antonio, TX) by a standard indirect immunoperoxidase technique with antigen retrieval. The stained slides were examined simultaneously by the authors with the use of a two-headed microscope, and given a score of 0, 1+, 2+, or 3+ based on the number of lymphocytes that stained in an aggregate, and the intensity of the staining. A score of 0 meant no staining was observed, 1+ indicated mild staining, 2+ indicated moderate staining, and 3+ meant that nearly all of the lymphocytes in the aggregate stained strongly (6). Differences in staining between the two groups were determined by a t-test.

The IRB Exempt Research Committee at our institution considered this study exempt from the Federal Policy for Protection of Human Subjects.

RESULTS

The lymphoid aggregates in the control group were generally smaller and more localized than the neoplastic aggregates (Fig. 1). Confirming previous results, the lymphoid aggregates from the benign control cases

tended to be CD3+, CD20-, and *bcl-2* weak+, whereas the malignant B-cell lymphoid aggregates tended to be CD3-, CD20+, and *bcl-2* strong+ (Figs. 2–4). Cases and controls showed a significant difference in scores for *bcl-2* staining ($P=0.001$, two-tailed t-test). As shown in Fig. 5, the majority (eight of 10) of the benign aggregates showed zero to mild staining, while 13 of 15 diagnosed B-cell lymphoma aggregates showed moderate to intense staining with the *bcl-2* antibody. On the other hand, survivin staining was virtually negative in nearly all samples, with trace staining in three cases (Fig. 6, Table 2). Trace staining of the

Fig. 1. Figures 1–4 and 6 are from the same control lymphoid aggregate (A) and mantle cell lymphoma (B). The reactive aggregate is less compact, and pushes megakaryocytes to the side. (H&E, original magnification: $\times 200$.)

Fig. 2. CD3 staining of benign (A) and malignant (B) bone marrow lymphoid aggregates. Several CD3+ cells are present in the benign aggregate, but the malignant one is largely negative. (Original magnification: $\times 200$.)

Fig. 3. CD20 staining of benign (A) and malignant (B) bone marrow lymphoid aggregates. Only scattered positive cells are present in the benign aggregate, whereas almost all of the cells in the mantle cell lymphoma are positive. (Original magnification: $\times 200$.)

TABLE 1. Control and malignant B-cell lymphoma cases

Diagnosis	Number of cases
Controls	
Acute myelogenous leukemia	2
Human immunodeficiency virus infection	2
Breast carcinoma	1
Thrombocytopenia	1
Anemia	1
Pyelonephritis	1
Viral infection	1
Plasma cell dyscrasia	1
B-cell lymphoma cases	
Mantle cell lymphoma	6
Follicular lymphoma	4
Diffuse large cell lymphoma	2
Small lymphocytic lymphoma	2
Marginal zone lymphoma	1

Fig. 4. *Bcl-2* staining of benign (A) and malignant (B) bone marrow lymphoid aggregates. Only scattered cells are positive for *bcl-2* in the reactive aggregate, whereas most of the neoplastic cells are positive for *bcl-2*. (Original magnification: $\times 200$.)

Fig. 5. Distribution of *bcl-2* staining for reactive control and malignant cases. The lymphoma cases have significantly ($P=0.001$, two tailed t-test) more staining for *bcl-2* compared to the controls.

Fig. 6. Survivin staining of benign (A) and malignant (B) bone marrow lymphoid aggregates. Both the benign and malignant aggregates are negative for survivin. (Original magnification: $\times 200$.)

TABLE 2. Staining for survivin

Survivin staining score	No. controls	No. cases
0	10	12
1+	0	3

lymphocytes in a dot-like pattern was observed in three cases (two mantle cell lymphomas and one small lymphocytic lymphoma).

Survivin demonstrated distinct staining of plasma cells in all samples, as well as occasional staining of

megakaryocytes. However, it was noticeably absent in the lymphoid aggregates.

DISCUSSION

Bcl-2 and survivin are intimately related in their anti-apoptotic function. A prior study (7) demonstrated survivin+/*bcl-2*+ cells in the germinal centers of reactive lymph nodes of patients with chronic lymphocytic leukemia (CLL). The relationship between the two proteins may be masked somewhat by the varying mechanisms of action of these two proteins. *Bcl-2* is known to be localized to mitochondria, and acts there to inhibit cytochrome c release. It also acts further downstream in other places in the cell to inhibit formation of the Aaf-1/procaspase-9 complex and the release of calcium from the endoplasmic reticulum (8). However, survivin has been described as acting more specifically to inhibit terminal effectors caspase-3 and caspase-7 (9). By this means, survivin functions to preserve the integrity of the mitotic spindle during normal cell cycling and hence prevent normal apoptosis (10).

Since apoptosis is an intrinsic way in which the body rids itself of damaged cells or cells that may be malignant, inhibition of this normal function can potentially have dramatic clinical effects. Survivin is unique among inhibitors of apoptosis proteins (IAPs) in that it also participates in cell cycle progression, and is thought to be the only IAP induced by CD40L (7). The prospects of interfering with survivin's activities appear promising clinically, considering that survivin expression is limited to cancerous cells (11,12).

Prior studies have been contradictory as to whether survivin expression is an unfavorable prognostic factor in diffuse large B-cell lymphomas (13,14). Increased survivin expression has been found in malignant hematopoietic neoplasms in general (15), and in diffuse large B-cell lymphomas (5,16), thyroid lymphomas (17), Hodgkin's lymphoma (18), and T-cell leukemias/lymphomas in particular (19). However, it is infrequently expressed in low-grade lymphomas (5,7). Most of the malignant bone marrow lymphoid aggregates in the current study were derived from cases of low-grade lymphoma. This most likely explains the lack of survivin expression in this cohort, and why determining survivin expression is not useful for distinguishing benign from malignant lymphoid aggregates.

Although malignant B-cell lymphoid aggregates in the bone marrow express the anti-apoptotic protein *bcl-2*, they do not appear to show significant expression of survivin. Therefore, determining survivin expression by immunohistochemistry does not help distinguish benign from malignant lymphoid aggregates in bone marrow biopsies. Perhaps a more sensitive system, such as

RT-PCR, could detect the mRNA expression (20) and demonstrate differences in survivin expression between benign and malignant bone marrow lymphoid aggregates.

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