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Immunohistochemical Expression of the Transcription Factor DP-1 and Its Heterodimeric Partner E2F-1 in Non-Hodgkin Lymphoma

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DP-1 is a G1 cell cycle-related protein that forms heterodimers with E2F, a family of transcriptional factors regulating the expression of genes important for G1 to S progression. Although the exact role of DP-1 is not well understood, it has been shown to stabilize DNA binding of E2F proteins. By immunohistochemistry, the authors examined the expression of DP-1 in lymphoid tissues, including 8 cases of reactive follicular hyperplasia and 69 cases of B-cell non-Hodgkin lymphoma. The expression of the cell cycle-related proteins E2F-1 and Ki-67 was also assessed. Scoring was based on the proportion of labeled nuclei (1–10%, 11–25%, 26–50%, and > 50%). In reactive follicular hyperplasia, staining for DP-1, E2F-1, and Ki-67 was largely confined to the germinal centers. All 25 cases of follicular lymphoma, regardless of grade, had a high proportion (> 50%) of DP-1–positive cells but a lower proportion of cells marking for E2F-1 and Ki-67 ($P < 0.001$). The diffuse large B-cell lymphomas (n = 24) had high DP-1 and Ki-67 scores but low E2F-1 scores ($P < 0.001$). Small lymphocytic (n = 10), marginal zone (n = 3), and mantle cell lymphomas (n = 5) contained relatively low proportions of cells labeled for all three markers. Precursor B-cell lymphoblastic lymphoma (n = 2) displayed high proportions of cells positive for DP-1, Ki-67, and E2F-1 (> 50% in both cases). Except in follicular center cell lesions, DP-1 expression generally correlated with that of Ki-67. However, the expression of DP-1 was discordant with that of E2F-1 in benign and malignant follicular center cells, suggesting that DP-1 may have functions other than facilitating E2F-1–dependent gene regulation and cell cycle progression in these neoplasms.
Key Words: B-cell non-Hodgkin lymphoma—DP-1—E2F-1—Ki-67.

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Cell cycle progression from G1 to S phase is regulated by complex protein–protein interactions. One of these involves the retinoblastoma protein, which acts as a suppressor of cell proliferation by binding and inhibiting E2F proteins. The E2F family of transcriptional factors modulates the expression of a broad array of genes important for cell cycle progression from G1 to S phase. Of the six members identified thus far, E2F-1 is the most extensively studied (1–7). When E2F-1 is released from the retinoblastoma protein, it forms a heterodimer with a member of the DP family (8). To date, two members of the DP family have been identified and cloned in mammalian cells: DP-1 and DP-2 (9,10). DP-1 is the more prevalent form (11). The exact biologic role of the DP family is unknown, although it has been shown that DP-1 stabilizes DNA binding of E2F-1 and thereby facilitates E2F-1–dependent cell cycle progression (12). The E2F-1/DP-1 heterodimer has been shown to regulate genes involved in DNA synthesis (i.e., dihydrofolate reductase, thymidine kinase, and DNA polymerase α) and those involved in cell growth (i.e., *N-myc*, *c-myc*, *IGF-1*, and cyclin A) (13–17).

Given the importance of the E2F-1/DP-1 heterodimer in cell cycle control, it is conceivable that abnormalities in either protein might contribute to the deregulation of cellular processes involved in tumor growth. Many studies have been performed to examine the oncogenic potential of E2F-1. For example, ectopic expression of E2F-1 in cell lines can override retinoblastoma protein-imposed cell arrest and propel cells from quiescence into S phase (18,19). In an interleukin-3–dependent myeloid cell line, E2F-1 can replace or overcome the requirement for growth factors and promote cell cycle progression (20). In previous studies, we demonstrated that E2F-1 expression is increased in some types of non-Hodgkin lymphoma (NHL), such as mantle cell lymphomas (21), and in approximately 30% of multiple myelomas (22). It is known that DP-1 is broadly but not uniformly expressed during murine embryogenesis (23). However, the deregulation and oncogenic properties of DP-1 have not been examined.

In this study, we demonstrate that DP-1 can be detected using immunohistochemical techniques applied to routinely fixed and processed, paraffin-embedded tissue sections. We used this method to survey the expression patterns of DP-1 and its heterodimeric partner E2F-1 in follicular hyperplasia and in B-cell NHL. To evaluate whether DP-1 is linked to E2F-1 expression and to cell proliferation, we also examined the expression of Ki-67.

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MATERIALS AND METHODS

Tissues

Sixty-nine morphologically and immunophenotypically defined cases of NHL were collected from the hematopathology and consultation files of the Department of Pathology at the Los Angeles County and University of Southern California Medical Center, Los Angeles, CA. Each case was diagnosed according to the World Health Organization classification (24). The distribution of chosen cases paralleled that of the major NHL subtypes seen in the United States (25), with follicular lymphoma and diffuse large B-cell lymphoma making up most of the cases studied. We also assessed eight cases of reactive follicular hyperplasia in four tonsils and four lymph nodes. All cases were fixed in 10% buffered formalin, routinely processed, and embedded in paraffin.

Immunohistochemistry

DP-1 was detected with the monoclonal antibody 1DP06 (Ab6, Neomarkers, Fremont, CA) at a dilution of 1:25. The monoclonal antibody reactive with E2F-1 protein (clone KH 95; Santa Cruz Biotechnology, Santa Cruz, CA), was used at 1:30 dilution. For detection of the Ki-67 antigen, a nuclear-associated proliferation antigen expressed in G1 through S phase, the monoclonal antibody MIB-1 (Immunotech, Westbrook, ME), was used at a dilution of 1:50. Control sections were immunostained under identical conditions, substituting buffer solution for the primary antibody.

Tissue sections were mounted onto charged slides (Surgipath, Richmond, IL), baked at 56°C for 60 minutes, deparaffinized with xylene, and rehydrated with graded ethanols to distilled water. Sections were placed in 0.01 mol/L citrate buffer at pH 6.0 and heated to boiling twice in a microwave oven for 5 minutes per cycle.

Reactivity was demonstrated by an avidin-biotin immunoperoxidase detection system employing 3′,3′diaminobenzidine-tetrahydrochloride dihydrate (Vector Laboratories, Burlingame, CA) as the chromogen.

Evaluation of Positive Cells

The tissue sections were initially scanned at low power to identify evenly labeled areas. In cases of NHL, residual reactive lymphoid follicles were avoided during the evaluation. For assessment of DP-1, only cells exhibiting strong homogeneous nuclear staining were considered positive; weak nuclear staining was not scored. Although cytoplasmic DP-1 staining was noted in some cases, no systematic attempt at assessment was performed because of the variable amounts of cytoplasm in different lymphoma types. For assessment of E2F-1 and Ki-67, homogeneous nuclear staining was considered positive, regardless of the staining intensity.

All cases were estimated independently by two authors (J.A.C. and R.K.B.). On the basis of these results, four grades of positivity were defined: 1 to 10%, 11 to 25%, 26 to 50%, and greater than 50% cells. These categories are similar to those used in our previous studies (21,22). Discrepancies in the assignment of grade and staining intensity were reconciled by joint review using a double-headed microscope. The differences in scores between DP-1 and Ki-67 or E2F-1 were assessed using Fisher exact test. Results with $P < 0.05$ were considered significant.

RESULTS

Follicular Hyperplasia

Nearly all (> 90%) of the follicular center cells in cases of follicular hyperplasia were strongly positive for DP-1 (Fig. 1), but only rare lymphoid cells in the mantle zone were strongly positive. Similarly, only a relatively small proportion of the small and large lymphoid cells in the interfollicular areas was strongly positive for DP-1. In tonsillar tissues, nuclei in the basal layer of the squamous epithelium were strongly positive. In tonsillar and nodal tissues, the endothelial cells were often strongly positive for DP-1.

The results for E2F-1 and Ki-67 staining patterns were similar to those of our previous study (21). Staining for both E2F-1 and Ki-67 was predominantly found in the germinal centers and was strongest in the polarized dark zones. Expression of Ki-67 was observed in greater than 50% of cells of all eight cases. In comparison, only three of eight cases stained for E2F-1 showed greater than 50% of cells positive, and this positivity was restricted to a small subset of the centroblasts. Table 1 shows the distribution of scores for the three markers studied.

Non-Hodgkin Lymphoma

Cases of small lymphocytic lymphoma (n = 10), marginal zone lymphoma (n = 3), and mantle cell lymphoma (n = 5) demonstrated relatively low scores for all three markers. In small lymphocytic lymphoma, staining for DP-1 was predominantly restricted to the prolymphocytes in the proliferation centers (Fig. 2). Mantle cell lymphoma tended to have intermediate scores for all three markers, but only one case had both DP-1 and Ki-67 scores of greater than 50%. In comparison, both cases of precursor B lymphoblastic lymphoma had high scores for all three markers.

Follicular lymphoma cases had high DP-1 scores and variable E2F-1 and Ki-67 scores. Regardless of the grade, follicular lymphoma showed greater than 50% cells positive for DP-1, while the E2F-1 and Ki-67 scores increased in parallel with the grade. Similarly, 23 of 24 diffuse large B-cell lymphomas showed greater than 50% cells positive for DP-1, with a lower but wider

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TABLE 1. *DP-1, E2F-1, and Ki-67 expression*

Diagnosis	Cases	Marker	Percentage of positive cells			
			1–10%	11–25%	26–50%	>50%
Follicular hyperplasia	8	DP-1	0	0	0	8
		E2F-1	0	0	5	3
		Ki-67	0	0	0	8
B-cell non-Hodgkin lymphomas						
Small lymphocytic lymphoma	10	DP-1	9	1	0	0
		E2F-1	9	1	0	0
		Ki-67	3	5	2	0
Marginal zone B-cell lymphoma	3	DP-1	2	1	0	0
		E2F-1	2	1	0	0
		Ki-67	1	1	1	0
Mantle cell lymphoma	5	DP-1	0	3	1	1
		E2F-1	0	2	3	0
		Ki-67	0	1	3	1
Lymphoblastic lymphoma	2	DP-1	0	0	0	2
		E2F-1	0	0	0	2
		Ki-67	0	0	0	2
Follicular lymphoma, grade 1	7	DP-1	0	0	0	7
		E2F-1	6	1	0	0
		Ki-67	0	3	3	1
Follicular lymphoma, grade 2	7	DP-1	0	0	0	7
		E2F-1	2	2	2	1
		Ki-67	0	1	4	2
Follicular lymphoma, grade 3	11	DP-1	0	0	0	11
		E2F-1	0	4	7	0
		Ki-67	0	1	3	7
Diffuse large B-cell lymphoma	24	DP-1	0	0	1	23
		E2F-1	4	1	9	10
		Ki-67	0	0	3	21

range of E2F-1 scores. Ki-67 scores in this group were consistently greater than 50%.

Since 50% positive cells appeared to represent a natural break point for the DP-1 data in follicular lymphoma and diffuse large B-cell lymphoma (Table 1), the groups of positive cells were collapsed into two categories: 1 to 50% and greater than 50%. When all 25 cases of follicular lymphoma were grouped, the proportion of cells positive for DP-1 was always greater than 50% (Fig. 3). In contrast, only 10 cases had greater than 50% Ki-67–positive cells ($P < 0.001$), and only one case had greater than 50% E2F-1–positive cells ($P < 0.001$). When the follicular lymphomas were divided by grade, the number of cases showing greater than 50% Ki-67 expression increased with grade. However, regardless of the grade, there were significant differences between the DP-1 scores compared with the Ki-67 and E2F-1 scores. Similar to follicular lymphoma, almost all (23 of 24) of the cases of diffuse large B-cell lymphoma had DP-1 scores greater than 50% (Fig. 4). In contrast, only 10 cases had greater than 50% E2F-1–positive cells ($P < 0.001$), and 21 cases had greater than 50% Ki-67–positive cells ($P = 0.30$).

DISCUSSION

The goal of this study was to assess the staining patterns of DP-1 in follicular hyperplasia and in various types of B-cell NHL, and to compare its expression with that of E2F-1 and Ki-67. Because DP-1 and E2F-1 act as a heterodimeric unit, we expected to find DP-1 levels upregulated in tandem with E2F-1 (23).

Not surprisingly, those lesions considered low-grade lymphomas, such as small lymphocytic and marginal zone lymphoma, had relatively low scores (1–50% positive) for all three markers, reflecting a lower proliferative rate. In mantle cell lymphoma, considered a more aggressive lymphoma, scores for all three markers were slightly higher. The one case with greater than 50% cells positive for E2F-1 and Ki-67 was a blastoid variant of mantle cell lymphoma thought to behave very aggressively (26). In high-grade lymphoblastic lymphoma, all three markers were highly expressed (> 50%), reflecting a high proliferative rate.

Surprisingly, in follicular lymphoma and diffuse large B-cell lymphoma, DP-1 expression did not parallel that of E2F-1. Follicular lymphoma had uniformly high DP-1 scores (> 50% cells positive), low E2F-1 scores, and variable Ki-67 scores (Table 1). In this lymphoma, the high level of DP-1 expression was independent of the cytologic grade, although, as expected, Ki-67 and E2F-1 scores rose with increasing tumor grade. It is noteworthy that high DP-1 scores were also observed in reactive follicular hyperplasia. Diffuse large B-cell lymphomas like follicular lymphoma had significantly higher DP-1 scores than E2F-1 scores. However, unlike follicular

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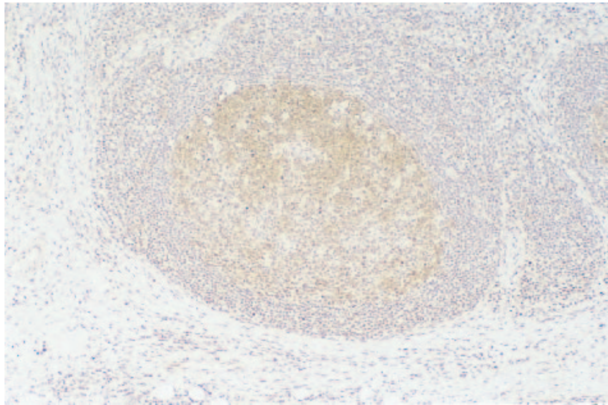

FIG. 1. Follicle in a reactive lymph node stained for DP-1. Note that staining is restricted to follicular center cell nuclei (immunoperoxidase).

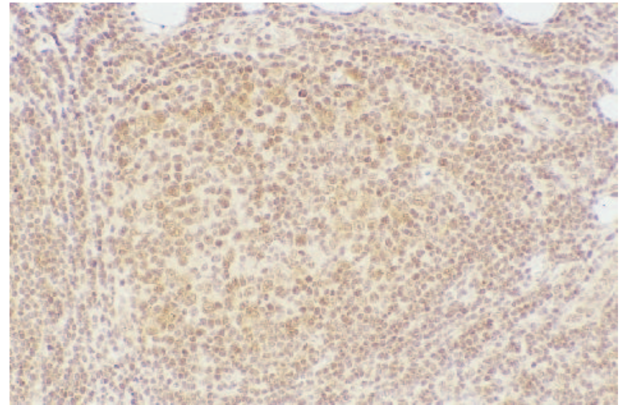

FIG. 3. Follicular lymphoma stained for DP-1 (immunoperoxidase).

lymphoma, their DP-1 scores were not significantly different from their Ki-67 scores. Therefore, the expression of DP-1 appeared to be closely associated with cell proliferation as measured by Ki-67 staining.

The discordance between the DP-1 and E2F-1 scores in reactive follicular hyperplasia, follicular lymphoma, and diffuse large B-cell lymphoma suggests that the expression of DP-1 was independent of E2F-1 in these lesions. The similarity in staining patterns for DP-1 and E2F-1 in reactive follicular hyperplasia and in follicular lymphoma is not surprising considering that both involve follicular center cells. It is possible that upregulation of DP-1 uncoupled from E2F-1 and Ki-67 expression is a unique feature of follicular center cells and lesions derived from them. This possibility suggests that DP-1 is important during normal development of germinal centers and has functions other than acting as a transcription regulator in cell cycle control.

Discordance between DP-1 and E2F-1 was also observed in a subset of diffuse large B-cell lymphoma. Even though almost all the diffuse large B-cell lymphomas showed high DP-1 scores, 14 out of the 24 cases had discordant E2F-1 scores. Since some diffuse large B-cell lymphomas are thought to arise from follicular center cells (27), perhaps these cases displaying discordant DP-1/E2F-1 scores developed from follicular center cells. However, since the DP-1 scores were not significantly different from Ki-67 scores, DP-1 in diffuse large B-cell lymphoma is probably involved in cell cycle control, but not in association with E2F-1.

Interestingly, in many small lymphoid cell neoplasms, DP-1 staining was localized to the nucleus despite a relatively low E2F-1 score. Since nuclear localization of DP-1 is dependent on its dimerization with an E2F partner and unbound DP-1 fails to localize in the nucleus (12), the possibility that DP-1 might be partnered with other E2F members in B-cell lymphoid lesions must be considered. In support of this concept, it has been shown that DP-1 can form heterodimers with all the other members of the E2F family (28).

In addition, although we scored only cells that were strongly positive for DP-1 in this study, most of the remaining lymphoid cells in both reactive and neoplastic

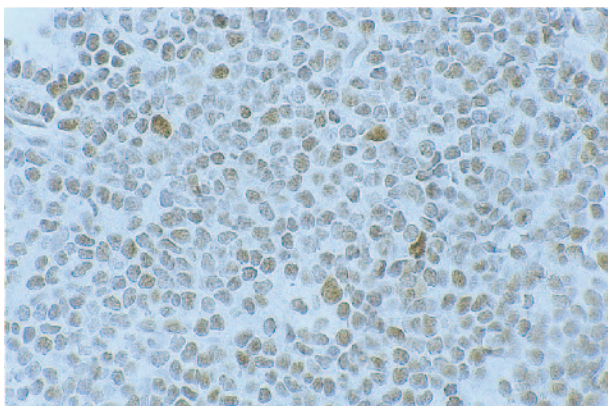

FIG. 2. Small lymphocytic lymphoma stained for DP-1. Note that expression is primarily restricted to the prolymphocytes (immunoperoxidase).

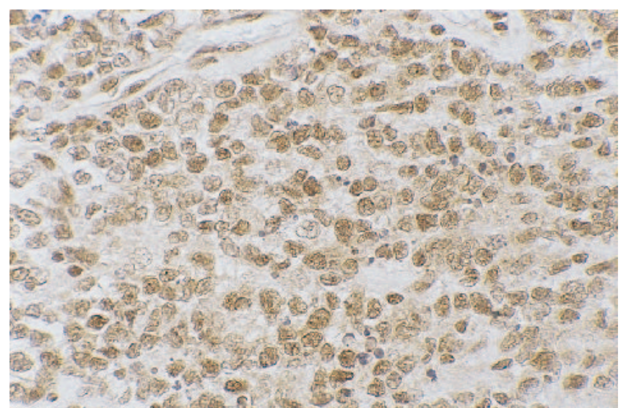

FIG. 4. Diffuse large B-cell lymphoma stained for DP-1 (immunoperoxidase).

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states showed uniformly weak nuclear DP-1 staining. This observation suggests a basal level of DP-1 expression in these cells and supports studies demonstrating that DP-1 is normally constitutively expressed and awaits dimerization with E2F-1 to exert its effects (29,30). Thus, normal E2F/DP activity is limited by the availability of the E2F protein and not the DP proteins (31).

In summary, with the exception of follicular lymphoma and diffuse large B-cell lymphoma, DP-1 expression paralleled that of E2F-1. The high level of DP-1 expression in reactive follicular hyperplasia and in follicular lymphoma suggests that DP-1 is probably important in follicular center cell development, but its exact role in these cells requires further study. The dissociation of the expression level of DP-1 and E2F-1 seen in some types of NHL suggests that DP-1 can be expressed independently of E2F-1 and that DP-1 may have functions other than serving as a transcription partner for E2F-1. □

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