Thymidine Phosphorylase Expression in B-cell Lymphomas and Its Significance

A New Prognostic Marker?

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OBJECTIVE: To explore thymidine phosphorylase (TP) expression in B-cell lymphomas (BCLs). TP is expressed by tumor and stromal cells in a variety of cancers.

STUDY DESIGN: Paraffin-embedded tissues from follicular lymphomas, diffuse large BCLs (DLBCLs), and benign lymph nodes were studied using immunohistochemical staining with antibodies for TP and CD68. Prognostic markers were used to stain DLBCLs. We correlated TP expression in DLBCLs indirectly with prognostic immunomarkers and directly with survival data.

RESULTS: TP expression in BCLs was noted in a subset of malignant B cells. TP expression in higher-grade lymphoma was identified in 66% of cases and 11% of lower-grade lymphomas. Macrophages/stromal cells demonstrated an intense cytoplasmic and/or nuclear staining pattern in both lymphoma and benign lymph nodes, confirmed by CD68 coexpression. Increased macrophage/stromal cells in higher-grade lymphomas are associated with enhanced TP expression in neoplastic B cells (observation only). Sixty-eight percent of TP-positive DLBCLs were of nongerminall center origin, indicating poorer prognosis.

CONCLUSION: TP is more likely expressed by malignant B cells in higher-grade lymphomas, and expression of TP possibly results from changes intrinsic to the tumor cells or interactions between microenvironment and tumor. TP positivity in DLBCL correlates with nongerminall center origin and worse outcome. (Anal Quant Cytopathol Histopathol 2013;35:301–305)

Keywords: B-cell lymphoma, diffuse large B-cell

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Follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) are hematogenous malignancies of B-cell origin. FL is graded between 1 and 3 based on histologic parameters related to the tumor’s biologic behavior. FL grade 3 and DLBCL are higher-grade BCLs, whereas FL grades 1 and 2 are considered lower-grade BCLs. DLBCLs can be subdivided into those of germinal center origin and those of non-germinal center origin through the application of well-established prognostic immunohistochemical (IHC) panels using CD10, Bcl-6, and Mum-1. Thymidine phosphorylase (TP), also known as platelet-derived endothelial cell growth factor, may be overexpressed in both neoplastic cells and tumor stromal cells in a variety of cancers, including breast, colorectal, gastric, esophageal, lung, bladder, and salivary gland. The role of TP in tumorigenesis is thought to be mediated by its ability to inhibit the apoptosis pathway and ensure tumor cell survival and to promote tumor angiogenesis through activation of the PI3K-mTOR pathway. TP expression has potential clinical utility as a biomarker to predict chemotherapy response and survival in a variety of malignancies, including esophageal squamous cell carcinoma, breast cancer, and other cancers. Few studies have explored the significance of TP expression in hematopoietic malignancies. Our previous study demonstrated that TP can be expressed by malignant T lymphocytes in mycosis fungoides. Here we explore the relationship of TP expression in cases of FL and DLBCL with various established prognostic indicators and demonstrate for the first time the relationship of TP expression and patient survival in these types of BCLs.

Materials and Methods

Archived paraffin tissue blocks from 55 patients with a diagnosis of BCL, including FL (grades 1–2: 8 cases; grade 3: 10 cases), DLBCL (28 cases), and 9 benign lymph nodes, were used to generate a tissue microarray. IHC staining using antibodies to TP and CD68 was applied to all the cases. In DLBCL cases the IHC prognostic markers CD10, Bcl-6, and Mum-1 were used.

For IHC staining, slides were deparaffinized and rehydrated in a series of alcohol solutions. IHC staining was performed using an automated system (Dako Autostainer, Universal Staining System Autostainer, Carpinteria, California, U.S.A.). The primary antibodies used were TP (1:1000; Novus Biologicals, Littleton, Colorado, U.S.A.); CD20 (1:2000; Dako); CD10 (1:200, Vector Laboratories, Burlingame, California); Bcl-6 (1:10; Dako); Mum-1 (1:20; Dako); and CD68 (1:2000; Dako). The IHC staining pattern of each case was assessed blindly by senior pathology residents, in duplicate, with any inconsistencies referred for analysis by a senior attending physician. Correlation of TP expression by various cell populations within these specimens was performed indirectly with prognostic immunomarkers and directly with retrospective survival data (survival data are available only in a subset of DLBCL cases). Positive staining of neoplastic B cells with TP was defined as strong cytoplasmic staining in ≥ 10% of neoplastic B cells in cases of FL and DLBCL. All experimental protocols for this retrospective study were approved by the Institutional Review Board of Drexel University College of Medicine.

Results

TP Staining Pattern in BCLs

In the specimens studied, TP stained macrophages, endothelial cells, and follicular dendritic cells with an intense cytoplasmic and/or nuclear staining pattern in both benign and neoplastic cases (Figure 1A). CD68 coexpression highlights this pattern (not shown). TP expression by neoplastic B cells in a subset of BCLs demonstrated a characteristic cytoplasmic staining pattern (Figure 1B). Cell-specific staining patterns are summarized in Table I. In lower-grade BCLs (FL grade 3 and DLBCL, particularly the latter), macrophages/stromal cells are relatively increased compared with lower-grade BCLs (FL1 and FL2). The increased presence of macrophages/stromal cells in higher-grade lymphomas appears to be associated with enhanced TP expression by neighboring neoplastic B cells (observation only). The complexity of this expression pattern observed using our chromogenic staining method precludes successful quantification of a possible relationship.

Distribution of Positive TP Staining by Neoplastic B Cells in Lymphoma Cases

In lower-grade BCLs (FL1 and 2), only 2 of 18 cases were found to be positive for TP (11%). TP expression by neoplastic B cells in FL3 and DLBCL was ob-
served in 6 of 10 cases (60%) and in 25 of 38 cases (66%), respectively. TP expression approaches statistical significance with a $\chi^2$ of 0.0307 when comparing positive TP expression by neoplastic B cells in lower- and higher-grade BCLs (Figure 2).

**Correlation of Patient Survival Data with TP-positive Staining in Neoplastic B Cells in DLBCL**

In DLBCL, a well-established prognostic panel utilizing CD10, Bcl-6, and Mum1 was applied to distinguish cases of germinal center origin (CD10+, Bcl-6+, Mum1−) from those of nongerminal center origin (CD10−, Bcl-6−, Mum1+) with the latter portending a poorer clinical prognosis. Among DLBCL cases with positive TP expression, a majority (68%, 13/19) demonstrated nongerminal center origin.

Finally, we directly correlated TP expression with patient survival data in DLBCL cases. As demonstrated in the Kaplan-Meier survival curve (Figure 3), positive TP expression (dotted line) was associated with a worse survival as compared with cases with negative TP expression (solid line). These findings suggest a direct correlation between increased TP immunoexpression and overall worse patient survival in DLBCL.

**Discussion**

TP expression in benign tissue (spleen, liver, lymph nodes, esophagus, and rectum) has been identified in a variety of cells (macrophages, stromal cells, glial cells, endothelial cells, and reticulocytes) with a cytoplasmic or nuclear staining pattern. Overexpression of TP by neoplastic cells has been documented in a variety of malignancies (breast, colorectal, gastric, esophageal, lung, and bladder).

Although TP expression has been reported in nonhematopoietic malignancies, studies of TP expression in hematopoietic diseases are few. In 1997 Doussis-Anagnostopoulou et al first demonstrated TP expression in a variety of tissues from patients with hematopoietic malignancies including peripheral T-cell lymphoma, intestinal T-cell lymphoma, anaplastic large T-cell lymphoma, Hodgkin’s lymphoma, small lymphocytic lymphoma, mantle cell lymphoma, FL, and DLBCL. TP expression in this limited study was noted only within the

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**Table 1**  
**Thymidine Phosphorylase Immunohistochemical Stain (Cytoplasmic/Nuclear)**

<table>
<thead>
<tr>
<th></th>
<th>Small lymphocytes</th>
<th>Neoplastic cells</th>
<th>Macrophages and dendritic cells</th>
<th>Endothelial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign lymph nodes</td>
<td>Negative</td>
<td>N/A</td>
<td>Positive</td>
<td>Positive</td>
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<tr>
<td>Diffuse large BCL/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>follicular lymphoma</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
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dendritic cells, macrophages, and endothelial cells in these hematopoietic malignancies. No studies since then have further explored TP expression in hematopoietic malignancies, including BCLs. Recently we have demonstrated that TP expression is present in neoplastic T cells of lymph nodes and skin involved with mycosis fungoides, or Sézary syndrome.19

In the current study we observed a pattern of TP expression similar to observations made by Doussis-Anagnostopoulou et al22 and in our own recent study19 within benign background lymph node stromal elements (macrophages, endothelial lining cells, and follicular dendritic cells) in a predictable pattern. In addition, TP expression by a subset of neoplastic lymphocytes varied in extent and intensity in cases of FL1–3 and DLBCL. This study demonstrates that TP is more likely expressed by neoplastic B cells in higher-grade lymphomas (DLBCL and FL grade 3 versus FL grades 1–2). Positive TP staining by neoplastic B cells is associated with higher-grade BCLs. Increased numbers of macrophages/stromal cells in higher-grade lymphomas are associated with enhanced TP staining in neoplastic B cells (observation only). In DLBCL, TP-positive expression by neoplastic B cells seems to correlate with nongerminal center origin, a pattern that often portends a worse clinical outcome. Further, we have demonstrated an inverse relationship between patient survival and TP expression by neoplastic B cells. Although these survival data were available for a limited subset of DLBCL patients, the potential use of TP as a prognostic marker in FL/DLBCL must be explored further.

The expression of TP by neoplastic lymphoma cells in higher-grade lymphomas may be secondary to changes intrinsic to the tumor cell itself or alternatively may reflect interactions between the tumor microenvironment and the lymphoma cells. Previous studies12,16 noted that the mechanism of TP in tumorigenesis proceeds by inhibition of the apoptosis pathway in tumor cells and stimulation of tumor angiogenesis by tumor and stromal cells via the PI3K/mTOR pathway. The observation of concomitant neoplastic B-cell expression and intense stromal cell expression in higher-grade lesions suggests that synergy/communication between the two cell populations may contribute to the malignant behavior. The exact mechanism of TP expression in BCLs, however, needs further investigation.

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