Immunohistochemical Expression of mTOR in Germinal Center and Nongerminal Center Group of Diffuse Large B-Cell Lymphoma: A Clinicopathological Study

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Abstract

Immunohistochemical expression of m-TOR was studied in a retrospective series of patients with diffuse large B-cell lymphoma. High m-TOR expression was associated with majority of patients with germinal center phenotype and high IPI in this sample. High m-TOR expression may offer prognostic value as well as a potential target for therapeutic intervention in patients with diffuse large B-cell lymphoma.

Background: The mammalian target of rapamycin (mTOR) pathway regulates many major cellular processes and is implicated in an increasing number of neoplasms, including lymphoma. **Patients and Methods:** We correlated immunohistochemical expression of mTOR with germinal center and nongerminal center phenotype, B cell lymphoma-2 (bcl-2) and cellular homolog of the retroviral v-myconcogene (c-myc) expression, and International Prognostic Index (IPI) score in 31 patients with diffuse large B-cell lymphoma (DLBCL). **Results:** Virtually all patients in our study with high mTOR scores had a germinal center phenotype. Furthermore within the germinal center subgroup, patients with high mTOR scores were associated with higher IPI scores ($P < .001$). **Conclusion:** Based on our results we propose that within the category of germinal center phenotype of DLBCL, mTOR expression might help identify a subset of patients with potentially more aggressive tumors who might benefit from use of targeted therapy using mTOR inhibitors.

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of Non-Hodgkin lymphoma and is associated with clinical and biological diversity. It displays heterogeneous clinical and molecular characteristics.[1] A number of constitutively activated growth signaling pathways have frequently been observed in DLBCL including the phosphatidylinositol 3-kinase (PI3K) pathway.[2] The mammalian target of rapamycin (mTOR) kinase, a key member of the pathway has emerged as a major player in many aspects of cell biology including cell cycle progression and survival.[3,4] In an earlier study done by our group we found that patients with a high total immunohistochemical mTOR score showed a trend toward shorter survival.[5] Per the World Health Organization classification of hematopoietic tumors, DLBCL are classified into 2 main subgroups based on the pattern of immunohistochemical staining of CD10, bcl-6, and multiple myeloma oncogene-1 (mum-1): the germinal center B-cell–like subgroup (GCB) and nongerminal center-like subtype (non-GCB).[6] The intent of this study was to investigate any correlation between tumors with GCB or non-GCB cell phenotypes with total immunohistochemical mTOR score. Further, we also compared the expression of these biomarkers with B cell lymphoma-2 (bcl-2) and cellular homolog of the retroviral v-myconcogene (c-myc) expression, and International Prognostic Index (IPI).

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Patients and Methods

This study was performed at SUNY Upstate Medical University, Syracuse with institutional review board approval. For this study we selected 31 cases of DLBCL of which sufficient material was present in the paraffin-embedded block to construct microarrays and for which mTOR results were already known from our earlier study.[5] Construction of tissue microarray (TMA) and immunohistochemical staining for CD20, CD10, bcl-6, bcl-2, and mum-1 were performed at the Department of Pathology, University of Rochester, Rochester, NY. Immunohistochemical staining for c-myc was performed at the Department of Pathology, SUNY Upstate Medical University, Syracuse, NY.

For construction of the TMA, hematoxylin and eosin-stained sections from each paraffin-embedded, formalin-fixed block were used to define diagnostic areas. Two to 4 (average, 3) random, representative 1-mm cores were obtained from each case and inserted in a grid pattern into a recipient paraffin block using a tissue arrayer (Beecher Instruments, Silver Spring, MD). Sections (5-μm) were then cut from the TMA and stained with antibodies to CD20 (L26, Ventana), CD10 (56C6, CM129, Biocare; 1:50 dilution), bcl-6 (GI191/A8, 227M, Cell Marque; 1:200 dilution), mum-1 (MUM1p, M7259, Dako; 1:50 dilution), bcl-2 (124, M0887, Dako; 1:50 dilution), and c-myc (Y69, Abcam; 1:50 dilution).

Immunohistochemical Analysis

A CD20 stain was performed to evaluate each core for involvement by tumor. For each case, the core with the highest percentage of tumor cells stained was used for analysis. For CD10, bcl-6, and mum-1 expression, cases were considered positive if 30% or more of the tumor cells were labeled. Cutoff values of 40% for c-myc and 70% for bcl-2 were established based on a previous study.[7] The intensity of staining was also evaluated, but was not used to determine positivity because the variability in tissue fixation and processing appeared to affect the intensity of staining. Immunohistochemical results for CD10, bcl-6, and mum-1 were used to subclassify the cases into GCB or non-GCB subtype as previously described.[8] Subsequently these results were correlated with immunohistochemical mTOR score (mTOR [7C10] XP rabbit monoclonal antibody from Cell Signaling Technology, Beverly, MA; 1:50 dilution) as described previously.[5] These findings were further correlated with clinical parameters whenever available, and IPI score.

Statistical Analysis

Continuous and discrete variables were summarized using mean/standard deviation/range and frequency (percentage), respectively. Standard 2-sample t tests and Pearson χ^2 tests were used to compare the difference between groups. Fisher exact tests were applied if 1 of the cell counts was < 5 for 2×2 tables.

Results

The study group consisted of 20 (64.5%) male and 11 (35.5%) female patients. Of the total patients, 13 patients (45%) had high IPI scores (> 3). Based on the pattern of staining of the 3 antibodies (CD10, bcl-6, and mum-1; Figures 1-3), we found 20 patients (64.5%) to have tumors with the GCB phenotype and 11 patients (35.4%) with a non-GCB phenotype (Table 1). In our series, 12 patients (38.7%) showed high total mTOR immunohistochemical scores (Figures 4 and 5). All tumors except 1 with a high mTOR score were of GCB phenotype (92%; $P = .02$; Table 1). mTOR-negative tumors (Figure 6) were distributed nearly equally among the GCB (9 out of 19 patients, 47.3%) and non-GCB (10 out of 19 patients, 52.6%) phenotype groups, respectively. Most of the cases in our series were bcl−2-positive (26 out of 31 patients, 83.8%) and c−myc-negative (27 out of 31 patients, 87.1%) and these findings were distributed in similar proportions between the GCB and non-GCB cell groups. A very small percentage of cases (3 out of 31 patients, 9.6%) were positive for bcl-2 and c-myc. Of the 31 patients, IPI score was available for 28 patients. DLBCL with the germinal center phenotype and higher mTOR score were also seen to be associated with higher IPI score and this difference was found to be statistically significant compared with patients with lower mTOR scores and germinal center phenotype ($P < .001$; Table 2). Low and high IPI scores were nearly equally distributed within the non-GCB subset of DLBCL cases and a very small percentage (1 out of 11 cases, 9.1%) of cases

Figure 1 CD10-Positive Tumor (Magnification ×50)

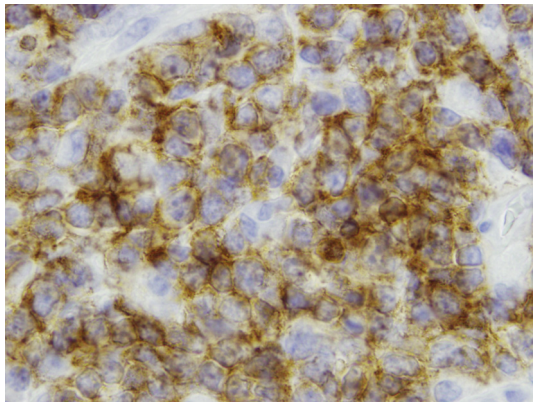

Figure 2 Bcl-6—Positive Tumor (Magnification ×50)