p63 Protein expression in high risk diffuse large B-cell lymphoma

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ABSTRACT

Background: p63 gene is a p53 homologue that encodes proteins with transactivation, DNA-binding and tetramerisation domains. The isoforms TAp63 and TAp73 transactivate p53 target genes and induce apoptosis, whereas the isoforms ΔNp63 and ΔNp73 lack transactivation and might have dominant-negative effects in p53 family members. p63 is expressed in germinal centre lymphocytes and can be related to the development of the lymphoma, but the prognostic significance of its expression in the survival of patients with diffuse large B-cell lymphoma (DLBCL) remains unclear.

Aims: To determine whether quantitative immunohistochemical (IHC) analysis of p63 protein expression correlates with CD10 antigen, Bcl-6 antigen and IRF4 antigen expression and to determine whether p63 is a surrogate predictor of overall survival in high–intermediate and high risk DLBCL populations.

Methods: CD10, Bcl-6 and IRF4 expression were retrospectively evaluated by IHC in 73 samples of high–intermediate and high risk DLBCL and were used to divide the lymphomas into subgroups of germinal centre B-cell-like (GCB) and activate B-cell-like (ABC) DLBCL. Similarly, p63 expression was evaluated by IHC and the results were compared with subgroups of DLBCL origin and with the survival rates for these patients.

Results: p63 was expressed in more than 50% of malignant cells in 11 patients and did not show correlation with subgroups of GCB-like DLBCL or ABC-like DLBCL, but p63(+) patients had better disease-free survival (DFS) than those who were negative (p = 0.01).

Conclusions: p63(+) high–intermediate and high risk DLBCL patients have a better DFS than negative cases.

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease. Previous studies have shown different survival rates for patients of the same age-adjusted international prognostic index (aIPI) score that underwent the same therapeutic protocol.1 2 Other variables may also influence the prognosis. The analysis of gene expression by DNA microarray revealed distinct prognostic groups of DLBCL.2 These subgroups can be divided by immunohistochemistry (IHC) with monoclonal antibody to CD10 and Bcl-6 that are expressed in germinal centre B-cell-like (GCB-like) DLBCL and IRF4 that are expressed in activate B-cell-like (ABC-like) DLBCL.3 Indeed, a p53 gene homologue (p63) was described recently and some authors related it to poor prognosis in malignant lymphoma. p65 is a p55 homologue gene located at 3q28, encoding different transcripts with various effects on p53 activation and apoptosis.4 5 Previous studies have shown the role of p65 gene in the growth and development of epithelial tumours.4 6 Although p63 is expressed in germinal centre lymphocytes and seems to be related to the development of the lymphoma, its function in the lymphoid tissue is not clear.7 8 A study from Japan showed expression of p63 protein in 34% of cases with poor overall survival (OS). A higher expression of p63 in Bcl-6(+) cases was also shown.6 However, this study evaluated patients with different international prognostic indices and did not therefore represent all subgroups of DLBCL.

We evaluated p63 expression by IHC for high–intermediate and high risk DLBCL only and verified its impact on survival rates, correlating it with GCB-like DLBCL and ABC-like DLBCL.

METHODS

Patients and treatment

From January 1992 to December 2005, 73 high–intermediate and high risk patients with DLBCL (aged 18–60 years, median 59.5 years) were treated with eight cycles of CHOP-like regimens with or without rituximab at the Clinical Hospital of Sao Paulo University (HC-FMUSP). The study was approved by the Institutional Review Board Committee. Rituximab was not used as the drug is still unavailable in our public health care units. Patients with immunodeficiency-associated tumours were excluded; clinical stage was performed using the Ann Arbor classification. Rituximab score was calculated for all patients; remission was defined according to Cheson’s criteria.10

Tissue samples were obtained from surgical specimens that were reviewed by a haematopathologist from the Department of Pathological Anatomy of HC-FMUSP according to the WHO classification.

Immunohistochemistry

Sections (4 μm thick) were cut, deparaffinised, rehydrated and pressure cooked in an EDTA buffer (pH 6.0) at 95°C for 5 min for antigen retrieval; for Bcl-6, Tris–EDTA buffer at pH 9.0 was used with 5 min pressure cooking. After cooling, the sections were immersed in 3% hydrogen peroxide for 20 min and incubated at room temperature for 1 h, followed by overnight incubation at 4°C with mouse monoclonal antibodies against p63 (clone 4A4, dilution 1:200; Dako, Glostrup, Denmark), Bcl-6 (IF6, dilution 1:40; Novocastra, Newcastle, UK), IRF4 (MUMIP dilution 1:500, Dako), CD10 (56C6 dilution 1:250; Novocastra). Clone 4A4 could detect all p63 isoforms as identified by Western blotting. Biotinylated rabbit antimouse immunoglobulin IgG was used as the secondary antibody (DakoCytomation) and 3,3-diaminobenzidine-hydrogen peroxide as the chromogen; for
Bcl-6, peroxidase-polymer (Novolink, Novocastra) was used. Sections were counterstained with haematoxylin, dehydrated, cleared and mounted. Five hundred neoplastic cells per specimen were evaluated at ×400 magnification and the ratio (%) of p63 immunoreactive neoplastic cells was recorded. We used a cut-off value of ≥10% immunoreactivity to Bcl-6, CD10 and IRF4 and ≥50% to p63. Classification in GCB-like DLBCL and ABC-like DLBCL was performed as previously reported.

Statistics

Overall survival was calculated as the interval between the date of the beginning of the treatment and either death or last follow-up. Disease-free survival (DFS) was defined as the interval between complete remission (CR) and first progression, relapse or death. Time-to-event endpoints were estimated according to the Kaplan–Meier method. Cox’s proportional hazards regression was used for multivariate analyses of OS and DFS; all statistical calculations were performed using SPSS V.13.0.

RESULTS

p63 Expression in high risk DLBCL

Eleven (15.1%) of the 73 patients were found to express p63, with no statistical difference in the distribution of the clinical data in both p63(−) and p63(+) groups (table 1). Staining in positive cases was visualised in the nuclei of neoplastic lymphocytes (fig 1).

We did not show correlation between p63 expression and OS (p = 0.09), although we demonstrated correlation between p63 expression and DFS. With a median follow-up of 123 months, DFS at 60 months was 49.6% for p63(−) cases and 100% for the p63(+) group (p = 0.01) (fig 2).

p63 Expression and correlation with CD10, Bcl-6 and IRF4 antigens

Bcl-6 antigen was positive in 36 (49.3%) patients; there was no significant correlation with p63 expression (p = 0.27). CD10 antigen was observed in 14 (19.2%) patients. IRF4 was positive in 39 (53.4%) patients; there was no significant correlation with p63 expression. p63 Was positive in 3 (12.5%) of the 24 GCB-like DLBCL cases and in 8 (16.3%) of the 49 ABC-like DLBCL group (tables 2 and 3). Furthermore, we did not observe a significant association between p63 expression pattern and subgroups of GCB-like DLBCL and ABC-like DLBCL.

DISCUSSION

Several reports have shown that p53 mutations and p53 overexpression are common events in DLBCL and are associated with a poor prognosis. The discovery of p53 homologues p63 and p73 has added additional levels of complexity to the study of p53 function. Both p63 and p73 encode numerous proteins

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Table 1  Expression of p63 according to clinical characteristics

<table>
<thead>
<tr>
<th>p63</th>
<th>Negative (%)</th>
<th>Positive (%)</th>
<th>Total (%)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B symptoms</td>
<td>54 (87.1)</td>
<td>9 (81.8)</td>
<td>63 (86.3)</td>
<td>0.468</td>
</tr>
<tr>
<td>Extranoval</td>
<td>27 (43.5)</td>
<td>5 (45.5)</td>
<td>32 (43.8)</td>
<td>0.58</td>
</tr>
<tr>
<td>BM+</td>
<td>13 (21)</td>
<td>3 (27.3)</td>
<td>16 (21.9)</td>
<td>0.451</td>
</tr>
<tr>
<td>Bulky</td>
<td>30 (48.4)</td>
<td>6 (54.5)</td>
<td>36 (49.3)</td>
<td>0.48</td>
</tr>
<tr>
<td>High LDH</td>
<td>56 (80.3)</td>
<td>10 (90.9)</td>
<td>66 (90.4)</td>
<td>0.717</td>
</tr>
<tr>
<td>Ann Arbor stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I and II</td>
<td>13 (21)</td>
<td>2 (18.2)</td>
<td>15 (20.5)</td>
<td>0.598</td>
</tr>
<tr>
<td>III and IV</td>
<td>49 (79)</td>
<td>9 (81.8)</td>
<td>58 (79.5)</td>
<td>–</td>
</tr>
<tr>
<td>aIPI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High–intermediate</td>
<td>40 (64.5)</td>
<td>7 (64.8)</td>
<td>47 (64.4)</td>
<td>0.603</td>
</tr>
<tr>
<td>High</td>
<td>22 (35.5)</td>
<td>4 (35.2)</td>
<td>26 (35.6)</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>62 (84.9)</td>
<td>11 (15.1)</td>
<td>73 (100)</td>
<td>–</td>
</tr>
</tbody>
</table>

BM, bone marrow; aIPI, age-adjusted international prognostic index; LDH, lactate dehydrogenase.
between p63 expression and GCB-like DLBCL. 

**Table 2 Immunohistochemical stain results**

<table>
<thead>
<tr>
<th></th>
<th>GCB-like DLBCL (n, %)</th>
<th>ABC-like DLBCL (n, %)</th>
<th>Total (n, %)</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD10</td>
<td>14 (58.3)</td>
<td>0 (0)</td>
<td>14 (19.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Bcl-6</td>
<td>20 (83.3)</td>
<td>16 (32.6)</td>
<td>36 (49.3)</td>
<td>NS</td>
</tr>
<tr>
<td>MUM-1</td>
<td>6 (25)</td>
<td>33 (67.3)</td>
<td>39 (53.4)</td>
<td>NS</td>
</tr>
<tr>
<td>p63</td>
<td>3 (12.5)</td>
<td>8 (16.3)</td>
<td>27 (37)</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>24 (32.6)</td>
<td>49 (67.1)</td>
<td>73 (89)</td>
<td></td>
</tr>
</tbody>
</table>

*χ² test. GCB, germinal centre B; ABC, activate B-cell; DLBCL, diffuse large B-cell lymphoma; NS, not significant.

with transactivation, DNA binding, and tetramerisation domains. The isoforms TAp63 and TAp73 transactivate p53 target genes and induce apoptosis, whereas other isoforms ΔNp63 and ΔNp73 lack transactivation and might convey dominant-negative effects in all p53 family members. In contrast to p53, p63 is expressed in normal tissues such as squamous epithelia and urothelium, the basal glandular cells of the prostate and breast, as well as basal bronchial cells of the lung, normal lymph nodes and peripheral blood lymphocytes. Several studies have investigated the role of p63 in neoplastic transformation and tumour progression. In transitional cell carcinomas of the bladder, loss of p63 expression is associated with tumour progression. Yamaguchi et al. identified mutation of p63 in blast crisis of chronic myelogenous leukemia. At the present time, few studies have investigated the prognostic significance of p63 in DLBCL, especially in a high–intermediate and high risk population. Di Como et al. showed high levels of p63 in a subset of DLBCL (25%) and in follicular lymphoma (22%). We have analysed the expression of p63 in high–intermediate and high risk DLBCL and observed p63 expression in 15.1% of cases, lower than that found by Fukushima et al., whose cut-off value was at 20%, and Park and Oh, whose cut-off value was at 5%. In contrast to Fukushima et al., who showed correlation between p63 expression and GCB-like DLBCL, we did not observe correlation between GCB-like DLBCL or ABC-like and p63 expression. Similarly, as opposed to what was described by Park and Fukushima, we did not show a relation between p63 expression and unfavourable prognosis in DLBCL. Surprisingly, we showed that p63 had a protective effect on DLBCL patients, especially in DFS. In our report we showed a DFS of 100% for patients with p63 expression (p = 0.01); however, we used a cut-off value for p63 higher than used by Park and Fukushima. Our results were similar to what has been observed for other tumours, such as gallbladder cancer, for which the absence of p63 was associated with tumour development. The cut-off values for the IHC prognostic markers have been defined at random, taking into account the presence or absence of a given antigen in either normal or neoplastic tissue. We believe, however, that the best criterion would be to determine the cell percentage with impact on survival rates. In our study, a cut-off value at 50% of the cells had an impact on survival; this should be tested in other studies to check its recurrence.

**REFERENCES**

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