Expression of p63 in Thymomas and Normal Thymus

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Abstract

The p63 gene, a member of the p53 family, is an epithelial marker expressed in embryonic ectoderm, breast myoepithelium, prostate, oral epithelium, epidermis, and urothelium. The ∆N-p63 isoforms of p63, which are believed to behave as oncogenes, are expressed in squamous cell carcinoma, basal cell carcinoma, and transitional cell carcinoma. Only a few authors have looked for p63 expression in thymomas and normal thymus. We, therefore, thought of undergoing such a search by taking advantage of our archival material. We studied 66 cases of thymoma (1 type A, 8 type AB, 12 type B1, 19 type B2, 12 type B3, and 14 type C/thymic carcinoma) and 10 specimens of normal human thymus arranged in tissue microarrays. All thymomas (including thymic carcinomas) were positive for p63 regardless of type. Most of the epithelial cells of the normal thymus were also positive for this marker.

Materials and Methods

All thymoma cases were retrieved from the consult files of one of us (J.R.) and arranged in tissue microarrays (36-40 cases per paraffin block). They were reviewed and reclassified according to the current World Health Organization classification16: type A, spindle cell or medullary; type AB, mixed; type B1, lymphocyte-rich, lymphocytic, predominantly cortical, or
organoid; type B2, cortical; type B3, epithelial, atypical, squamoid, or well-differentiated thymic carcinoma; and type C thymoma, thymic carcinoma. Table 1. Thymic carcinomas were further subdivided into squamous cell carcinoma and mucoepidermoid carcinoma.

Immunohistochemical staining was performed using the 4A4 monoclonal antibody (DAKO A/S, Glostrup, Denmark), which recognizes all 6 p63 isoforms, on 5-µm-thick paraffin-embedded tissue sections mounted on poly-L-lysine-coated glass slides. After heat drying, sections were deparaffinized in xylene (twice for 5 minutes each time) and sequentially rehydrated in 100%, 70%, and 50% ethanol. Sections were pretreated with an antigen-retrieval solution (0.01 mol/L of citrate buffer, pH 6) at 99°C for 40 minutes and then incubated with the antibody 4A4 at a dilution of 2 µL/mL in tris(hydroxymethyl)aminomethane-buffered saline for 1 hour at room temperature. Detection steps were performed using a commercially available kit (EnVision Plus-HRP, DAKO) according to the manufacturer’s instructions. Peroxidase activity was developed with 3-3’-diaminobenzidine-copper sulfate (Sigma Chemical, St Louis, MO) to obtain a brown-black end product. Sections were counterstained in weak hematoxylin (30

Table 1
Thymomas Classified According to the Current World Health Organization Classification

<table>
<thead>
<tr>
<th>Thymoma Type</th>
<th>No. of Cases Available for p63 Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td>AB</td>
<td>8</td>
</tr>
<tr>
<td>B1</td>
<td>12</td>
</tr>
<tr>
<td>B2</td>
<td>19</td>
</tr>
<tr>
<td>B3</td>
<td>12</td>
</tr>
<tr>
<td>C</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
</tr>
</tbody>
</table>

seconds) and dehydrated in 100% alcohol (twice for 5 minutes each time) and xylene (twice for 5 minutes each time) before mounting for light microscopy. Staining for thyroid transcription factor-1, a marker of pulmonary and thyroid epithelium, was performed as a negative control experiment.15,18-22

Only nuclear expression was accepted as positive in the analysis of p63-stained sections. A semiquantitative assessment of the degree of p63 expression was performed according to the following criteria: negative, no evidence of nuclear staining; weak, nuclear staining present, but chromatin pattern and nucleoli still discernible; and intense, nuclei completely dark brown, with nuclear features not identifiable Image II.

**Results**

There were 66 thymomas available for evaluation: 1 type A, 8 type AB, 12 type B1, 19 type B2, 12 type B3, and 14 type C thymomas (thymic carcinomas) (Table 1). The thymic carcinomas were subdivided into 13 squamous cell carcinomas and 1 mucoepidermoid carcinoma.

In all specimens, p63 positivity was restricted to normal or neoplastic epithelial cells. In the normal thymus, p63 was identified in the epithelial cells from all major compartments, ie, subcapsular, cortical (dendritic), medullary, and Hassall corpuscle–related areas.13 In the Hassall corpuscles, p63 expression was prominent in the peripherally located cells, with progressive loss of reactivity toward the more central and better-differentiated (keratinized) regions.

All thymomas (including the thymic carcinomas) expressed p63 Table 2. The population of neoplastic, spindle-shaped, thymic epithelial cells in the 1 case of pure type A thymoma was easily identified with this marker Image 21.

<table>
<thead>
<tr>
<th>Thymoma Type</th>
<th>Location of p63 Labeling</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>Spindle-shaped cells</td>
</tr>
<tr>
<td>AB</td>
<td>Type A areas</td>
</tr>
<tr>
<td>B1</td>
<td>Few epithelial cells</td>
</tr>
<tr>
<td>B2</td>
<td>Scattered, plump epithelial cells</td>
</tr>
<tr>
<td>B3</td>
<td>Epithelial cells with mild atypia</td>
</tr>
<tr>
<td>C</td>
<td>Epithelial cells with clear-cut atypia</td>
</tr>
</tbody>
</table>

* All cases were positive for p63.

In type AB thymomas, p63 staining was helpful in distinguishing type A areas (positive) from connective tissue septa (negative) Image 31. In type B1 thymomas, there was p63 positivity of the neoplastic epithelial cells of stellate shape scattered in a lymphocyte-rich background Image 41. Type B2 thymomas showed positivity in the plump epithelial cells with vesicular nuclei and distinct nucleoli. A perivascular arrangement of these cells was highlighted by the stain in some cases Image 51. The positivity for p63 in most type B3 thymomas and thymic carcinomas was particularly strong and generally more intense than in the other thymoma types Image 61 and Image 71. The nonneoplastic lymphocytes present in thymomas and normal thymus were invariably negative for p63 Image 81.

**Discussion**

In this report, we document the constant expression of p63 in normal and neoplastic thymic epithelial cells.12-15 The prevalence of p63 staining in the peripheral portions of
Hassall corpuscles of normal thymus with progressive loss in the central, more mature regions is of interest. A similar phenomenon has been described in the normal epidermis, in the sense of there being a loss of p63 expression in the more mature components. It has been hypothesized that p63 induces expression of differential markers, shortly after which its own levels are progressively reduced.6,10

In types A and AB thymoma, p63 was helpful in delineating the spindle-shaped epithelial cells and to distinguish them from connective tissue septa. In type B2 thymomas, p63 highlighted the perivascular distribution of the tumor cells, making their recognition easier. Type B3 thymomas and thymic carcinomas exhibited a more intense positivity for p63 compared with the other thymoma types.

Conceivably, this may be due to p63 gene amplification with overexpression of the ΔN-p63α, supporting the hypothesis that the latter may behave as an oncogene.2,3 Di Como et al13 speculated that the expression of certain p63 isoforms, such as dominant-negative ΔN-p63, could result in binding and inhibition of transactivation by p53 and TAp63 (isoforms capable of transactivating p53 target genes and inducing apoptosis). It has also been proposed that p63 expression could drive the transcription of oncogenic proteins.

Frequently, p63 is present in the stem cell compartment of many epithelial tissue types,2,5,13 but the strong and universal positivity we found for this marker in normal thymus and thymomas is of interest. Further studies will be necessary to understand the significance of this finding and
explore this gene’s possible role as an oncogene and as a potential therapeutic target.

From a practical diagnostic viewpoint, p63 immunoreactivity may be useful for identifying the component of epithelial cells in normal or diseased thymuses as an adjunct to cytokeratins, considering the complete lack of immunoreactivity in thymocytes. It is interesting that p63 is expressed in B-cell lymphomas but rarely (if ever) in T cell–precursor lymphomas, a neoplasm that can arise within the thymus.14

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References


