TCL1 Protein Expression in Testicular Germ Cell Tumors

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Abstract

We immunohistochemically studied TCL1 protein expression in different histologic types of 63 testicular germ cell tumors: 23 seminomas, 14 embryonal carcinomas, 4 teratomas, 2 yolk sac tumors, and 20 mixed germ cell tumors. The 20 mixed germ cell tumors contained components of seminoma (n = 10), embryonal carcinoma (n = 18), teratoma (n = 16), yolk sac tumor (n = 7), and choriocarcinoma (n = 3). We also examined 40 cases of intratubular germ cell neoplasia, unclassified type (IGCNU). Positive immunoreactivity for TCL1 was observed in 91% of the seminoma samples (30/33) and all IGCNU cases. In contrast, no TCL1 expression was detected among the nonseminomatous germ cell tumors. In the context of testicular germ cell neoplasia, the presence of TCL1 protein appears restricted to IGCNU and seminoma, suggesting association with an undifferentiated state and loss of protein expression with tumor differentiation. Immunohistochemical evaluation of TCL1 expression may have usefulness in substantiating a diagnosis of IGCNU or seminoma and in the separation of seminoma from nonseminomatous germ cell tumors.

Materials and Methods

We identified 63 cases of testicular germ cell tumor from the files of the Department of Pathology, City of Hope, Duarte, CA. H&E-stained slides from all cases were reviewed to confirm the diagnoses. The testicular germ cell tumors were classified according to World Health Organization criteria. The T-cell leukemia/lymphoma 1 (TCL1) oncogene was initially identified through analysis of chromosomal alterations frequently associated with T-cell prolymphocytic leukemia. In addition to its involvement in the development of T-cell neoplasia, TCL1 expression has also been observed in a subset of normal B cells and B-cell lymphomas at specific developmental stages, suggesting a role in B-cell differentiation. From a functional standpoint, the TCL1 protein is thought to bind and increase effector functions of AKT proteins, leading to enhanced cell proliferation and survival.

Although expressed predominantly in cells of lymphoid lineage, the TCL1 gene has also been detected in normal testicular tissue and the TCL1 protein identified in seminomas by immunohistochemical methods. The expression of the TCL1 antigen in nonseminomatous germ cell tumors of the testis has, however, not been extensively evaluated.

The objectives of the present study were to characterize the expression pattern of TCL1 protein in a variety of histologic types of testicular germ cell tumors and to assess the potential diagnostic usefulness of TCL1 in the immunohistochemical evaluation of testicular germ cell neoplasia.
cell tumors. The 20 cases of mixed germ cell tumor included the following components: 10 seminomas, 18 embryonal carcinomas, 16 teratomas, 7 yolk sac tumors, and 3 choriocarcinomas. Foci of intratubular germ cell neoplasia, unclassified type (IGCNU) were present in the adjacent parenchyma of 40 of the 63 testicular germ cell tumors. Normal testicular tissue samples from 6 adults who had undergone routine autopsy were also analyzed.

Tissue samples from each of the cases had been fixed in 10% neutral buffered formalin and embedded in paraffin. A representative tissue block was selected from each case for immunohistochemical study. Immunohistochemical study was performed using a monoclonal antibody directed against TCL1A (dilution 1:750; Upstate Cell Signaling Solutions, Lake Placid, NY). Sections were deparaffinized in xylene and rehydrated in a graded ethanol series. Antigen retrieval was performed by heating slides in EDTA buffer (pH 8.0) in a pressure cooker (Biocare Medical, Concord, CA) at 98°C for 45 minutes. Staining was performed using an automated immunostainer (DAKO, Carpinteria, CA) followed by antibody detection using the DAKO EnVision+ System (DAKO) and 3,3′-diaminobenzidine as a chromogen. The slides were counterstained with hematoxylin and coverslipped. Appropriate positive and negative tissue controls were used throughout.

**Results**

The immunohistochemical results are summarized in **Table 1**. TCL1 was expressed in 20 of 23 cases of pure seminoma and in the seminomatous areas of mixed germ cell tumors in each of 10 cases evaluated immunohistochemically **Image 1**. TCL1 immunoreactivity in all samples was localized primarily to the nucleus and membrane of the neoplastic cells, with a lesser degree of cytoplasmic staining also observed. Conversely, all histologic types of nonseminomatous germ cell tumors, including embryonal carcinoma, yolk sac tumor, teratoma, and choriocarcinoma, showed no evidence of TCL1 expression in their pure forms or as components of mixed germ cell tumors.

IGCNU present in the vicinity of the germ cell tumors demonstrated positive immunoreactivity for TCL1 in 40 of 40 samples **Image 2**. The tumor cells showed a pattern of staining similar to that observed in seminomas. Nonneoplastic testicular components present in the index specimens and from separately analyzed autopsy tissues did not stain with the TCL1 antibody.

**Table 1**

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>No. of Positive Cases/Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure germ cell tumor</td>
<td></td>
</tr>
<tr>
<td>Seminoma</td>
<td>20/23</td>
</tr>
<tr>
<td>Embryonal carcinoma</td>
<td>0/14</td>
</tr>
<tr>
<td>Yolk sac tumor</td>
<td>0/2</td>
</tr>
<tr>
<td>Teratoma</td>
<td>0/4</td>
</tr>
<tr>
<td>Mixed germ cell tumor component</td>
<td></td>
</tr>
<tr>
<td>Seminoma</td>
<td>10/10</td>
</tr>
<tr>
<td>Embryonal carcinoma</td>
<td>0/18</td>
</tr>
<tr>
<td>Yolk sac tumor</td>
<td>0/7</td>
</tr>
<tr>
<td>Teratoma</td>
<td>0/16</td>
</tr>
<tr>
<td>Choriocarcinoma</td>
<td>0/3</td>
</tr>
<tr>
<td>Intratubular germ cell neoplasia</td>
<td>40/40</td>
</tr>
</tbody>
</table>

**Image 1** TCL1 immunoreactivity in seminoma. A, Representative example of seminoma characterized by a proliferation of uniform cells with clear cytoplasm, well-defined cell borders, and an associated lymphocytic infiltrate (H&E ×200). B, The neoplastic cells exhibit strong expression of TCL1 (×200).
Discussion

Although known primarily as an oncogene involved in the development of T-cell neoplasia, recent studies have also suggested an important role for TCL1 in murine embryogenesis, as well as in embryonic stem cell regulation and self-renewal. In this context, TCL1 has been identified as one of the downstream target genes transcriptionally regulated by OCT3/4 that controls proliferation of embryonic stem cells. As in leukemic cells, TCL1 is thought to promote embryonic stem cell proliferation through enhancement of AKT1 kinase activity, resulting in inhibition of apoptosis. The OCT3/4-TCL1-AKT1 signal pathway has also been shown to maintain the survival of cancer stem cell–like cells in somatic tumor models.

There exist relatively few data regarding TCL1 expression patterns in human embryonic stem cells and germ cells. However, Narducci et al. using an antihuman TCL1 monoclonal antibody, observed TCL1 protein expression in a number of testicular seminomas, leading them to suggest that dysregulation of the TCL1 gene may contribute to the development of human germ cell tumors.

In the present immunohistochemical study, TCL1 was found to be highly expressed in seminomas, with positive immunoreactivity observed in 91% of the samples analyzed. Conversely, all histologic types of nonseminomatous germ cell tumors, including embryonal carcinoma, yolk sac tumor, teratoma, and choriocarcinoma, lacked expression of TCL1. These findings are comparable to those obtained by Narducci et al. who reported TCL1 immunoreactivity in 10 (77%) of 13 seminomas, with no evidence of expression in 2 teratocarcinomas. The data obtained in the present investigation also demonstrate consistent expression of TCL1 protein in IGCNU, with positive staining of the neoplastic cells constituting this lesion present in all cases evaluated. IGCNU and seminoma are considered undifferentiated precursor lesions with the capacity to evolve into other histologic types of testicular germ cell tumors. The finding that TCL1 is expressed preferentially in IGCNU and seminoma as compared with nonseminomatous germ cell tumors suggests that loss of protein expression occurs with tumor differentiation.

The characteristic distribution pattern of TCL1 protein expression among testicular germ cell tumors suggests a potential role for use of the anti-TCL1 antibody in the clinical setting to facilitate classification of neoplasms of germ cell origin. Specifically, TCL1 immunohistochemical study can be used as a tool to distinguish seminomas from nonseminomatous germ cell tumors. From a clinical perspective, this distinction is important because there are established differences between these groups of tumors with regard to biologic behavior and treatment. In most circumstances, appropriate classification of testicular germ cell tumors can be made based on morphologic features. Seminoma, however, can be occasionally confused with embryonal carcinoma, particularly when the former exhibits atypical histologic features or in the context of a small biopsy sample or poor tissue fixation. Indeed, studies demonstrating the value of central histologic review of previously diagnosed testicular malignancies have shown most discrepancies to be the result of errors in distinguishing seminoma from embryonal carcinoma. The use of immunohistochemical study may therefore be of value in differentiation of these entities.

A number of immunohistochemical markers with potential usefulness in discriminating seminoma from embryonal carcinoma have been previously evaluated, among which are antibodies directed toward KIT (CD117), CD30, and SOX transcription factors. KIT immunoreactivity in a membrane distribution has been shown to be limited largely to seminomas, with nonseminomatous germ cell tumors, including embryonal carcinoma, generally lacking KIT expression. Conversely, CD30 is regarded as a sensitive and specific marker for embryonal carcinoma, with positivity in seminomas considered uncommon. An antibody panel using CD30 in combination with KIT has demonstrated value in the distinction of seminoma from embryonal carcinoma, with a KIT+/CD30– immunophenotype characteristic of seminoma and a KIT+/CD30+ immunophenotype indicative of embryonal carcinoma.
More recently, studies addressing expression of the SOX family of transcription factors in testicular germ cell tumors have shown differential expression of SOX2 and SOX17 proteins among seminomas and embryonal carcinomas.\(^{28-30}\) SOX2 immunoreactivity has been demonstrated in 100% of cases of embryonal carcinoma by previous investigators, whereas the majority of seminomas have been shown to lack SOX2 expression,\(^{28-30}\) with the exception of a single reported case exhibiting very focal immunoreactivity.\(^{28}\) In contrast, SOX17 has been reported to be highly expressed in all seminoma samples, with embryonal carcinomas essentially lacking SOX17 positivity.\(^{29,30}\)

Although a direct comparison with these aforementioned markers was not performed in the current study, TCL1 appears to show similar efficacy in the context of differentiating seminoma from embryonal carcinoma, with an observed sensitivity of 91% and specificity of 100% in this regard. TCL1 thus offers an additional marker for the immunohistochemical distinction of these particular entities.

The results of the present study also suggest a role for TCL1 as a biomarker for the diagnosis of IGCNU. All samples of IGCNU analyzed expressed TCL1, with no immunoreactivity detected in nonneoplastic germ cells. The high sensitivity and specificity of TCL1 immunohistochemical study has potential value for detecting the presence of IGCNU in testicular biopsy specimens in patients at high risk for the development of germ cell tumors, although the diagnostic usefulness of this approach in clinical practice remains to be determined.

Data yielded by the current investigation indicate that similar to other embryonic stem cell regulatory factors such as OCT3/4, NANOG, SOX2, SOX17, and SALL4,\(^{28-35}\) the presence of TCL1 protein is detectable in testicular germ cell tumors by immunohistochemical methods. In the context of testicular germ cell neoplasia, TCL1 expression appears limited to IGCNU and seminoma, with no immunoreactivity present in nonseminomatous germ cell tumors. This restricted pattern of TCL1 expression may be diagnostically useful in the clinical setting for distinguishing seminoma from nonseminomatous germ cell tumors, in particular embryonal carcinoma.

Reference: