Germ Cell Origin of Testicular Carcinoid Tumors

Phillip H. Abbosh,1 Shaobo Zhang,1 Gregory T. MacLennan,3 Rodolfo Montironi,4 Antonio Lopez-Beltran,6 Joseph P. Rank,6 Lee Ann Baldridge,1 and Liang Cheng1,2

Abstract

Purpose: Carcinoids are neuroendocrine tumors and most frequently occur within tissues derived from the embryonic gut. These tumors can occur in any organ site but are rare in the testis. The cell type giving rise to testicular carcinoid is unknown. We hypothesized that testicular carcinoid may have a germ cell origin.

Experimental Design: We describe our analysis of protein and genetic markers of germ cell neoplasia, using immunohistochemistry and fluorescence in situ hybridization, in four testicular carcinoid tumors.

Results: All four cases of testicular carcinoid tumor arose in a background of mature teratoma. Isochromosome 12p was identified in carcinoid tumor cells in all four samples. 12p overrepresentation was also observed in three cases. Isochromosome 12p and 12q overrepresentation were present in cells of coexisting mature teratoma in three cases. Carcinoid tumors showed strong immunoreactivity for synaptophysin and chromogranin, but no immunoreactivity for OCT4, CD30, c-kit, TTF-1, and CDX2. Membranous and cytoplasmic staining for β-catenin was detected in three cases.

Conclusion: Our findings suggest that testicular carcinoid represents a phenotypic expression of testicular teratoma and is of germ cell origin.

Materials and Methods

Patients. We analyzed four cases of testicular carcinoid tumor. All four cases were carcinoids arising in mature teratomas in postpubertal males (age range, 20-38 years; mean, 32 years). No patient had symptoms or signs of excess hormone secretion or a history of familial cancer syndrome.

Fluorescence in situ hybridization. Sections of 4-μm thickness were stained with H&E and reviewed, and the original diagnosis was confirmed. Sections were prepared from buffered formalin-fixed, paraffin-embedded tissue blocks. FISH analysis was done as previously described (6, 7). Dual-color FISH was done by using a mixture of Spectrum Orange–labeled centromeric α satellite DNA probe (CEP12) and Spectrum Green–labeled subtelomeric (Tel12) DNA probes for chromosome arm 12p (Vysis), which were diluted with 18U.S.C. Section1734 solely to indicate this fact.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Liang Cheng, Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, 350 West 11th Street, Clarian Pathology Laboratory, Room 4010, Indianapolis, IN 46202. Phone: 317-491-6442; Fax: 317-491-6419; E-mail: liang.cheng@yahoo.com.

© 2008 American Association for Cancer Research. doi:10.1158/1078-0432.CCR-07-4146

Testicular carcinoid tumor is rare. It was originally reported in 1954 by Simon (1) who described it as part of a cystic teratoma, and additional cases have been subsequently reported. All reported cases have occurred in peripubertal or postpubertal males (2, 3). The origin of testicular carcinoid is a matter of controversy. Some investigators have hypothesized that testicular carcinoid tumor arises from germ cells and represents a monodermal teratoma or simply a component of a mature teratoma (4), but definitive proof of this hypothesis has not been provided in previous studies. Others have postulated that testicular carcinoid tumors are derived from Leydig cells (5). In this study, we did fluorescence in situ hybridization (FISH) analysis to evaluate several testicular carcinoid tumors for the presence of a chromosomal abnormality commonly present in testicular germ cell tumors, and also evaluated the immunoreactivity of these tumors to antibodies against a number of well-established neuroendocrine and testicular germ cell tumor markers.
A classic seminoma specimen was used as a positive control for FISH analyses. Lymphocytes within each tumor were used as negative controls for each tumor.

**Immunohistochemistry.** Sections were cut at 4-μm thickness and deparaffinized for immunohistochemical studies, which were done using an automated immunostainer. Antigen retrieval was carried out by heating sections in DAKO Target Retrieval Solution S1699 for 15 min. Endogenous peroxidase activity was inactivated by incubation in 3% H2O2 for 15 min. Nonspecific binding sites were blocked using Protein Block (DAKO Corporation) for 20 min. Tissue sections were then incubated with the purified rabbit polyclonal antibody against c-kit (1:50 dilution; Oncogene), goat polyclonal antibody against OCT4 (1:500 dilution; Santa Cruz Biochemical), rabbit polyclonal antibody to synaptophysin (prediluted, DAKO), rabbit polyclonal antibody to chromogranin (prediluted, DAKO), mouse monoclonal antibody to CD30 (prediluted, DAKO), mouse monoclonal antibody to thyroid transcription factor-1 (TTF-1; 1:100 dilution, clone 8G7G3/1, DAKO), mouse monoclonal antibody to CDX2 (clone CDX2-88, prediluted, Biogenex), or mouse monoclonal antibody to β-catenin (1:150 dilution, DAKO) for 30 min at room temperature, followed by biotinylated secondary antibody (DAKO) and peroxidase-labeled streptavidin. 3,3-Diaminobenzidine was used as the chromogen in the presence of hydrogen peroxide. Positive and negative controls were used and stained appropriately.

**Results**

All four cases showed histologic features typical of carcinoid tumor. Tumors were composed of monomorphic polygonally shaped cells with modest amounts of eosinophilic cytoplasm and uniform bland nuclei with finely dispersed chromatin (Fig. 1). The architectural arrangement of tumor cells was typical of that seen in carcinoid tumors in other sites, with insular and trabecular structures and with gland formation in some tumors. One tumor was entirely insular, one was insular with gland formation, one was insular with gland formation...
and trabeculae, and one was composed only of trabeculae and small glands, lacking any insular architecture. Mitotic figures were not seen in any case. Intratubular germ cell neoplasia was identified in one case (case 4). Coexisting mature teratomas were present in all four cases.

FISH was done to assess the state of chromosome 12p within these samples (Table 1; Fig. 1). Carcinoid tumors contained i(12p) in all four cases. In the coexisting teratomatous cells, i(12p) was detected in three of four cases. Chromosome 12 overrepresentation was present within the carcinoid tumor cells in three of four cases, and was also identified in tumor cells of the adjacent teratomatous elements in three of four cases.

Carcinoid tumors of the testis showed strong immunoreactivity for chromogranin and synaptophysin, both of which are neuroendocrine markers (Table 2). Positive immunostaining for this antigen was both membranous and cytoplasmic and was noted in 25% to 80% of the carcinoid carcinoids (10), was observed in three cases (cases 2, 3, and 4). Immunostaining for this antigen was both membranous and cytoplasmic and was noted in 25% to 80% of the carcinoid tumor cells. Testicular carcinoid tumor cells showed no immunoreactivity to antibodies against OCT4 staining in a single case (4). Our study provides definitive evidence that testicular carcinoid tumors exhibit the presence of the classic genetic alterations that characterize germ cell tumors: 12p isochromosomy and overrepresentation.

Carcinoid tumors also occur in the ovary and it is interesting to compare ovarian carcinoid tumor to testicular carcinoid tumor. Carcinoid tumors are ~15 times more common in the ovary than in the testis. Ovarian carcinoid tumors are most often associated with mature cystic teratomas (dermoid cysts), which constitute the most common type of ovarian germ cell tumor up to the age of 30 years (31, 32). Cytogenetic studies of benign ovarian teratomas reveal that these tumors typically do not harbor chromosome 12p alterations (33), but that malignant ovarian germ cell tumors frequently do. In contrast, testicular teratomas (with the exception of those pure teratomas that arise in young males, typically 5 years old or less) are considered malignant, and exhibit the same 12p abnormalities that characterize other malignant germ cell tumors in postpubertal males. Determining the frequency of i(12p) in ovarian carcinoids may provide evidence for a transformed germ cell origin of these tumors as well, although it seems unlikely to be found because benign ovarian teratomas do not harbor this genetic abnormality. Ovarian carcinoids, because they typically arise within benign teratomas, are considered benign, in contrast to testicular carcinoids. Carcinoid of the ovary is often associated with struma ovarii, whereas testicular carcinoid is not associated with a second somatic carcinoma.

In our study, we found that the classic genetic alterations that characterize germ cell tumors, 12p isochromosomy and overrepresentation, are also demonstrable in testicular carcinoid tumors. The fact that testicular carcinoid exhibits this genetic abnormality is evidence against the hypothesis that testicular carcinoid tumors are of Leydig cell origin, because it is well documented that classic Leydig cell tumors rarely harbor 12p abnormalities (34). These tumors showed uniform immunohistochemical expression of neuroendocrine markers, but lacked expression of CD30, OCT4, CDX2, TTF-1, and c-kit. Our findings suggest that testicular carcinoid is a phenotypic expression of teratoma and is of germ cell origin.

### Table 1. FISH analysis for isochromosome 12p and 12p overrepresentation in testicular carcinoid and coexisting teratomas

<table>
<thead>
<tr>
<th>Case</th>
<th>Carcinoid</th>
<th>Teratoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i12p</td>
<td>12p OvR</td>
</tr>
<tr>
<td>1</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: i12p, isochromosome 12p; 12p OvR, chromosome 12p overrepresentation.

### Table 2. Immunohistochemical staining results of testicular carcinoid tumors

<table>
<thead>
<tr>
<th>Case</th>
<th>ChroA</th>
<th>Syn</th>
<th>Oct-4</th>
<th>CD30</th>
<th>c-kit</th>
<th>TTF1</th>
<th>CDX2</th>
<th>β-Cat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: ChroA, chromogranin A; Syn, synaptophysin; β-Cat, β-catenin.
References


