FOXL2 Mutations in Granulosa Cell Tumors Occurring in Males

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● Context.—Granulosa cell tumors comprise less than 5% of ovarian tumors in women and are much rarer in men, with only about 20 cases reported, to our knowledge. Recently, a somatic mutation of FOXL2 was reported in virtually all adult-type granulosa cell tumors in women.

Objective.—To investigate FOXL2 mutations in granulosa cell tumors occurring in males.

Design.—Five cases of an adult-type granulosa cell tumor from males were selected from the files of the Mayo Clinic. Nine other testicular tumors (1 juvenile granulosa cell tumor, 5 Leydig cell tumors, and 3 Sertoli-Leydig cell tumors) were evaluated for comparison.

Results.—All 5 cases had classic histopathologic features of the adult-type granulosa cell tumor. Inhibin was diffusely positive in all cases. FOXL2 402C→G (C134W) was identified in 40% (2 of 5) of the male, adult-type granulosa cell tumors. Of the 2 tumors positive for the mutation, 1 occurred in the testes of a man, and the other one affected the abdominal ovaries of a phenotypically male patient. All other testicular tumors were negative for the mutation.

Conclusions.—The FOXL2 402C→G (C134W) mutation is also present in adult-type granulosa cell tumors occurring in men, although in a smaller proportion when compared with the rates reported in women. FOXL2 mutational analysis can be a helpful in the diagnosis of granulosa cell tumors of the testis.

Clinicopathologic and Molecular Genetic Features of 5 Adult-Type Granulosa Cell Tumors (aGCTs) Occurring in Males

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age, y/Sex</th>
<th>Tumor Site</th>
<th>Tumor Size, cm</th>
<th>Histopathologic Features</th>
<th>FOXL2 Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77/M</td>
<td>R testis</td>
<td>2.5</td>
<td>aGCT with abundant luteinized cells</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>22/M</td>
<td>L testis</td>
<td>1</td>
<td>aGCT diffuse type</td>
<td>Absent</td>
</tr>
<tr>
<td>3</td>
<td>38/M</td>
<td>Abdominal ovary</td>
<td>NA</td>
<td>aGCT</td>
<td>Present</td>
</tr>
<tr>
<td>4</td>
<td>4 moa/M</td>
<td>R testis</td>
<td>NA</td>
<td>Cystic aGCT</td>
<td>Absent</td>
</tr>
<tr>
<td>5</td>
<td>40/M</td>
<td>L testis</td>
<td>2.1</td>
<td>aGCT</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not available.

The housekeeping gene β-actin was amplified in all samples to check the DNA quality, using the PCR primers as follows: 5′-ACAGACCTCCATCCCAAGAC (forward) and 5′-GAG-GCGTACGGGATAGCAC (reverse).

The PCR cycling parameters were as follows: initial denaturation at 94°C for 2 minutes, 40 cycles of denaturation at 94°C for 30 seconds, annealing at 62°C for 30 seconds, and extension at 72°C for 1 minute, followed by a final extension at 72°C for 10 minutes. The PCR products of FOXL2 (269 base pair [bp]) and β-actin (198 bp) were separated on a 3% agarose gel and visualized by ethidium bromide staining. An aliquoted FOXL2 PCR product was used for bidirectional DNA sequencing. Mutation surveyor software was used to analyze the data.

RESULTS

Five cases of aGCT arising in males were identified. Clinicopathologic features are presented in the Table. The median age at diagnosis was 35 years old (range, 4 months to 77 years). Four GCTs (80%) were located in the testis, but one patient (case 3) had a GCT in the intra-abdominal ovaries, although he was phenotypically male. Tumor sizes ranged from 1 to 2.5 cm in 3 cases (60%). In 2 cases (40%; cases 3 and 4), the size was not available, but on the glass slide, the lesions measured at least 2 cm each. All cases had classic histopathologic features of aGCTs and are illustrated in Figure 1, A through E. Cytologically, all had the typical characteristics of aGCTs and contained a homogeneous population of bland cells with regular nuclei and focal nuclear grooves. Case 1 had numerous luteinized cells. Case 2 was a diffuse-type GCT with a prominent fibrothecomatus component, and case 4 was a cystic GCT. Cases 3 and 5 showed areas of insular and diffuse growth. Inhibin immunostain was diffusely positive in all cases. Cases 3 and 5 showed areas of insular and diffuse growth. Case 2 was a diffuse-type GCT with a prominent fibrothecomatus component, and case 4 was a cystic GCT. Cases 3 and 5 showed areas of insular and diffuse growth. Inhibin immunostain was diffusely positive in all cases.

Independent of the location, the diagnosis of sex cord–stromal tumors is mostly based on microscopic, morphologic features. Most cases have typical histopathologic findings, posing little obstacle to a precise diagnosis. On the other hand, cases with unusual microscopic characteristics represent a significant diagnostic challenge. Immunohistochemistry is usually helpful in distinguishing sex cord–stromal tumors from other tumor types but often lacks the specificity to differentiate among the various subtypes of sex cord–stromal tumors. Recently, ovarian aGCTs were shown to have a point mutation in FOXL2. That gene is a transcription factor essential to the development of granulosa cells and is the earliest marker of ovarian differentiation in mammals. Various studies have confirmed the high sensitivity and specificity of that molecular finding, indicating the potential for using this alteration in the diagnosis of aGCT. This study investigated whether a similar scenario could be applied to aGCT occurring in males.

Hes et al recently reported the absence of FOXL2 402C→G in 3 testicular aGCTs and 4 incompletely differentiated, testicular, sex cord–stromal tumors. Here, we show that FOXL2 402C→G (C134W) was also present in aGCTs occurring in males. In one case, the tumor involved the testis, and in another case, the abdominal ovary of a phenotypically male patient. The latter cannot be considered a testicular tumor, yet the evidence suggests this mutation can be also found in men with female gonads, which, to our knowledge, has not been previously reported. The mutation was absent in the testicular, juvenile GCT; the Leydig cell tumors; and the Sertoli-Leydig cell tumors. Although the number of cases in this study was too small to assess the frequency, it is likely that the alteration was found in a smaller proportion of aGCTs in men, when compared with the rates reported in females. This discrepancy between the frequency of mutations found in the ovaries and the testis begs the question as to whether all the tumors currently considered to be testicular aGCTs are, indeed, aGCTs or whether they could represent another histologic subtype, either presently characterized or still unrecognized. The differentiation of aGCT from other sex cord–stromal subtypes can be difficult or impossible in some cases, both in the testis and ovaries, and misclassification in this study or the study by Hes et al cannot be dismissed, even though strict diagnostic criteria were applied. Current investigations in the ovary have been highlighting the occurrence of misclassification of aGCT, if the presence of FOXL2

DISCUSSION

Sex cord–stromal tumors comprise a heterogenous group of neoplasms that include GCTs of the juvenile and adult types, thecomas, fibromas, and tumors of Sertoli and Leydig cells in isolation or combination, among others. Granulosa cell tumors affect both the ovaries and testes. They are generally more common in the former. Various studies have also reported the absence of FOXL2 mutations in granulosa cell tumors. The presence of FOXL2 mutations in cases of granulosa cell tumors in men suggests that these tumors may be considered aGCTs.

Figure 1. Photomicrographs of granulosa cell tumors in men. Case 1 (A) had numerous luteinized cells. Case 2 (B) was a diffuse-type GCT. Cases 3 and 5 (C and E) showed areas of insular and diffuse growth. Case 4 (D) was a cystic GCT (hematoxylin-eosin, original magnifications ×100 [A through E]).
mutation is considered the diagnostic gold-standard, although this issue is still controversial.\textsuperscript{8,10} However, aberrant expression of the FOXL2 nuclear protein has been demonstrated in testicular juvenile GCTs by immunofluorescense.\textsuperscript{9} FOXL2 immunostaining was also found in ovarian, juvenile GCTs that did not correlate with the presence of FOXL2 mutations, an occurrence that can be also seen with other types of tumors.\textsuperscript{11} To our knowledge, this is the first time that mutations have been shown in a GCT occurring in males. This finding indicates that FOXL2 mutational analysis may be helpful in the diagnosis of GCTs in men and in the differential diagnosis of sex cord–stromal tumors of the testis.

References