SALL4 Is a Novel Diagnostic Marker for Testicular Germ Cell Tumors

Dengfeng Cao, MD, PhD,* Jianping Li, BS,* Charles C. Guo, MD,† Robert W. Allan, MD,‡ and Peter A. Humphrey, MD, PhD*

Abstract: The diagnosis of testicular germ cell tumors (GCTs) sometimes can be challenging without ancillary markers. Here we performed an immunohistochemical study of a novel stem cell marker SALL4 in a large series of 110 primary testicular GCTs (65 pure and 45 mixed) containing the following types of tumors and/or tumor components: 50 intratubular germ cell neoplasias (ITGCNs), 62 classic seminomas, 2 spermatocytic seminomas, 39 embryonal carcinomas (EC), 5 pediatric and 26 postpubertal yolk sac tumors (YST), 7 pediatric and 25 postpubertal teratomas, and 5 choriocarcinomas. We compared SALL4 with OCT4 in all GCTs, and SALL4 to α-fetoprotein (AFP) and glypican-3 in all YSTs. To test SALL4 specificity, 23 testicular non-GCTs (10 Leydig cell tumors, 4 Sertoli cell tumors, 3 adenomatoid tumors, 3 paratesticular rhabdomyosarcomas, 2 diffuse large B-cell lymphomas, and 1 rete testis papillary cystadenoma) and 275 nontesticular tumors were also stained for SALL4. All ITGCNs, classic seminomas, and ECs demonstrated strong SALL4 staining in more than 90% tumor cells. Of 275 nontesticular tumors, only 10 carcinomas and 1 sarcoma showed focal (<25% tumor cells) SALL4 staining, 14 (45%) show staining in less than 30% tumor cells. Our findings indicate that SALL4 is a novel sensitive and relatively specific marker for testicular GCTs. SALL4 is a more sensitive marker than AFP and glypican-3 for YST.

Key Words: SALL4, testis, germ cell tumor, yolk sac tumor, diagnostic marker

More than 90% testicular tumors are of germ cell origin. Although most are malignant, testicular GCTs are curable with modern therapy. Testicular GCTs of different histologic types differ in their clinical behavior and prognosis, and therefore are managed differently. For example, seminoma is generally treated with low-dose radiation, whereas nonseminomatous GCTs are not treated with radiation therapy. Therefore, accurate pathologic diagnosis and proper classification of testicular GCTs is critical for selecting appropriate treatment and determining prognosis. The diagnosis of testicular GCT tumor is usually straightforward based on examination of hematoxylin and eosin sections, but sometimes it can be challenging because about half of the adult testicular GCTs contain more than one type of tumor and different types of testicular GCTs often show overlapping morphologic features and can mimic one another and mimic non-GCTs. In these difficult cases, ancillary diagnostic markers are often needed.

Several diagnostic markers have been used to facilitate the diagnosis of testicular GCTs. Earlier markers included placental-like alkaline phosphatase, CD30, CD117 (C-KIT), and α-fetoprotein (AFP). Although these markers are useful for testicular GCTs, they show only moderate sensitivity and/or specificity. In addition, their expression may be lost in metastatic foci or after treatment. Recently, glypican-3 has been proposed as a new marker for yolk sac tumor (YST), but its staining is typically focal. For these reasons, novel markers are urgently needed. Recently, novel embryonic stem cell markers such as OCT4, NANOG, and SOX2 have emerged as more sensitive and specific markers for testicular GCTs. For example, OCT4 and
NANOG label seminoma and embryonal carcinoma (EC). SOX2 labels EC OCT4, NANOG, and SOX2 are sensitive and specific markers for testicular GCTs, but they are only expressed in a subset of testicular GCTs: intratubular germ cell neoplasia (ITGCN), seminoma, and EC. None of them is expressed in other types of testicular GCTs such as spermatocytic seminoma, YST, and choriocarcinoma, and thus in certain circumstances their diagnostic utility is limited.

OCT4, NANOG, and SOX2 function to maintain the pluripotency and self-renewal of embryonic stem cells. Recent studies have shown that the maintenance of the pluripotency and self-renewal of embryonic stem cells is regulated by a group of genes not only including OCT4, NANOG, SOX2 but also SALL4. Within this network, SALL4 regulates transcription of OCT4, indicating that SALL4 acts upstream of OCT4. The relationship between SALL4 and OCT4, NANOG and SOX2 suggests that SALL4 might also be a marker for testicular GCTs. The aim of this study is to investigate the potential diagnostic utility of SALL4 in a large series of 110 primary testicular GCTs (65 pure and 45 mixed). To test SALL4 specificity for testicular GCTs, 23 testicular non-GCTs and 275 nontesticular tumors from various organs and sites were also stained for SALL4. In addition, 275 nontesticular tumors from various organs and sites were also stained for SALL4: 158 metastatic carcinomas (6 head and neck squamous cell carcinomas, 8 thyroid carcinomas, 12 lung carcinomas, 8 breast carcinomas, 7 hepatocellular carcinomas, 3 cholangiocarcinoma, 2 ampullary adenocarcinomas, 10 pancreatic adenocarcinomas, 18 gastric adenocarcinomas, 15 esophageal carcinomas, 10 renal-cell carcinomas, 10 urothelial carcinomas, 12 prostatic adenocarcinomas, 18 ovarian carcinomas, 6 uterine carcinomas, 13 colonic adenocarcinomas), 12 metastatic melanomas, 11 primary and 2 metastatic mesotheliomas (5 abdominal, 8 pleural), and 72 primary and 20 metastatic sarcomas (3 primary and 4 metastatic alveolar soft part sarcomas, 6 primary and 1 metastatic angiosarcomas, 7 chondrosarcomas, 2 clear-cell sarcomas of soft tissue, 3 primary and 2 metastatic epithelioid sarcomas, 7 fibrosarcomas, 6 primary and 2 metastatic leiomyosarcomas, 5 primary and 1 metastatic liposarcomas, 9 malignant fibrous histiocytomas, 7 primary and 1 metastatic malignant peripheral nerve sheath tumors, 6 primary and 1 metastatic osteosarcomas, 2 primary and 2 metastatic primitive neuroectodermal tumors/Ewing’s sarcomas, 8 primary and 1 metastatic rhabdomyosarcomas, and 1 primary and 5 metastatic synovial sarcomas). The immunohistochemical status of SALL4 in these carcinomas and melanomas was also reported in a previous study.

Immunohistochemical Staining and Evaluation
For each case, 1 to 3 formalin-fixed paraffin-embedded tissue blocks were retrieved to generate 4μm unstained slides for immunohistochemical staining with a SALL4 monoclonal antibody (clone 6E3, dilution 1:100, Abnova Corporation, Taiwan, Republic of China). For GCTs, OCT4 immunostain was also performed with a SALL4 monoclonal antibody (clone 6E3, dilution 1:100, Abnova Corporation, Taiwan, Republic of China). Appropriate positive and negative controls were included for each run of immunostains. For both SALL4 and OCT4, only nuclear staining was considered positive. The staining was scored using the same criteria as described above. The percentage of tumor cells labeled with SALL4 or OCT4 was semiquantitatively scored as 0 (no tumor cells staining), 1≤30% tumor cells), 2+ (31% to 60%), 3+ (61% to 90%), and 4+ (>90%). For YSTs, we also performed AFP (prediluted antibody, Ventana Medical systems, Tuscon, AZ) and glypican-3 (prediluted antibody, Biomosaic, Burlington, VT) immunostain. The percentage of cells stained with AFP or glypican-3 (cytoplasmic and/or membranous) was scored using the same criteria as described above.

Statistical Analysis
Fisher exact test was used to compare the staining results of SALL4 to those of OCT4 in GCTs. Chi-Square test was used to compare SALL4 staining to that of...
AFP and glypican-3 in YSTs. A $P < 0.05$ is considered statistically significant.

RESULTS

**SALL4 in ITGCNs (N = 50)**

Among the 110 testicular GCTs, 50 have associated ITGCN on sections used for SALL4 immunohistochemical staining. All 50 ITGCNs showed strongly $4+$ SALL4 staining (Table 1, Fig. 1). All OCT4-positive ITGCN tumor cells were strongly positive for SALL4 (Fig. 1).

**SALL4 in Pure Classic Seminomas and Classic Seminoma Components (N = 62)**

All 62 classic seminomas (44 pure seminomas and 18 as a component in mixed GCTs) showed strong $4+$ SALL4 staining in tumor cells (Table 1, Fig. 2).

**SALL4 in Spermatocytic Seminomas (N = 2)**

Two spermatocytic seminomas (patient age 52 and 64 years old, respectively) were stained for SALL4. The percentage of tumor cells stained with SALL4 was 80% to 85% ($3+$) and 90% to 95% ($4+$), respectively (Table 1). The staining intensity in both cases was weak-to–moderate, similar to that in spermatogonia but weaker than that (strong) in ITGCN, classic seminoma, EC, and YST. All 3 types of cells (intermediate cells, small cells, and larger mononuclear or multinucleated cells) were positive for SALL4 (Fig. 3).

**SALL4 in Pure EC and EC Components (N = 39)**

All 39 ECs (5 pure and 34 as a component in mixed GCTs) showed strong $4+$ SALL4 staining in tumor cells (Table 1, Fig. 4).

---

**TABLE 1. Immunohistochemical Staining of SALL4 in Testicular Germ Cell Tumors**

<table>
<thead>
<tr>
<th>Tumor Type (Pure and Component)</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intratubular germ cell neoplasia (N = 50)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>50 (100%)</td>
</tr>
<tr>
<td>Classic seminoma (N = 62)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>62 (100%)</td>
</tr>
<tr>
<td>Spermatocytic seminoma (N = 2)</td>
<td>0</td>
<td>0</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
<td></td>
</tr>
<tr>
<td>Embryonal carcinoma (N = 39)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>39 (100%)</td>
<td></td>
</tr>
<tr>
<td>Pediatric yolk sac tumor (N = 7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5 (100%)</td>
<td></td>
</tr>
<tr>
<td>Postpubertal yolk sac tumor (N = 26)</td>
<td>3</td>
<td>4 (57%)</td>
<td>0</td>
<td>26 (100%)</td>
<td></td>
</tr>
<tr>
<td>Pediatric teratoma (N = 7)</td>
<td>4</td>
<td>3 (85%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Postpubertal teratoma (N = 27)</td>
<td>0</td>
<td>1 (20%)</td>
<td>3 (60%)</td>
<td>1 (20%)</td>
<td></td>
</tr>
<tr>
<td>Choriocarcinoma (N = 5)</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**FIGURE 1.** Immunohistochemical staining of SALL4 in ITGCN. ITGCN cells are characterized by large nuclei with irregular contour and prominent nucleoli, and clear cytoplasm (A1, B1) and they are strongly positive for SALL4 (A2, B2). These ITGCNs cells are confirmed by their strong OCT4 staining (A3, B3). ITGCN indicates intratubular germ cell neoplasia.
SALL4 in Pediatric Pure Yolk Sac Tumors and Yolk Sac Tumor Components (N = 5)

Among the 31 YSTs included in this study, 5 occurred in pediatric patients (mean age 17 mo, age 6 to 25 mo). Four YSTs were pure and 1 occurred in association with a mature teratoma component. The histologic patterns displayed by these 5 YSTs included solid in 2, microcystic in 5, macrocystic in 1, glandular in 5, polyvitelline vesicular in 4, and endodermal sinus pattern in 1. In all 5 YSTs, the tumor cells showed 4+ SALL4 staining in all histologic patterns (Table 1, Fig. 5). The staining intensity was strong in all histologic patterns except the glandular area in 2 of 5 cases showed mixed strong and focally weak-to–moderate staining (cells with weak-to–moderate staining accounting for less than 5% SALL4-positive tumor cells).

SALL4 in Postpubertal Pure Yolk Sac Tumors and Yolk Sac Tumor Components (N = 26)

 Twenty-six of 31 YSTs in this study were in postpubertal patients including 1 pure tumor (in a 19-year-old male) and 25 as a component in mixed GCTs (mean age 28.5 y, range: 15 to 52 y). The histologic patterns exhibited by these 26 YSTs included solid in 15, microcystic in 22, macrocystic in 1, glandular 3, papillary in 2, endodermal sinus in 3, parietal in 7, myxoid in 5, polyvesicular vitelline in 1, and hepatoid in 1. In all 26 cases the tumor cells showed 4+ (>90% tumor cells) SALL4 staining (Table 1, Fig. 5). All histologic patterns were strongly positive for SALL4 staining, but focal (<5% positive cells) weak-to–moderate staining was seen in the myxoid pattern in 2 of 5 cases and in the glandular pattern in 1 of 3 cases, respectively.

FIGURE 2. Immunohistochemical staining of SALL4 in classic seminoma. Seminoma can grow as solid (A1, A2), intertubular (B1, B2), and intratubular (C1, C2) patterns. It may also invade rete testis (D1, D2). Strong SALL4 staining is seen in more than 95% tumor cells (A3–D3). These tumor cells are also strongly positive for OCT4 (A4–D4).
SALL4 in Pediatric Pure Teratomas and Teratomatous Components (N = 7)

We included 7 pediatric teratomas including 6 pure and 1 as a component in a mixed GCT (mature teratoma and YST) (Table 1). The mean age for these patients was 2.8 years (age range: 4 mo to 8 y). Two of these 7 tumors contained immature elements (primitive neuroepithelial tissue). The immature elements in 1 of these 2 cases showed 1+ moderate SALL4 staining (10% immature elements stained) (Fig 6) and the mature elements in both cases were negative for SALL4. In the 5 tumors with only mature elements, the enteric type glands showed weak 1+ staining (1%, 1%, and 10% cells in enteric glands stained, respectively) in 3 cases including the one in association with YST (Fig. 6). Other mature elements were negative for SALL4 staining in all these 5 cases.

SALL4 in Postpubertal Pure Teratomas and Teratomatous Components (N = 27)

This study included 27 teratomas in postpubertal patients including 3 pure and 24 as a component in mixed GCTs. All 3 pure teratomas were morphologically mature. Thirteen of the latter 24 teratomas contained only mature elements (13/24); eleven contained both mature and immature elements (11/24).

Of the 3 pure mature teratomas, only one showed 1+ weak SALL4 staining in the enteric type glands (1% cells stained in these glands). Among the teratomas occurring as a component of a mixed GCT, the only mature elements showing SALL4 staining were the teratomatous glands (most were enteric-type glands, few were nonmucinous glands) in 22 of 24 (92%) cases (Fig. 6). The percentage of total teratomatous glandular epithelial cells stained with SALL4 ranged from 1% to 20% (mean 7%). Most of the SALL4-positive glands were focally stained with SALL4 in the lining epithelial cells but occasionally as many as 70% to 80% lining cells in a gland were stained with SALL4. The staining intensity was usually weak except in 1 case the nonmucinous glands showed focal strong staining (Fig. 6).

As mentioned above, immature elements were identified in 11/24 teratomas occurring as a component of mixed GCTs. They contained 2 types of immature tissue: primitive neuroepithelial tissue with blastema-like mesenchyme (n = 5) and low-grade immature mesenchyme (n = 11). The primitive neuroepithelial tissue and blastema-like stroma showed 1+ weak to focally strong SALL4 staining in 4 of 5 cases (percentage of SALL4 positive cells in immature elements is approximately 20% in 3 cases and 1% in 1 case) (Fig. 6). The low-grade mesenchyme showed 1+ weak SALL4 staining in 7 of these 11 cases (percentage of cells stained with SALL4 ranges from 1% to 5%, mean 4%).

FIGURE 3. Immunohistochemical staining of SALL4 in spermatocytic seminoma (A1, B1). The staining intensity of SALL4 in spermatocytic seminoma (A2, B2) is similar to that of adjacent normal spermatogonia (B2). Few primary spermatocytes also show dot-like staining (B2). All 3 types of tumor cells (small, intermediate, and large) are stained with SALL4 (A2, B2). In contrast, all tumor cells are negative for OCT4 (A3, B3).
FIGURE 4. Immunohistochemical staining of SALL4 in embryonal carcinoma (A1 papillary, B1 glandular, C1 solid, and D1 intratubular growth pattern). Strong SALL4 staining is seen in more than 95% tumor cells (A2–D2). These tumor cells are also strongly positive for OCT4 (A3–D3). Rarely embryonal carcinoma grows intimately with yolk sac tumor in a double-layered pattern (E1), in which yolk sac tumor grows beneath the embryonal carcinoma. Both embryonal carcinoma and yolk sac tumor components are strongly positive for SALL4 (E2) but only embryonal carcinoma is positive for OCT4, whereas yolk sac tumor is not (E3).
SALL4 in Choriocarcinomatous Components (N = 5) and Scattered Syncytiotrophoblast in Other Types of GCTs (N = 13)

Choriocarcinoma was identified as a component in 5 mixed GCTs. Mononucleated trophoblastic cells were positive for SALL4 in all 5 cases: 1+ (1% to 2% tumor cells) in 1, 3+ in 3, and 4+ in 1 (Table 1, Fig. 7). The staining intensity was moderate in 2 of 5 cases, moderate with focally strong in 1, and weak in the remaining 2 cases (Fig. 7). Syncytiotrophoblastic cells were negative for SALL4 in all 5 cases (Fig. 7). In addition, scattered syncytiotrophoblastic cells were identified in 13 nonchoriocarcinomatous GCTs and all of them were negative for SALL4 staining (Fig. 7).

SALL4 in Primary Testicular Non-GCTs

All 10 Leydig cell tumors, 4 Sertoli cell tumors, 3 adenomatoid tumors, 3 paratesticular rhabdomyosarcomas, 2 diffuse large B-cell lymphomas, and 1 rete papillary cystadenoma were negative for SALL4 staining.

SALL4 in Nontesticular Tumors

Two hundred seventy-five nontesticular tumors from various organs and sites including 158 metastatic carcinomas, 12 metastatic melanomas, 13 mesotheliomas (11 primary and 2 metastatic) and 92 sarcomas (72 primary and 20 metastatic) were also stained with SALL4. SALL4 result on the carcinoma and melanoma staining has been previously reported.6 Of all these
tumors, only 10 (6 esophageal, 3 gastric, and 1 colonic) metastatic carcinomas and 1 primary sarcoma (malignant fibrous histiocytoma) showed 1+ weak nuclear SALL4 staining. The mean percentage of tumor cells positive for SALL4 was 10% (range: 0.5% to 25%) in these 10 carcinomas and 5% to 10% in the sarcoma.

SALL4 in Non-neoplastic Testicular Tissue

The non-neoplastic seminiferous tubules were present on the sections for SALL4 staining in 50 cases including 9 pediatric testes and 41 postpubertal testes. Positive SALL4 staining was observed in spermatogonia in all 50 testes (both pediatric and postpubertal) (Fig. 8). The staining intensity in most of spermatogonia was weak-to–moderate (Fig. 8) but rarely spermatogonia showed moderate-to–strong SALL4 staining (still weaker than that in neoplastic cells). In addition, a few (<5%) primary spermatocytes showed dot-like weak SALL4 staining (Fig. 3). Secondary spermatocytes, spermatids, spermatozoa, and Sertoli cells were negative for SALL4. Leydig cells, rete testis, epididymis, spermatic cord, fibroblasts, blood vessels, and hematopoietic cells showed no staining for SALL4. OCT4 was negative in all components of non-neoplastic testicular tissue.

Comparison of SALL4 Stain to That of OCT4 in Testicular GCTs

To compare SALL4 with OCT4, we performed immunostain for OCT4 in all testicular GCTs. Table 2
summarized the comparison of SALL4 result to that of OCT4. OCT4 was also 4+ strongly positive in all ITGCNs, classic seminomas and ECs. No OCT4 staining was observed in any spermatocytic seminoma, YST, teratoma, or choriocarcinoma.

Comparison of SALL4 to α-fetoprotein and Glypican-3 in Yolk Sac Tumors

To compare SALL4 with currently used YST markers AFP and glypican-3, we performed AFP and glypican-3 immunostains for all 31 YSTs. Twenty-nine of 31 (94%) YSTs were positive for AFP including 1+ in 14 cases, 2+ in 7 cases, and 3+ in 8 cases (Table 3, Fig. 5). No YST showed 4+ AFP staining (>90% tumor cells). Two of 31 cases (6%) were negative for AFP. There was often a high background staining in AFP staining. All 31 YSTs (100%) showed glypican-3 staining including 1+ in 14 (45%), 2+ in 5 (16%), 3+ in 7 (23%), and 4+ in 5 (16%) (Table 3, Fig. 5). Of the 14 (45%) YSTs showing only 1+ glypican-3 staining, 4 (2 pure, 2 as a component in mixed GCTs) showed staining in less than 5% tumor cells. In contrast to AFP and glypican-3, SALL4 staining in more than 90% tumor cells was seen in all 31 YSTs (100% sensitivity) (Table 3, P < 0.0001 for both SALL4 vs. AFP and SALL4 vs. glypican-3).

DISCUSSION

SALL4 is a zinc finger transcription factor that shares homology to the Drosophila spalt (sal) gene. In Drosophila sal acts as a region-specific homeotic gene with an important role in the specification of head and tail regions during embryonic development. In the mouse, SALL4 is essential to early embryogenesis and homozygous mutant mice exhibit early embryonic lethality. SALL4 forms a regulatory circuit with OCT4, NANO1, and SOX2 to maintain embryonic stem cell pluripotency and self-renewal. In this self-stabilizing network SALL4 regulates OCT4 transcription, suggesting action upstream of OCT4. In this study, we have shown that by immunohistochemical staining SALL4 protein was detected in all types of testicular GCTs. In contrast, OCT4 only labeled ITGCNs, classic seminoma, and EC, as observed by us and others. Therefore SALL4 had a broader expression than OCT4 in testicular GCTs. In embryonic stem cells SALL4 acts upstream of OCT4, it is unknown whether similar relationship exists in testicular GCTs.

Our findings have clearly demonstrated diagnostic utility of SALL4 for testicular GCTs. In the current study, SALL4 stained more than 90% tumor cells in all ITGCNs, classic seminomas, and YSTs (both pediatric and postpubertal). In 2 spermatocytic seminomas, approximately 80% to 85% and 90% to 95% tumor cells were stained with SALL4, respectively. Therefore SALL4 demonstrated 100% sensitivity for ITGCNs, seminomas (classic and spermatocytic), and ECs. In addition, SALL4 also stained some (27 of 34) teratomas and the mononucleated trophoblastic cells in all 5 choriocarcinomas. In contrast, all 23 testicular non-GCTs showed no SALL4 staining. Among 275 nontesticular tumors (158 metastatic carcinomas, 12 metastatic melanomas, 11 primary and 2 metastatic mesotheliomas, 72 primary and 20 metastatic sarcomas) from various organs and sites, only 1 carcinoma and 1 sarcoma showed weak SALL4 staining in less than 25% of tumor cells. In the literature, the only other types of tumors that have been reported to show positive SALL4 staining were precursor B-cell lymphoblastic lymphoma and acute myeloid leukemia, whereas other types of hematopoietic tumors were negative for SALL4. All these indicate that SALL4 is a novel sensitive diagnostic marker for testicular GCTs especially for ITGCN, classic and spermatocytic seminomas, EC, and YST with relatively high specificity (96.3% if using any tumor cells stained with SALL4 as the cutoff, 100% if using at least 25% tumor cells stained with SALL4 as the cutoff in our study). In a previous study, we have demonstrated that...
all 22 metastatic seminomas, 20 metastatic ECs and 7 of 8 metastatic YSTs from testis showed strong SALL4 staining in more than 90% tumor cells. The remaining 1 metastatic YST showed strong SALL4 staining in 80% tumor cells.

Therefore the high (100%) sensitivity of SALL4 for seminoma, EC and YST not only holds for their primary forms within testis but also for their metastatic forms in extragonadal sites.6

FIGURE 8. Immunohistochemical staining of SALL4 in non-neoplastic testicular tissue. Spermatogonia both in children (A1) and postpubertal males (B1) are weakly to moderately positive for SALL4 stain (A2, B2). The SALL4 staining intensity in spermatogonia is less intense or weaker than that in adjacent intratubular germ cell neoplasia cells (C1 H&E; C2 SALL4 stain) and infiltrating seminoma cells (D1 H&E; D2 SALL4 stain). Spermatogonia are negative for OCT4 stain (A3–D3). H&E indicates hematoxylin and eosin.

1074 | www.ajsp.com © 2009 Lippincott Williams & Wilkins
Besides its role in distinguishing a GCT from a non-GCT, SALL4 is also useful for classifying difficult GCTs. For example, it is sometimes difficult to distinguish glandular EC from glandular YST and glandular YST from glandular teratoma. A panel including SALL4, OCT4, and epithelial membrane antigen (EMA) will help ease the difficulty. The glands in EC will be SALL4+/OCT4+/EMA−, whereas those in YST will be SALL4+/OCT4−/EMA−. Teratomatous glands are typically positive for EMA but negative for OCT4. SALL4 staining in teratomatous glands is typically weak if present. Another example is that morphologically “atypical” seminoma might mimic the solid variant of EC and YST. In this scenario SALL4 clearly also demonstrates its utility in distinguishing them when it is used in conjunction with other markers such as OCT4 and CD30. Seminoma has a profile characterized by SALL4+/OCT4+/CD30−, whereas EC is positive for all these 3 markers. In contrast, YST is positive for SALL4 but negative for OCT4 and CD30.

Compared with other types of testicular GCTs, YST is notoriously for displaying multiple histologic patterns mimicking many other types of tumors, and thus pose the greatest diagnostic challenges. Current markers for YST include AFP and glypican-3, but both are lack of adequate sensitivity. Here, we compared SALL4 with AFP and glypican-3 in all 31 YSTs. Positive AFP staining was seen in 94% (29 of 31) of YSTs, in keeping with that (range: 50% to 100%) reported in the literature. However, as observed by others, we also found that AFP staining in YSTs was typically focal and patchy: 1+ (<30% tumor cells) staining in 14 (45%) YSTs including 7 with no more than 10% tumor cells stained. In our study, all 31 YSTs showed positive glypican-3 staining, similar to that reported in 2 previous studies. However, 1+ (<30% tumor cells) glypican-3 staining was seen in 14 of our 31 (45%) YSTs. In the 2 previous studies, positive glypican-3 staining in less than 50% tumor cells was seen in 50% and 12% YSTs, respectively. These findings indicate that although AFP and glypican-3 are good markers for YST, the fact that their staining was seen in less than 30% tumor cells in nearly half of YSTs limit their diagnostic utility in certain conditions such as limited biopsy material in metastatic sites. Furthermore, AFP and glypican-3 are not specific for YST, either. AFP was often positive in teratomatous enteric type glands. Glypican-3 also labeled EC, choriocarcinoma, and immature teratoma. In contrast to AFP and glypican-3, SALL4 strongly stained more than 90% tumor cells in all 31 YSTs. Even in metastatic sites, such high sensitivity of SALL4 with staining in more than 80% to 90% tumor cells was still maintained for metastatic testicular YST. Although SALL4 is not entirely specific for YST; our findings do indicate that SALL4 is a more sensitive marker than AFP and glypican-3 for YST.

Besides demonstrating diagnostic utility of SALL4 in testicular GCTs, our findings that SALL4 was expressed in all types of GCTs, both in pediatric and postpubertal patients, might help shed some light on the pathogenesis of testicular GCTs. Testicular GCTs can be divided into 3 genetically distinct groups: teratoma and YST in neonates and infants, classic seminoma and nonseminomatous GCTs in postpubertal patients, and spermatocytic seminoma in old patients. Gene imprinting study has suggested that the neoplastic transformation of pediatric GCTs (teratoma and YST) initiates during germ cell migration, whereas this occurs at a later stage in adult testicular GCTs. Spermatocytic seminoma is thought to originate in primary spermatocyte.

### Table 2. Comparison of SALL4 Immunohistochemical Staining to That of OCT4 in Testicular Germ Cell Tumors

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>SALL4 Positive</th>
<th>OCT4 Positive</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intratubular germ cell neoplasia (N = 50)</td>
<td>50 (100%)</td>
<td>50 (100%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Classic seminoma (N = 62)</td>
<td>62 (100%)</td>
<td>62 (100%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Spermatocytic seminoma (N = 2)</td>
<td>2 (100%)</td>
<td>0</td>
<td>0.33 (but only 2 cases)</td>
</tr>
<tr>
<td>Embryonal carcinoma (N = 39)</td>
<td>39 (100%)</td>
<td>39 (100%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Pediatric yolk sac tumor (N = 5)</td>
<td>5 (100%)</td>
<td>0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Postpubertal yolk sac tumor (N = 26)</td>
<td>26 (100%)</td>
<td>0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pediatric teratoma (N = 7)</td>
<td>4 (57%)</td>
<td>0</td>
<td>0.02</td>
</tr>
<tr>
<td>Postpubertal teratoma (N = 27)</td>
<td>23 (85%)</td>
<td>0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Choriocarcinoma (N = 5)</td>
<td>5 (100%)</td>
<td>0</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

### Table 3. Comparison of Immunohistochemical Staining of SALL4 to Those of AFP and Glypican-3 in 31 Testicular Yolk Sac Tumors

<table>
<thead>
<tr>
<th>SALL4 Staining</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SALL4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>31 (100%)</td>
<td>&lt;0.0001 (SALL4 vs. AFP)</td>
</tr>
<tr>
<td>AFP</td>
<td>2 (6%)</td>
<td>14 (45%)</td>
<td>7 (23%)</td>
<td>8 (25%)</td>
<td>0</td>
<td>&lt;0.01 (SALL4 vs. OCT4)</td>
</tr>
<tr>
<td>Glypican-3</td>
<td>0</td>
<td>14 (45%)</td>
<td>5 (16%)</td>
<td>7 (23%)</td>
<td>5 (16%)</td>
<td>&lt;0.001 (SALL4 vs. glypican-3)</td>
</tr>
</tbody>
</table>
patients ITGCN is considered the precursor to classic seminoma and nonseminomatous GCTs, whereas pediatric YST and teratoma are thought not to originate from ITGCN but from either embryonic stem cells or early migrating primordial germ cells.\textsuperscript{1,2,42} One of the key events in the malignant transformation of germ cells is the block in germ cell differentiation. It has been proposed that dysregulation of embryonic pluripotency genes might play some role in this process.\textsuperscript{35,36} In this study, we have demonstrated SALL4 protein in all 3 types of GCTs that arise from different mechanisms at different stages of germ cell development, suggesting that dysregulation of SALL4 might be a common factor in the pathogenesis of all 3 types of testicular GCTs. Of interest, the protein expression level of SALL4 (evidenced by semiquantitative immunohistochemical staining) varied in different types of GCTs and seemed to correlate with the degree of tumor differentiation. For example, the staining intensity of SALL4 in the more poorly differentiated tumors such as ITGCN, classic seminoma, EC, and YST (both pediatric and postpubertal patients) was diffuse and strong (stronger than that in normal spermatogonia), whereas in the more differentiated tumors such as teratomas and spermatocytic seminomas the staining was focal and/or weak (weaker than that in normal spermatogonia, stronger in immature elements than mature elements). These observations suggest that SALL4 might be important for these tumors to maintain their poorly differentiated status. Previous studies have shown that depletion of SALL4 targets in embryonic stem cells and extraembryonic endoderm cells results in differentiation.\textsuperscript{22} In addition, it is also possible that SALL4 might be also involved in the apoptotic pathways by preventing apoptosis in testicular GCTs to maintain tumor growth as its does in acute leukemia.\textsuperscript{54} Further studies to investigate SALL4 in testicular GCTs will be helpful for us to better understand its possible role in these tumors.

Finally, besides having a broader expression profile in testicular GCTs than OCT4, SALL4 also differs from OCT4 in that SALL4 is expressed in normal spermatogonia in both in children and postpubertal males and a few primary spermatogonia in postpubertal testis. In contrast, OCT4 is not expressed in any germ cells after 3 to 4 months of postnatal age (the final differentiation of gonocytes into infantile spermatogonia).\textsuperscript{37} It is unknown, what mechanism turns off SALL4 expression as spermatogonia differentiate into more mature germ cells. However, our finding does raise an interesting question: does SALL4 play a role in self-renewal of spermatogonia, both in children and adults, as it does in embryonic stem cells?\textsuperscript{49,52,53,55,56} In human testis, type A spermatogonia are thought to be stem cells in renewal of spermatogonia and in spermatogenesis.\textsuperscript{30,32,33} Our study is the first one to demonstrate SALL4 protein in normal spermatogonia and testicular GCTs, so further studies to explore this aspect of SALL4 function would be interesting.

In summary, we investigated the expression of a novel stem cell marker SALL4 by immunohistochemistry in a large series of 110 primary testicular GCTs, 23 testicular non-GCTs, and 275 non-testicular tumors. Our results indicate that SALL4 is a novel sensitive and relatively specific diagnostic marker for testicular GCTs.

**REFERENCES**


