Expression of Glypican 3 in Ovarian and Extragonadal Germ Cell Tumors

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Abstract

Germ cell tumors (GCTs), rare malignancies that occur in a wide range of locations and display variable histologic patterns, may pose diagnostic challenges. Glypican 3 (GPC3), a membrane-bound heparan sulfate proteoglycan, has been shown to be a novel diagnostic marker in testicular GCT. However, GPC3 expression in ovarian and extragonadal GCT has not been reported. We evaluated GPC3 immunoreactivity in GCTs from 63 patients (57 children and 6 adults), including 14 ovarian and 20 extragonadal primary GCTs and 8 metastases along with 21 primary testicular GCTs for comparison. All 33 yolk sac tumors (YSTs) and both choriocarcinomas were immunoreactive for GPC3. In contrast, a minority of immature (4/10) and mature (4/35) teratomas were positive. No positivity was seen in 6 embryonal carcinomas or 5 germinomas. GPC3 is differentially expressed in ovarian and extragonadal GCTs, with expression predominantly observed in YSTs and choriocarcinoma.

Germ cell tumors (GCTs) are a histologically diverse entity that can occur in a wide variety of locations and age groups. The majority of GCTs have a gonadal origin and comprise a large portion of tumors of the testicles and ovaries, with a 95% and 30% prevalence of neoplasms in these organs, respectively.[1,2] However, a small number of these lesions can be extragonadal, reported as between 1% and 5%.[3,4] The mediastinum is the most common site outside the gonads for GCT, with lesions in the retroperitoneum, pineal gland, and sacral region constituting most other areas affected.[1,3,4] Despite their location, these neoplasms are speculated to have a similar histogenesis and are thought to be derived from surviving germ cell precursors sequestered during developmental migration.[4] Although their origins are similar, GCTs have different prognoses and treatments based on histologic subtype, tumor location, and age group.[4]

GCTs are divided into histologic subtypes based on differentiation and range from totipotent to well-differentiated.[5] Germinoma, including dysgerminoma and seminoma, is undifferentiated and considered to give rise to the more differentiated subtypes. Embryonal carcinoma (EC) is a poorly differentiated, primitive-appearing subtype. Yolk sac tumor (YST) and choriocarcinoma (CC) are subtypes with extraembryonic differentiation. Mature teratoma (MT) and immature teratoma (IT) are differentiated subtypes that resemble adult or fetal tissue. Because the subtypes can often be admixed, the diagnosis of GCT and identification of individual components can be challenging.[2,4,5] In particular, YST has a wide variety of growth patterns that can be misinterpreted,[2,4,5] which is especially problematic because YST is often intermixed with teratomatous components and when overlooked in the prepubescent population can mistakenly lead to a benign

diagnosis.[2,4] Therefore, an immunohistochemical marker that reliably highlights YST and ideally distinguishes it from other components would be beneficial.

Recently, microarray data identified GPC3 as a marker that was overexpressed in YST compared with other testicular GCT components.[6] Similarly, GPC3 was reported to have high immunohistochemical expression in adult testicular YST.[7,8] However, GPC3 has not been reported in GCTs from other organs or in the pediatric age group. As such, we sought to investigate whether GPC3 could detect YSTs originating in ovarian and various extragonadal locations in the adult and pediatric populations and in metastatic lesions.

Materials and Methods

Archived, formalin-fixed, paraffin-embedded tissue blocks from 57 pediatric and 6 adult patients with GCT accessioned between 1976 and 2006 were obtained from the surgical pathology files of Northwestern Memorial Hospital, Chicago, IL (6 cases) and Children's Memorial Hospital of Chicago (57 cases). The 63 cases included 20 extragonadal (8 central nervous system, 6 sacrococcygeal, 3 mediastinal, 1 retroperitoneal, 1 kidney, and 1 stomach), 14 ovarian, and 21 testicular primary GCTs and 8 metastases (7 lymph node and 1 lung). The 63 cases of GCT consisted of the following histologic subtypes: YST, 33; MT, 35; IT, 10; EC, 6; germinoma, 5; and choriocarcinoma, 2 **Table 1**. (Note that many cases contained more than 1 histologic subtype, and therefore column totals in Table 1 do not equate with the number of cases.) MT histologically closely resembled normal adult tissue and included squamous, glandular, and respiratory epithelium, hyaline cartilage, and bone. IT was narrowly defined as areas composed of neuroepithelium.

Sections (5 μm) from 1 representative block from each case were deparaffinized, rehydrated in graded alcohols, and subjected to heat-induced epitope retrieval in 0.1 mol/L of citrate buffer at pH 6.0 in a microwave for 20 minutes. The slides were then incubated with a primary monoclonal antibody specific for GPC3 (Biomosaics, Burlington, VT) with a dilution of 1:200 for 1 hour at room temperature. After incubation with rabbit antimouse secondary antibody, a subsequent reaction was performed with the biotin-free horseradish peroxidase enzyme–labeled polymer of EnVision+ detection system (DAKO, Carpinteria, CA). 3,3′-diaminobenzidine was used as the chromogen (DAKO), and the sections were counterstained with hematoxylin.

Each histologic subtype was independently analyzed for cytoplasmic and membranous staining. Immunoreactivity was semiquantitatively evaluated as negative (0, <5% of cells stained), focally positive (1+, 5%-10% of cells stained), positive (2+, 11%-50% of cells stained), or diffusely positive (3+, >50% of cells stained). Staining intensity for each subtype was graded from 0 to 3+, and a mean intensity was calculated. Adjacent benign tissue, present in sections from 31 cases, was also assessed for the percentage of cells stained and strength of staining. Placental tissue was used as a positive control sample.

Results

Although previous studies focused on adult testicular GCTs, the cohort in this study was composed predominantly of pediatric patients.[7,8] The age of patients ranged from 2 days to 34 years (mean, 8.7 years).

Immature and mature placental tissue, used as a positive control sample, demonstrated strong GPC3 staining in syncytiotrophoblasts with weaker staining in cytotrophoblasts. Benign tissue adjacent to tumor, including testicle, ovary, skeletal muscle, adipose, and stomach, was negative. Only benign kidney showed weak 1+ expression of GPC3 within renal tubules.

All YST components had GPC3 staining (33/33 [100%]), with 32 cases positive or diffusely positive (3+, 28 [85%]; 2+, 4 [12%]) and 1 focally positive (1+ [3%]) **Image 1A** and **Image 1B**. The border between YST and other histologic components, including germinoma, EC, MT, and IT, was sharp and well-defined. All cases exhibited strong

Table 1
Histologic Subtypes of Germ Cell Tumors

	YST	Choriocarcinoma	IT	MT	EC	Germinoma
Ovary	4	0	4	9	0	3
Testis	16	1	3	9	5	1
Extragonadal						
Sacrococcygeal	5	0	2	5	0	0
Central nervous system	2	0	0	6	1	0
Other	2	0	1	4	0	0
Metastases						
Lymph node	4	0	0	2	0	1
Lung	0	1	0	0	0	0
Total	33	2	10	35	6	5

EC, embryonal carcinoma; IT, immature teratoma; MT, mature teratoma; YST, yolk sac tumor.

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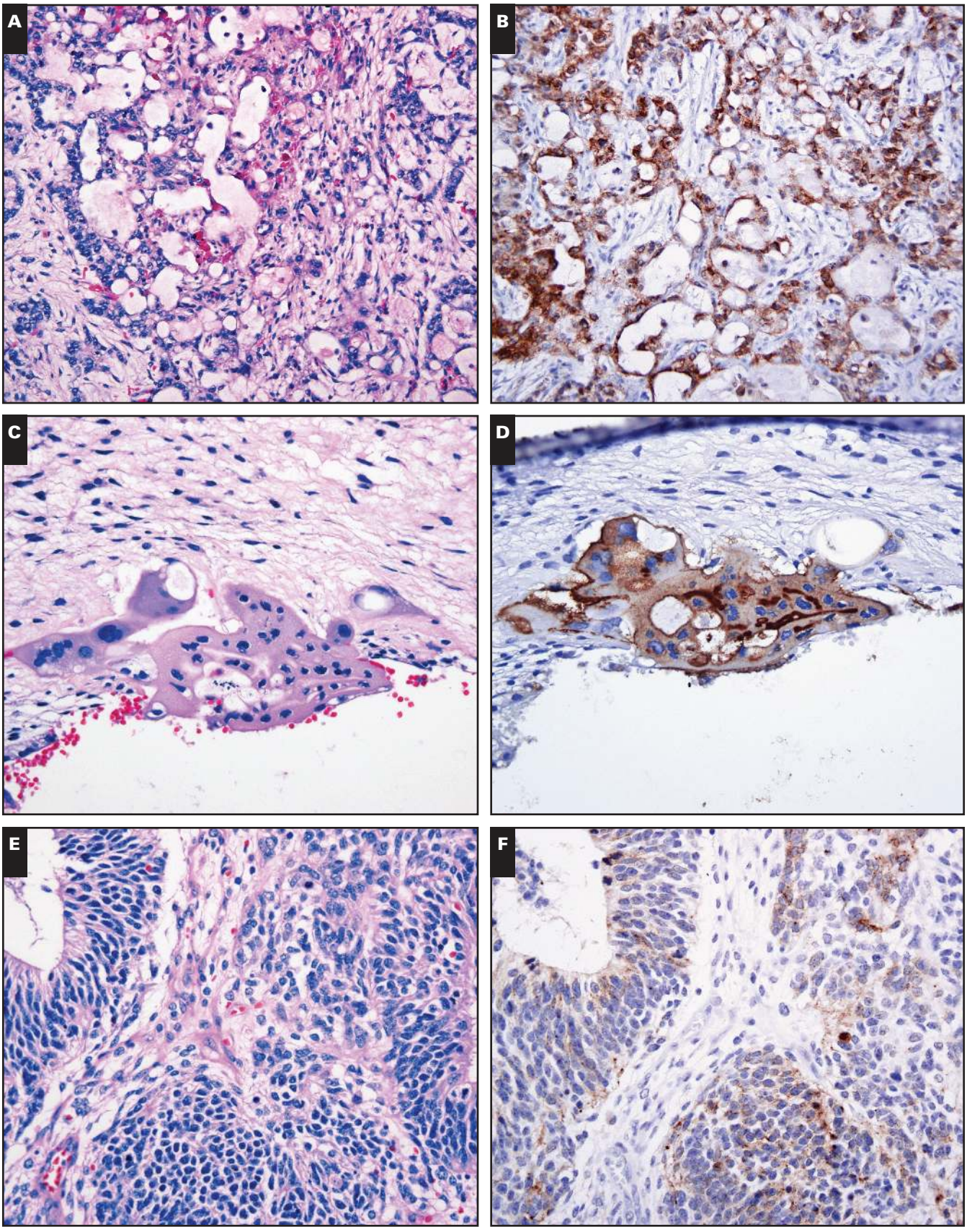

Image 1 Glypican 3 (GPC3) immunohistochemical analysis in germ cell tumor subtypes. **A** and **B**, Yolk sac tumor. Strong and diffuse reactivity (**A**, H&E, ×20; **B**, GPC3 immunostain, ×20). **C** and **D**, Choriocarcinoma. Strong positivity within syncytiotrophoblasts (**C**, H&E, ×40; **D**, GPC3 immunostain, ×40). **E** and **F**, Immature teratoma. Weak reactivity within primitive neural elements (**E**, H&E, ×20; **F**, GPC3 immunostain, ×20).

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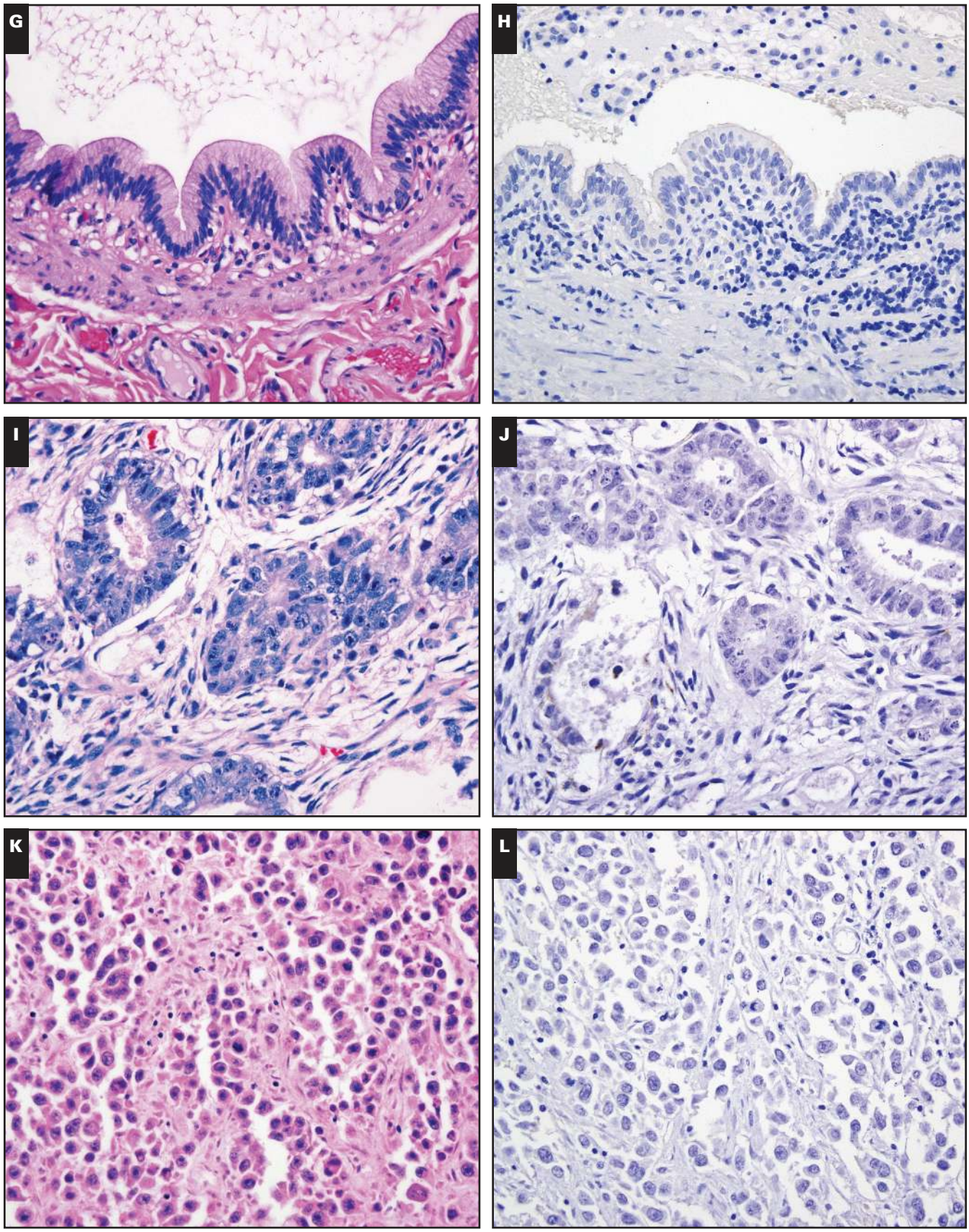

G and **H**, Mature teratoma. No staining seen within mature glandular epithelium (**G**, H&E, ×20; **H**, GPC3 immunostain, ×20). **I** and **J**, Embryonal carcinoma in a pseudoglandular arrangement (**I**, H&E, ×40) and negative GPC3 immunostain (**J**, ×40). **K** and **L**, Germinoma (**K**, H&E, ×20) and negative GPC3 immunostain (**L**, ×20).

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or moderate staining intensity with a mean intensity of 2.9. YST was examined for different histologic growth patterns. Patterns of YST included microcystic (32), macrocystic (21), endodermal sinus (19), solid (12), papillary (5), polyvesicular vitelline (3), and glandular-alveolar (3), all of which showed uniform, similar GPC3 positivity.

Choriocarcinoma, present in 2 cases, was also positive (3+ [50%]; 2+ [50%]) **Image 1C** and **Image 1D**. Strong staining (2.5) was observed in malignant syncytiotrophoblasts and weaker staining in cytotrophoblasts, similar to staining in benign placental tissues.

Of the 10 cases containing IT, defined as neuroepithelium, 6 (60%) were negative and 4 cases were positive (3+, 1 [10%]; 2+, 3 [30%]) **Image 1E** and **Image 1F**. Staining in the positive cases was predominantly weak, and the overall mean intensity was 0.9. Of 35 MTs, 31 were negative with 4 cases showing positivity (2+, 1 [3%]; 1+, 3 [9%]) **Image 1G** and **Image 1H**. The elements positive in these 4 cases were primitive-appearing spindle cells (4 cases) and columnar epithelium (2 cases). The 6 cases (100%) with EC **Image 1I** and **Image 1J** and the 5 cases (100%) with germinoma were negative **Image 1K** and **Image 1L**. **Table 2** summarizes the results of the immunohistochemical stains.

Discussion

Glypicans are a family of heparan sulfate proteoglycans that are attached to the exocytoplasmic cell surface by a glycosylphosphatidylinositol anchor. Six glypicans have been identified in mice and humans, of which GPC3 is located on Xq26.[9,10] Because of the high level of expression mostly in fetal tissue, GPC3 is theorized to function in the regulation of growth and differentiation, with data revealing interactions with Wnts, hedgehogs, and bone morphogenic proteins.[11-14] Mutations in *GPC3* cause Simpson-Golabi-Behmel syndrome, a rare X-linked disorder characterized by prenatal and postnatal overgrowth, numerous developmental abnormalities, and an increased risk for embryonal malignancies, including Wilms tumor, neuroblastoma, gonadoblastoma, hepatoblastoma, and hepatocellular carcinoma.[15-17]

The role of GPC3 in tumor formation is controversial. Experimental data using tumor cell lines describe silencing of GPC3 within ovarian, mesothelial, lung, and breast carcinoma tissue consistent with a tumor suppressor function.[18-22] However, studies using tumoral tissue have detected increased expression. In tumors with increased expression, GPC3 is thought to act as an oncofetal protein.[23-26] The previously reported expression of GPC3 in testicular GCT seems to be a part of the latter category because no expression was observed in adult benign testicular tissue.[7,8] Immunohistochemical studies are limited and will need to be extended to further define the expression of GPC3 in normal and tumoral tissue. Currently, immunohistochemical studies describe high expression of GPC3 in almost all hepatocellular carcinomas, hepatoblastomas, testicular YSTs, and testicular choriocarcinomas and approximately two thirds of ovarian clear cell carcinomas.[7,8,23,27-29]

YST is a frequently overlooked subtype of GCT.[5,30] Small islands of YST often grow closely with other subtypes, such as EC and MT, and may not be seen.[5,30,31] Distinguishing between YST and EC may have particular clinical significance because the presence or an increased percentage of EC is associated with initial metastases and recurrences, and lack of YST has been correlated with more relapses.[32-36] Because of these issues, an immunostain that is positive in YST and negative in other histologic subtypes would be useful and is not currently available. In our study, GPC3 was consistently positive in all cases of YST tested, in gonadal and extragonadal locations and in adult and pediatric age groups in a manner similar to adult testicular GCT. GPC3 stained more than 50% of tumor cells and had a strong intensity in the majority of cases. The various growth patterns of YST showed similar staining. The boundary between YST and germinoma, EC, and MT was sharp with little to no background seen. These features allowed easy interpretation of the immunostain, and GPC3 reliably distinguished YST from germinoma, EC, and MT in almost all cases.

Owing to the numerous growth patterns, YST can be difficult to identify.[4] Specifically, endodermal sinus and papillary YST resemble papillary EC, and discerning YST from EC may be important because an increased proportion

Table 2
Glypican 3 Immunoreactivity in Germ Cell Tumor Subtypes

Score (Percentage of Cells Stained)	YST	Choriocarcinoma	IT	MT	EC	Germinoma
3+ (>50)	28 (85)	1 (50)	1 (10)	0 (0)	0 (0)	0 (0)
2+ (11-50)	4 (12)	1 (50)	3 (30)	1 (3)	0 (0)	0 (0)
1+ (5-10)	1 (3)	0 (0)	0 (0)	3 (9)	0 (0)	0 (0)
0 (<5)	0 (0)	0 (0)	6 (60)	31 (89)	6 (100)	5 (100)
Total No.	33	2	10	35	6	5
Mean intensity	2.9	2.5	0.9	0.3	0	0

EC, embryonal carcinoma; IT, immature teratoma; MT, mature teratoma; YST, yolk sac tumor.

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of EC is suggested to have a worse prognosis.[32,33] Sometimes α-fetoprotein is used to highlight YST, although its positivity is highly variable, and up to a third of ECs can be positive for this marker.[33,37,38] As such, authors report that α-fetoprotein is not a reliable marker for YST.[39] We demonstrated that GPC3 is a reliable marker for YST and does not stain EC, similar to results in testicular GCT.[7,8] In addition, some patterns of YST, such as enteric, glandular-alveolar, and polyvesicular vitelline, and small areas of microcystic YST can be mistaken for MT.[2,4,40] Recognizing small foci of YST in a background of MT can mean the difference between a benign and a malignant diagnosis, particularly in the prepubescent population, for which MT is often seen in conjunction with YST.[4,40] Currently, no marker differentiates YST from MT. In this study, we found that approximately 90% of MTs were negative for GPC3 and, in most cases, GPC3 differentiated MT from YST. Last, solid YST can appear similar to germinoma, although identifying YST in a background of germinoma is important because the treatment modalities differ for pure germinoma vs mixed GCT.[2] In our study, GPC3 distinguished these 2 subtypes because all cases of YST were positive and germinoma was consistently negative, similar to previous testicular GCT data.[7,8] Future studies are needed to compare GPC3 with cytokeratin AE1/AE3 for differentiating YST from germinoma.

A relationship between GPC3 expression and level of differentiation was demonstrated in our study in multiple organs. The least differentiated components of GCT, germinoma and EC, did not have any expression of GPC3, whereas the extraembryonic subtypes, YST and choriocarcinoma, expressed GPC3 in all cases examined. Most MTs were negative, whereas occasional ITs had weak expression of GPC3. Because all components of GCT are thought to be derived from a common cell of origin, our results suggest that GPC3 may be involved in extraembryonic lineage-specific differentiation. These findings are similar to those we previously described in testicular GCT and further confirm the possible importance of GPC3 in a similar differentiation pathway of GCT throughout the body.[7]

Our data showing that GPC3 is highly expressed in primary and metastatic YST from a variety of anatomic locations support the clinical usefulness of this marker. Future research is needed to assess the sensitivity of GPC3 in comparison with other currently used markers. Because of the consistent high expression, our data suggest that GPC3 has the potential to be a serum marker for gonadal and extragonadal GCT. In cell culture, GPC3 has been shown to be a secreted protein.[41] A serum marker for GPC3 has been developed and is commercially available. Detection of GPC3 in the serum has been shown to be more sensitive and specific in identifying hepatocellular carcinoma than α-fetoprotein, which is promising for its possible use in diagnosing or monitoring YST.[42]

We demonstrated increased expression of GPC3 in ovarian and extragonadal GCT and in pediatric testicular GCT, with highest expression in YST and choriocarcinoma components. GPC3 distinguished YSTs from most germinomas, ECs, and MTs, which is a feature unique to this marker. Based on our findings, GPC3 seems to have diagnostic value for identifying not only testicular but also ovarian and extragonadal GCTs in the pediatric and adult populations.

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