Expression of Glial Cell Line–Derived Neurotropic Factor Receptor $\alpha$-1 in Immature Teratomas

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Abstract

The immaturity of teratomas is usually manifested as immature neuroepithelium. The amount of immature neuroepithelium has been correlated with the survival of adult patients with ovarian immature teratoma. To date, no immunohistochemical marker has been found to facilitate the identification of immature teratoma. In this study, we evaluated the expression of glial cell line–derived neurotropic factor receptor $\alpha$-1 (GFR$\alpha$-1) for this purpose. We retrieved 38 cases of germ cell tumors: 26 cases contained immature teratoma, of which 24 had immature neuroepithelium and showed strong membrane staining for GFR$\alpha$-1. No significant staining was seen in other components including embryonal carcinoma, seminoma, yolk sac tumor, choriocarcinoma, immature mesenchyme, and intratubular germ cell neoplasia. Immunohistochemical staining for GFR$\alpha$-1 in immature neuroepithelium may facilitate its identification.

Teratomas are tumors with tissues derived from all 3 germ cell layers—endoderm, mesoderm, and ectoderm. Mature and immature teratomas are diagnosed based on the absence or presence of immature components. Teratoma can arise in ovaries and prepubertal and postpubertal testis. Evidence suggests that ovarian and prepubertal testicular teratomas are derived from benign germ cells. In contrast, postpubertal testicular teratomas derive from malignant germ cells. Therefore, teratomas in prepubertal boys are clinically benign, whereas in postpubertal men, they are malignant, independent of their degree of immaturity. On the other hand, immaturity is an important finding in adult ovarian teratomas. Immaturity of teratomas is usually manifested as immature neuroepithelium, which consists primarily of primitive neurotubes, neuroepithelial rosettes, rarely immature glial elements, and immature ependyma. The amount of immature neuroepithelium has been shown to correlate with survival of adults with ovarian immature teratoma, which forms the basis for the grading of these tumors.

Identification of these immature components is usually based on the recognition of embryonic-appearing neuroepithelium, which shows active mitosis and apoptosis. No immunohistochemical marker has been found to facilitate the identification of these components.

Glial cell line–derived neurotrophic factor (GDNF) is a member of the transforming growth factor $\beta$ family, which exerts trophic effects on several neuronal populations and developing kidney. GDNF family ligands interact with membrane receptors, one of which is GDNF receptor $\alpha$-1 (GFR$\alpha$-1), that, in turn, mediate stimulation of the Ret receptor tyrosine kinase. GDNF is expressed in Sertoli cells, while its receptors (RET and GFR$\alpha$-1) have been located in...
spermatogonia. GDNF-induced cell signaling has a central role in spermatogonial stem cell self-renewal.

Given that many neural crest derivatives rely on the GDNF family of ligands for survival and function, we undertook an analysis of the immature components of teratoma as a potential site of GDNF action. In this study, we evaluated the expression of GFRα-1, a receptor for GDNF in immature teratomas.

Materials and Methods

We retrieved 38 cases of germ cell tumors (GCTs) from the database of the Hospital of the University of Pennsylvania, Philadelphia Table 1. This research was approved by the University of Pennsylvania Institutional Review Board.

Of the 38 cases, 26 contained immature teratomatous components, of which 24 cases had immature neuroepithelium. Among the 38 cases, 6 were metastases from prior testicular mixed GCTs (4 to lymph nodes, 1 to brain, and 1 to lung), 2 primary mediastinal mixed GCTs, 20 testicular mixed GCTs, 4 primary testicular seminomas, and 6 ovarian immature teratomas, of which 5 were primary and 1 was recurrent.

We used 5-µm-thick sections of formalin-fixed and paraffin-embedded surgical specimens. Antigen retrieval was performed in 10 mmol/L of sodium citrate (pH 7.6) in a microwave for 4 minutes twice at 70% power level. Endogenous peroxidase was inactivated by incubation in 5% hydrogen peroxide for 5 minutes. Nonspecific binding sites were blocked by incubating with 2% normal horse serum for 20 minutes. GFRα-1 immunohistochemical stains were performed with a polyclonal anti–GFRα-1 goat antibody (R&D Systems, Minneapolis, MN) with 1:50 dilution and incubation for 20 minutes. GFRα-1 showed diffuse membranous staining. The staining intensity was classified as negative (0), weak (1+), moderate (2+), or strong (3+).

Results

Of 5 primary ovarian tumors, 3 had primary immature teratomas ranging from 17 to 30 cm with an average of 24 cm. Two were grade 2, and one was grade 1. The other 2 primary ovarian tumors were mixed GCTs with grade 2 immature teratomatous components. One patient had undergone resection of an ovarian teratoma and had grade 1 recurrent immature teratoma in the pelvis.

Two patients had primary mediastinal mixed GCTs with immature teratomatous components. Both were male patients with a diagnosis of primary mixed GCTs with immature components.

The remainder of the patients had testicular GCTs, of which 20 cases were primary testicular mixed GCTs and 4 were pure seminomas. Six cases were metastases from a known history of mixed testicular GCT.

Morphologically, 24 cases showed immature neuroepithelium with a mixture of adult structures or other germ cell components. Immature neuroepithelial components included primitive microtubules with well-demarcated inner cytoplasmic membranes and solid areas of primitive undifferentiated neuroepithelial cells. All cases with immature neuroepithelium showed strong, diffuse (3+) membranous GFRα-1 staining in the immature neuroepithelium Image 1. No significant staining was seen in other components, including other mesenchymal components. Two cases, in which the only immature foci were composed of immature mesenchyme, were negative. Most of the mature components of the teratoma were negative except for strong staining (3+) in the hair follicles in 3 cases and patchy staining in the basal layer of pseudostratified epithelium in 2 cases. Not surprisingly, there was weak GFRα-1 staining (1+) in the mature neural elements in 3 cases of mature teratoma (Image 1). The other nonteratomatous components such as embryonal carcinoma, seminoma, choriocarcinoma, yolk sac tumors, and intratubular germ cell
Image 1

Staining in immature neural components of immature teratoma, embryonal carcinoma, and mature neural elements. The immature neural components of immature teratoma (A, C, and E) were strongly positive (3+) for glial cell line–derived neurotropic factor receptor α-1 (GFRα-1) (B, D, and F); the embryonal carcinoma was negative (D and F).
neoplasia, unclassified were all negative for GFRα-1 staining.

Table 2. In addition, the spermatogonia of the seminiferous tubules and Leydig cells in the nonneoplastic tissue from the testicular mixed GCTs with immature teratomatous components showed weak GFRα-1 staining (1+), consistent with prior observations reported in the literature. 7,8

Discussion

Teratomas are biologically diverse tumors. Ovarian and prepubertal testicular teratomas may derive from benign germ cells. In contrast, postpubertal testicular teratomas derive from malignant germ cells, representing differentiation within a preexistent nonteratomatous malignant component. Therefore teratomas in boys are clinically benign, and teratomas in men are malignant, irrespective of the contents of immature components. Immaturity, however, is an important finding in ovarian teratomas. The amount of immature teratoma has been correlated with patient survival 9 and thereby forms the basis for the grading of these tumors. 4 It is critical not to confuse cellular but differentiated elements with immature elements.

Immature teratomatous components are usually recognized on the basis of embryonic-appearing neuroepithelium, which shows mitotic activity and apoptosis in contrast with differentiated neuroepithelial tissue. Sometimes, however, the recognition of immature teratomatous elements represents a challenging task.

No reliable immunohistochemical marker for immature teratoma has been previously identified. Giall fibillary acid protein (GFAP) and neurofilament immunoreactivities were studied in 9 cases of immature teratoma. 10 Discrete fibrillary cell process staining for GFAP was present at the margins of glial masses showing different stages of maturation or mixtures of different cell types. Primitive neuroepithelial tubules and areas simulating neuroblastoma were always negative. Neurofilament immunoreactivity was seen only focally in 1 case, in an area resembling ganglioma. Steeper and Mukai reported GFAP staining results in 13 pure immature teratomas of the ovary. GFAP facilitated the identification of glial tissue not evident on H&E-stained sections in 3 cases; however, neuroepithelial tissue, regardless of degree of differentiation, was uniformly negative for GFAP.

GFRα-1 is the receptor for GDNF, a factor initially identified in 1993 as a growth factor promoting the survival of...
of the embryonic dopaminergic neurons of the midbrain. Subsequent studies showed that GFRα-1 was also critical for the survival of spinal motoneurons and central noradrenergic neurons. GDNF signals through a receptor complex composed of the Ret tyrosine kinase and a glycosylphosphatidylinositol-anchored cell surface coreceptor that may be GFRα-1 or GFRα-2. GDNF first binds to the homodimer of GFRα-1, which, in turn, binds to 2 molecules of RET together, resulting in transphosphorylation of specific tyrosine molecules in their tyrosine kinase domains and intracellular signaling. RET activates several intracellular signaling pathways, including the phosphoinositide 3 kinase (PI3K) pathway. The PI3K pathway is crucial for neuronal survival and neurite outgrowth. In addition, GDNF can signal independently of RET through GFRα-1. The association of GFRα-1 with the neural cell adhesion molecule (NCAM) facilitates the binding of GDNF to the NCAM, which results in the activation of cytoplasmic Src-like kinase Fyn and focal adhesion kinase.

By binding to NCAM, GDNF stimulates Schwann cell migration and axonal growth in hippocampal and cortical neurons in a RET-independent manner. Targeted deletion of GDNF, GFRα-1, and RET have marked similar defects in kidney organogenesis and motoneurons in the spinal cord.

Because GDNF is crucial for neuronal survival, we reasoned that GFRα-1 would be present in immature teratoma. The specific expression of GFRα-1 in primitive neuroepithelium suggests that GDNF may have a role in its survival and that further study of its function is warranted. The specific immunohistochemical staining pattern of GFRα-1 in the immature neuroepithelium may facilitate the identification of such components in teratomas.

References


