Diagnostic significance of soluble c-kit in the cerebrospinal fluid of patients with germ cell tumors

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Object. Overexpression of the protooncogene c-kit has been suggested in a gonadal germ cell tumor (GCT). Recently, the soluble isoform of c-kit (s-kit) has been expressed in a variety of cell types. The goal of this study was to investigate the expression of c-kit and the clinical significance of s-kit in patients with GCTs.

Methods. The authors first conducted an immunohistochemical investigation of the expression of the c-kit protein in 27 surgical specimens. In all 18 specimens that contained germinomas, c-kit was diffusely expressed on the cell surface of the germinoma cells, but was not found on lymphocytes or interstitial cells. In seven of eight immature teratomas, only some mature components, such as cartilage and glands, were immunoreactive for c-kit. Syncytiotrophoblastic giant cells (STGCs) demonstrated negative findings as well, suggesting that primarily germinoma cells express c-kit. Next, 47 cerebrospinal fluid (CSF) samples collected from 32 patients with GCTs (15 samples from patients with pure germinomas, 14 from patients with teratomas, and two from a patient with a choriocarcinoma) were analyzed using a sandwich enzyme-linked immunosorbent assay. The level of s-kit was significantly higher in CSF collected from patients with germinomas and STGC germinomas than in CSF collected from patients with teratomas or non–germ cell brain tumors, or in CSF collected from controls. The concentration of s-kit in CSF was correlated with the patient’s clinical course; it was significantly higher in pretreatment samples obtained before and in samples obtained at the time of tumor recurrence than in samples collected from patients in whom the tumor was in remission. The level of s-kit was remarkably high in CSF collected from patients with subarachnoid tumor dissemination.

Conclusions. These results indicate that the concentration of s-kit in CSF may be a useful clinical marker for germinomas, especially for detecting recurrence or subarachnoid dissemination of these lesions.

Key Words • germ cell tumor • central nervous system • s-kit • dissemination • cerebrospinal fluid • tumor marker

The protooncogene c-kit encodes a transmembrane tyrosine kinase receptor for the SCF that is related to the platelet-derived growth factor receptor subfamily. Following binding to the SCF, dimerization, and phosphorylation, c-kit begins a signaling cascade that regulates cell growth. Physiologically, the c-kit protein is expressed in normal human tissues such as skin, breast, bone marrow, brain, and testis. In particular, c-kit has a significant role in the development of germ cells. During testicular development, the interaction between SCF and c-kit plays an important role in primordial germ cell migration and survival and in spermatogonial adhesion, proliferation, and survival. Alterations of c-kit expression have been reported in a variety of human neoplasms such as malignant melanoma, breast cancer, small cell and non–small cell lung cancer, thyroid tumors, and testicular GCTs. The soluble form of the c-kit receptor (s-kit), which consists of only the extracellular ligand-binding domain, can be generated by proteolytic cleavage or by alternative splicing. Serum levels of s-kit correlate with distinct subtypes of hematopoietic disorders of the French-American-British classification and reflect the pathological state of AML. Central nervous system GCTs are rare intracranial tumors that primarily occur in young patients. Because they are preferentially located along the ventricular system, that is, in the pineal and suprasellar regions, these lesions usually are in contact with the CSF. Therefore, if CNS tumors express s-kit, its presence in the CSF may represent a useful tumor marker, as is the case for other known oncoproteins such as α-fetoprotein, carcinoembryonic antigen, and β-HCG.
We confirmed immunohistochemically the expression and localization of c-kit in the CNS of patients with GCTs. We also compared the CSF concentration of s-kit in healthy volunteers and in patients with various types of brain GCTs and non-GCTs, and examined the correlation between the concentration of s-kit in the CSF and the clinical course of patients with GCTs. Our results indicate that the level of s-kit in the CSF may represent a novel clinical marker for GCTs and may aid in their differentiation from other CNS tumors. In addition, it may be especially useful for the early detection of recurrent germinomas and their subarachnoid dissemination.

**Materials and Methods**

**Tissue Samples and Immunohistochemical Analysis**

Tissues were obtained from 27 GCTs of the primary CNS that were surgically treated between 1986 and 1998 at either Kagoshima or Kumamoto University Hospital (Table 1). The surgical specimens were fixed in 10% formalin and embedded in paraffin. Thin sections of tumor (4–5 μm thick) were prepared, deparaffinized with xylene, rehydrated in a graded series of ethanol, and soaked in 0.3% methanolic hydrogen peroxide to eliminate endogenous peroxidase activity. The sections were then incubated overnight with 1:200 diluted polyclonal antibody against human c-kit. The avidin–biotin complex method was used in this study; for detection we used 0.05% diaminobenzidine tetrahydrochloride. To provide a negative control, tumor sections were incubated with normal rabbit serum instead of primary antibody. Slides were counterstained with hematoxylin. Tissue sections from testicular seminomas were used as a positive control. The intensity of c-kit staining in germinomas was categorized as positive or negative. Samples with fewer than 5% positive cells and those with weak positive staining were considered negative. In teratomas and choriocarcinomas, however, each histological component was separately evaluated for immunoreactivity.

**Samples of CSF**

Because CSF samples were not always available from the same tumor-tissue donors, many of the CSF samples used in this study were obtained from different patients treated at Kagoshima or Hokkaido University Hospital. The CSF was collected by lumbar puncture, ventricular drainage performed to manage hydrocephalus, or directly from cerebral cisterns or open ventricles at the time of surgery. We examined 47 samples of CSF collected from 32 patients with GCTs (15 samples collected from patients with pure germinomas, 14 from patients with teratomas [nine from patients with immature teratomas and five from patients with mixed lesions [germinoma plus immature teratoma]], and two from a patient with a choriocarcinoma). We also analyzed 19 CSF samples collected from 15 patients with non–germ cell primary brain tumors (seven samples from patients with medulloblastomas, three from patients with glioblastomas multiforme, four from patients with meningiomas, two from patients with schwannomas, and one sample each from patients with malignant lymphoma, metastatic brain tumor, and pituitary adenoma). The additional CSF samples were collected from nine patients who did not suffer from neoplastic disorders of the CNS (five samples from healthy volunteers, four from patients with hydrocephalus, and one from a patient with moyamoya disease). This latter group served as a control, and the samples collected in that group were analyzed to determine the baseline level of s-kit in CSF (Table 2). No patient with signs of inflammation (fever, leukocytosis or increased coreactive serum protein) was included in this study. The CSF samples were centrifuged at 1500 G and the supernatants were passed through filters (0.45-μm pore size) and stored at −70°C until use. In eight of 32 patients with GCTs, CSF cytology (10 CSF samples), computerized tomography and/or magnetic resonance imaging were used to diagnose subarachnoid dissemination.

**Quantification of Soluble c-kit by Sandwich ELISA**

We measured the serum level of s-kit by performing a sandwich ELISA using sKIT EIA. Briefly, standard human, soluble c-kit solutions (0–800 AU/ml) or CSF samples were mixed with alkaline phosphatase–conjugated monoclonal antibody against human s-kit and then inoculated into microtitre plates coated with another s-kit monoclonal antibody. After a 3-hour incubation at room temperature, the plates were washed six times with washing buffer and incubated for 1 hour at room temperature with paranitrophenyl phosphate. The enzymatic reaction was terminated by adding ethylenediamine tetraacetic acid. Absorbance was measured at 450 nm by using a microplate reader.

**Statistical Analysis**

The results of our investigation are expressed as means ± SEMs. The correlation between pairs of parameters was determined by linear regression analysis. The Welch t-test was used to evaluate the statistical significance of differences. The correlation between the concentration of s-kit and β-HCG in a CSF sample from a patient with a β-HCG–producing GCT was analyzed using the Pearson correlation coefficient. A probability value less than 0.05 was considered to indicate significance.

**Sources of Supplies and Equipment**

The polyclonal antibody against human c-kit (CD117) was purchased from Kako Japan (Kyoto, Japan) and the Vectastain Elite ABC (avidin–biotin complex) kit from Vector Laboratories (Burlingame, CA). The ELISA was performed using an sKIT EIA, purchased from Nichirei (Tokyo, Japan) and the microplate reader was acquired from BioRad (Hercules, CA).

**Results**

**Immunohistochemical Detection of c-kit in Primary GCTs of the CNS**

To confirm the expression of c-kit in GCTs of the CNS, we performed an immunohistochemical study to detect c-kit protein by using samples collected at surgery from 27 patients with GCTs (14 cases of germinoma, three cases of STGC germinoma, eight cases of immature teratoma, one case of mixed type [germinoma plus teratoma], and one case of choriocarcinoma). All samples from patients with germinoma demonstrated a strongly positive reaction for c-kit protein. The cell surface of the tumor cells was strongly positive for c-kit (Fig. 1A), which is consistent with its being a membrane receptor. Nontumor components, such as

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**Table 1**

Immunohistochemical staining of c-kit in GCTs of the CNS

<table>
<thead>
<tr>
<th>Histological Type of Lesion</th>
<th>No. of Tumors</th>
<th>No. W/ Expression of c-kit (%)</th>
<th>Positive Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>germinoma</td>
<td>14</td>
<td>14 (100)</td>
<td>germinoma component (diffusely)</td>
</tr>
<tr>
<td>germinoma &amp; STGC</td>
<td>3</td>
<td>3 (100)</td>
<td>germinoma component (diffusely; STGCs were negative)</td>
</tr>
<tr>
<td>immature teratoma</td>
<td>8</td>
<td>7 (87.5)</td>
<td>mature components (moderately)</td>
</tr>
<tr>
<td>germinoma &amp; teratoma (mixed)</td>
<td>1</td>
<td>1 (100)</td>
<td>germinoma component (diffusely)</td>
</tr>
<tr>
<td>choriocarcinoma</td>
<td>1</td>
<td>0 (0)</td>
<td>none</td>
</tr>
<tr>
<td>total</td>
<td>27</td>
<td>25 (92.6)</td>
<td>—</td>
</tr>
</tbody>
</table>

* — = not applicable.
A novel tumor marker for germinoma: s-kit

infiltrated lymphocytes and stromal cells, displayed negative reactions for c-kit. The staining pattern was similar to that collected from patients with testicular germinoma (Fig. 1B). Figure 1C and D demonstrates that STGCs that produced β-HCG in STGC germinomas and a choriocarcinoma displayed negative reactions for c-kit, whereas the surrounding germinomatous components exhibited strongly positive reactions in STGC germinomas, suggesting that the STGCs themselves do not express c-kit. The expression of c-kit was also detectable in seven of eight immature teratomas, although immunoreactivity was limited to some well-differentiated teratomatous structures such as cartilage, smooth muscle, skin, and glands (Fig. 1E and F). In one mixed-type tumor (germinoma plus immature teratoma), c-kit was expressed only in the germinomatous component (data not shown). Thus, in GCTs of the CNS, the germinomatous component is the predominant producer of c-kit and well-differentiated teratomatous components are minor producers of c-kit.

**Soluble c-kit Levels in CSF**

The results obtained from our immunohistochemical analysis led us to analyze the concentration of s-kit in CSF samples collected from patients with GCTs (Table 2). Initially, we determined the baseline concentration of s-kit in CSF obtained from control individuals, five patients with nonneoplastic diseases (hydrocephalus in four cases and moyamoya disease in one case), and five healthy volunteers. The mean concentration ± SEM in the control group was 7.68 ± 1.91 AU/ml. In patients with non–germ cell brain tumors (seven CSF samples from cases of medulloblastomas, three from cases of gliomas, four from cases of meningiomas, two from cases of schwannomas, and one each from cases of malignant lymphoma, pituitary adenoma, and metastatic brain tumor), the level was 7.23 ± 1.42 AU/ml, which was considered comparable to levels in normal controls. The s-kit level in all 47 samples of CSF collected from 32 patients with GCTs was 28.88 ± 8.14 AU/ml, which was significantly higher than the level in the control group (p < 0.015) and in other intracranial tumor groups (p < 0.012) (Table 2). This suggests that the measurement of s-kit in CSF is useful for the differential diagnosis of GCTs of the CNS. Next, to determine the correlation between the histological characteristics of GCTs and the level of s-kit in CSF, we categorized the tumors into the following five subgroups: pure germinoma (15 samples of CSF), STGC germinoma (16 samples of CSF), immature teratoma (nine samples of CSF), mixed type (germinoma plus immature teratoma, five samples of CSF) and choriocarcinoma (two samples of CSF). Because the clinical course may alter the s-kit level in the CSF, we excluded 17 CSF samples obtained at the time of tumor remission due to adjuvant therapy. The mean s-kit levels in the CSF were 28.94 ± 6.58 AU/ml for patients with germinomas (five CSF samples), 67.68 ± 24.55 AU/ml for those with STGC germinomas (14 CSF samples), 13.31 ± 1.72 AU/ml for those with immature teratomas (seven CSF samples), 15.7 ± 1.67 AU/ml for those with mixed type (three CSF samples), and 2.1 AU/ml for one with a choriocarcinoma (one CSF sample) (Fig. 2). Although the s-kit level in CSF was significantly higher in patients with STGC germinomas than in patients with immature teratomas (p < 0.05), the difference between patients with STGC germinomas and those with pure germinomas was not statistically significant. The small number of available samples does not permit us to draw firm conclusions; however, we noted that the mean level of s-kit in the CSF of patients with immature teratomas and mixed-type tumors was low; and it was lowest in the CSF of patients with choriocarcinoma. These results indicate that patients harboring GCTs with a germinoma component(s) exhibited higher CSF levels of s-kit than patients with tumors with a teratomatous component(s) or STGCs. This is consistent with our immunohistochemical finding that germinoma cells predominantly express untruncated c-kit.

**Correlation Between CSF Level of s-kit and the Clinical Course of the Disease**

We examined the correlation between patients’ clinical course and CSF levels of s-kit to determine whether s-kit could be a useful clinical marker for germinoma or STGC germinoma. We divided patients into four subgroups according to their clinical status. The patients in Group A (10 CSF samples) had not received adjuvant therapy; the patients in Group B (12 CSF samples) were currently receiving or had finished adjuvant therapy and their diseases were in partial or complete remission, the patients in Group C (three CSF samples) experienced tumor recurrence without dissemination, and Group D patients (eight CSF samples) harbored tumors in dissemination. The mean ± SEM was 19.58 ± 1.69 AU/ml in Group A, 6.79 ± 1.71 AU/ml in Group B, 18.82 ± 1.42 AU/ml in Group C, and 109.53 ± 37.41 AU/ml for Group D (Fig. 3). Our results show that during remission, the CSF level of s-kit returned to the control level and was significantly lower than that measured before adjuvant therapy (p < 0.00004). In patients in whom there was dissemination of tumor, the CSF level of s-kit was significantly increased (p < 0.05). These results indicate that the concentration of s-kit is well correlated with the

**Table 2**

<table>
<thead>
<tr>
<th>Histological Findings</th>
<th>No. of CSF Samples</th>
<th>s-kit Level (AU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>10</td>
<td>7.68 ± 1.91†</td>
</tr>
<tr>
<td>normal (healthy volunteers)</td>
<td>5</td>
<td>1.42†</td>
</tr>
<tr>
<td>hydrocephalus</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>moyamoya disease</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>non-germ cell brain tumor</td>
<td>19</td>
<td>7.23 ± 1.42†</td>
</tr>
<tr>
<td>medulloblastoma</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>glioma</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>meningioma</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>schwannoma</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>malignant lymphoma</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>pituitary adenoma</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>metastatic brain tumor</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>GCT</td>
<td>47</td>
<td>28.88 ± 8.14</td>
</tr>
<tr>
<td>germinoma</td>
<td>15</td>
<td>15.03 ± 3.51</td>
</tr>
<tr>
<td>germinoma w/ STGC</td>
<td>16</td>
<td>59.25 ± 22.14</td>
</tr>
<tr>
<td>immature teratoma</td>
<td>9</td>
<td>12.90 ± 1.39</td>
</tr>
<tr>
<td>mixed type (germinoma &amp; immature teratoma)</td>
<td>5</td>
<td>10.72 ± 3.34</td>
</tr>
<tr>
<td>choriocarcinoma</td>
<td>2</td>
<td>7.05 ± 4.95</td>
</tr>
</tbody>
</table>

* Data are expressed as the means ± SEMs.
† The mean values were only collected for the group as a whole and not for subgroups.
clinical course of germinomas with or without STGCs. Figure 4 shows a representative case of an STGC germinoma. In this patient, an increase of s-kit was detected in the CSF, leading us to suspect tumor recurrence with CSF dissemination. At its onset, the tumor was located in the suprasellar region and there was a mild increase in the CSF concentration of s-kit (13.18 IU/ml). Complete tumor remission was achieved after the patient underwent radiation therapy and chemotherapy, and the level of s-kit in the CSF returned to the normal range (0.31 IU/ml). Fourteen months later, however, the s-kit level again increased to more than 300 IU/ml and MR images disclosed tumor recurrence with dissemination around the ventricular wall.

**Correlation Between CSF Levels of s-kit and β-HCG**

The correlation between these levels was analyzed in 15 patients who harbored GCTs that produce β-HCG by using...
A novel tumor marker for germinoma: s-kit

Discussion

Our immunohistochemical study showed that c-kit is highly expressed in germinoma cells as well as in a limited number of differentiated teratoma components, but not in STGCs. The CSF concentration of s-kit is significantly higher in patients with germinomas (with or without STGCs) than in those harboring GCT subtypes. It is also correlated with the clinical course of the disease. We found the concentration of s-kit to be significantly higher in samples obtained before treatment and in those collected at the time of tumor recurrence. The CSF level of s-kit was dramatically increased in patients with subarachnoid tumor dissemination. These lines of evidence suggest that the concentration of s-kit in CSF may represent a valuable tumor marker of germinomatous components. The s-kit is produced by both proteolytic cleavage and alternative splicing and has been identified in culture supernatant of human leukemic cell lines and human umbilical vein endothelial cells. Because s-kit receptors may block the binding of SCF to c-kit receptors, they appear to act as receptor antagonists.13 Tajima, et al.,23 reported that, in patients with AML, the serum s-kit level was elevated and related to the stage of differentiation of the AML blast cells. In our study of GCTs, we assayed the levels of s-kit in CSF rather than in the serum because the expression of c-kit protein has been reported in malignant testicular GCTs,2 and because GCTs of the CNS are in direct contact with CSF and tend to spread by subarachnoid dissemination. Therefore, we argue that the level of s-kit in CSF is more sensitive than its level in serum. The serum level of s-kit in patients with AML was 457 ± 67 AU/ml (mean ± SEM), which is considerably higher than the CSF levels of s-kit that we measured.23 The only comparable samples in our study were obtained from patients with subarachnoid dissemination. When tumor cells are growing in a mass, cells inside the clump may not have contact with the CSF. Therefore, the s-kit concentration in CSF may be low unless the tumor is near a ventricle or a subarachnoid space. We noted a similar phenomenon in cases in which there was dissemination of malignant glioma cells through the subarachnoid space; monocyte chemoattractant protein-1 was significantly increased in the CSF of these patients.11 In addition, because the baseline CSF level of s-kit is less than 10 AU/ml (compared with a serum level of 224 ± 15 AU/ml in healthy individuals23), the s-kit concentration in CSF represents a more sensitive and specific marker.
Assays of serum and CSF for selected oncoproteins is standard practice in the preoperative evaluation of patients suspected of harboring GCTs of the CNS and in monitoring the treatment response of confirmed cases. The most useful markers are H9251-fetoprotein, normally synthesized by yolk sac endoderm, H9252-HCG, a glycoprotein normally secreted by syncytiotrophoblasts, and placental alkaline phosphatase, a cell surface glycoprotein elaborated by syncytiotrophoblasts and produced by primordial germ cells. Elevated levels of any of these markers constitute compelling presumptive evidence that a CNS mass is a GCT and that pattern of marker elevation is somewhat predictive of the histological characteristics of the tumor. We also investigated whether there was a correlation between s-kit and H9252-HCG, but found no significant correlation. This indicates that tumor cells that produce H9252-HCG do not express s-kit and is consistent with the immunohistochemical analysis of c-kit in STGCs. Furthermore, the Scatchard binding analysis failed to detect c-kit in human placental syncytiotrophoblasts. Therefore, we propose that β-HCG and s-kit are independent markers for different components of GCTs. Although teratomas clearly expressed c-kit, the CSF levels of s-kit were remarkably low in immature teratomas, probably because the c-kit–producing cells were restricted to some mature teratomatous components and, thus, the total number of s-kit–producing cells was smaller than that found in germinomas. In addition, clusters of s-kit–positive cells were surrounded by thick interstitial tissues, further inhibiting contact with the CSF. Our preliminary results suggest that the CSF levels of s-kit may represent a valuable marker for the diagnosis and treatment of germinomas. Before firm conclusions can be drawn, however, large numbers of samples must be studied to determine the actual value of s-kit for the diagnosis of these tumors and their dissemination. In addition, c-kit may be available as a molecular target for the treatment of germinomas. Studies are underway to clarify the biological roles of c-kit and s-kit and to determine their value for treating patients with GCTs.

Conclusions

Our study indicated that germinoma cells primarily express c-kit, whereas STGCs proved negative for the presence of c-kit. The level of s-kit was significantly higher in CSF samples obtained from patients with GCTs that contain germinomas. The concentration of s-kit in a patient’s CSF was correlated with that patient’s clinical course; it was significantly higher in pretreatment samples and in samples obtained at the time of tumor recurrence than in samples obtained from patients in whom the tumor was in remission. The level of s-kit was remarkably high in patients with subarachnoid tumor dissemination. Therefore, the concentration of s-kit in CSF may be a useful clinical marker for...
A novel tumor marker for germinoma: s-kit
germomas, especially for detecting tumor recurrence or subarachnoid dissemination.

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References


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