c-Kit Expression in Desmoid Fibromatosis
Comparative Immunohistochemical Evaluation of Two Commercial Antibodies

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Key Words: c-Kit; Tyrosine kinase receptor; Desmoid fibromatosis; Soft tissue neoplasms; Gastrointestinal stromal tumor; STI571; Imatinib mesylate; Gleevec; Immunohistochemistry

DOI: 10.1309/AN4E2ETCJAR6JJUY

Abstract

To determine the frequency of c-Kit staining in desmoids and optimize an assay for clinical use, we stained 19 desmoids from various sites at various dilutions with 2 commonly used rabbit polyclonal, anti-c-Kit antibodies (A4502, DAKO, Carpinteria, CA; C-19, Santa Cruz Biotechnology, Santa Cruz, CA), with and without heat-induced epitope retrieval (HIER) in citrate buffer. Appropriate external and internal control samples were evaluated for each test condition. At dilutions of 1:50 both antibodies stained substantial numbers of desmoids: with/without HIER, A4502, 89%/63%; C-19, 37%/74%. The staining was cytoplasmic without cell membrane accentuation. However, background stromal staining and nonspecific staining of endothelium and smooth and striated muscle were problematic with both antibodies at 1:50. At higher dilutions, C-19 stained no desmoid; however, diminished staining of external and internal control samples made it unreliable. A4502 similarly stained many fewer desmoids at higher dilutions. However, it retained strong staining of both external and internal control samples and showed much less nonspecific staining. Best results were achieved at 1:250 without HIER; only weak focal staining was present in 1 desmoid. With a simple immunohistochemical method optimized for clinical use, desmoid can be regarded as a c-Kit–negative tumor.

Desmoid fibromatosis (desmoid) behaves in a locally aggressive manner, often with multiple local recurrences and substantial patient morbidity. Because local control is known to be difficult, a number of chemotherapeutic interventions have been attempted with variable claims of success.¹⁻⁵ To date, however, no agent has proven reliably effective for routine clinical use. Because of its success in treating gastrointestinal stromal tumor (GIST), the drug imatinib mesylate (STI571, or Gleevec, Novartis, Basel, Switzerland), a c-Kit tyrosine kinase inhibitor, has piqued the interest of oncologists as a potential therapeutic agent for other mesenchymal neoplasms, including desmoid.

Yantiss et al⁶ reported c-Kit immunoreactivity in a high percentage (75%) of intra-abdominal desmoids. This finding raises the possibility that desmoid is a c-Kit–driven tumor and, if so, that it might respond to imatinib. It also calls into doubt the usefulness of c-Kit staining for distinguishing desmoid from GIST in tissue sections. In contrast with the findings of Yantiss et al,⁶ Miettinen et al⁷ found no c-Kit staining in 7 intra-abdominal desmoids, and, more recently, Hornick and Fletcher⁸ reported focal weak staining in only 1 (5%) of 20 desmoids. Miettinen⁹ addressed the issue of c-Kit staining in desmoid in a letter, in which he concluded that the presence or absence of staining in desmoid depends on the choice of antibody and the method used.

In an attempt to resolve the disparate results from these studies, we evaluated 2 commonly used anti–c-Kit antibodies at various dilutions with and without heat-induced epitope retrieval (HIER) in a series of desmoids. Our aims were to determine the incidence of c-Kit expression in desmoid and to optimize the immunohistochemical assay for clinical use.
Materials and Methods

Formalin-fixed, paraffin-embedded tissue blocks from 19 desmoids were retrieved from the archives of Detroit Medical Center Hospitals, Detroit, MI. The cases consisted of 11 extra-abdominal (including 2 mammary), 6 intra-abdominal, and 2 abdominal fibromatoses. Multi–tissue array blocks were created from 5-mm punches from each desmoid. Two commercial, rabbit polyclonal, anti-c-Kit antibodies were evaluated: A4502 (DAKO, Carpinteria, CA), which recognizes epitope 963-973, and C-19 (Santa Cruz Biotechnology, Santa Cruz, CA), which recognizes epitope 959-973. Every tumor was run with 3 different dilutions of each antibody (A4502 diluted to 1:50, 1:150, and 1:250; C-19 diluted to 1:50, 1:100, and 1:200), with and without HIER pretreatment.

For immunohistochemical analysis, 4-µm sections were collected on gelatin-coated slides. After deparaffinization, the tissue sections designated for HIER were placed in a 10-mmol/L concentration of citrate buffer, pH 6.0, steamed for 20 minutes, and allowed to stand for an additional 20 minutes in the hot buffer. All tissue sections then were placed on the Ventana automated stainer (Ventana Medical, Tucson, AZ) using a titration protocol and aminoethylcarbazol as the detection system. Primary antibodies to c-Kit were placed on the tissue sections and incubated for 32 minutes. After the staining run was complete, the tissue sections were removed from the stainer and counterstained with hematoxylin.

A multitissue block section containing 4 GISTs served as the positive external control with each run. Mast cells within the desmoids served as positive internal controls. Each tumor also was stained with Giemsa to identify mast cells for comparison with the c-Kit results. A negative control sample was included with each run by omitting the primary antibody and yielding no signal.

For each tumor, the percentage of positive cells was estimated, intensity of staining was scored with a semiquantitative scale (+++, strong; +, weak; ±, weak to negative [for multi–tissue positive external control sample when weak positive staining was present in less than all 4 GISTs]; or 0, none), and distribution of staining within the cell (membranous, cytoplasmic, or Golgi) was noted. Other observations tabulated for each tumor were the presence or absence of background stromal staining and whether normal mast cells, breast epithelium, endothelium, or smooth or skeletal muscle stained.

Results

Desmoids

At a dilution of 1:50, both antibodies stained substantial numbers of the 19 desmoids. The A4502 antibody stained 17 (89%) and 12 (63%), while the C-19 antibody stained 7 (37%) and 14 (74%) with and without HIER, respectively. The staining reaction involved the majority of cells in most tumors and showed a cytoplasmic pattern with no evidence of cell membrane or Golgi accentuation Image 18. With the C-19 antibody, staining intensity was regarded as weak in most cases Image 21, while the A4502 antibody was more likely to show strong staining Table 11.

However, at a dilution of 1:50, both stains showed a background blush due to light nonspecific stromal staining in many of the tumors. This problem was more prevalent with the C-19 antibody, which also more frequently stained endothelium and smooth and striated muscle Image 31 and Image 41. At the 1:50 dilution, both antibodies stained intratumoral mast cells in most of the 14 desmoids in which mast cells were identified by Giemsa stain: 13 of 14 for A4502, and 14 of 14 for C-19.

At dilutions of 1:100 and 1:200, the C-19 antibody no longer stained any desmoid. However, intratumoral mast cell staining concomitantly decreased to only 2, 3, and 1:0, and 3 of 14 desmoids at 1:100, 1:100 with HIER, 1:200, and 1:200 with HIER, respectively. Thus, at these dilutions, the reliability of staining with the C-19 became suspect.

At dilutions of 1:150 and 1:250, the numbers of desmoids stained by the A4502 antibody also decreased; 1 (5%) at 1:150, 5 (26%) at 1:150 with HIER, 1 (5%) at 1:250, and 0 (0%) at 1:250 with HIER. All were categorized as weakly positive, and the single positive desmoid (same tumor) at 1:150 and 1:250 showed only weak, very focal staining. At these dilutions with the A4502 antibody, the background was clean and there was no staining of endothelium or smooth or striated muscle Image 51. In addition, the staining of intratumoral mast cells was better preserved compared with the C-19 antibody: 9, 12, 12, and 9 desmoids had c-Kit–positive mast cells at 1:150, 1:150 with HIER, 1:250, and 1:250 with HIER, respectively, in the 14 tumors in which mast cells were identified with Giemsa stain. Staining of breast epithelium, which is normally positive for c-Kit, in the mammary fibromatosis cases also was preserved with the A4502 antibody at these higher dilutions.

GIST Control Samples

The 4 tumor multitissue GIST control slides showed intense diffuse staining with the A4502 antibody in all runs with no detectable differences among test conditions Image 68 and Image 71. By contrast, staining intensity with the C-19 antibody diminished from strongly positive at the 1:50 dilution Image 81, to weakly positive at the 1:100 dilution Image 91, to weakly and inconsistently positive at 1:200. Thus, at higher dilutions, the C-19 antibody showed diminished staining in GISTs, and this result was independent of whether HIER was used.
**Discussion**

c-Kit is a transmembrane tyrosine kinase receptor, which when activated signals a cascade of intracellular reactions that culminates in cell proliferation. In some forms of human cancer, notably GISTs, acute myelogenous leukemia, and mastocytosis, the c-Kit receptor is activated by somatic mutation. In other cancers, such as pulmonary small cell and ovarian carcinomas, paracrine or autocrine activation is postulated. c-Kit is expressed strongly in GISTs, where it is detected readily by immunohistochemical analysis. It also is detected in a variety of normal human cells, including mast cells, breast epithelium, basal cells of the skin, melanocytes, germ cells, and interstitial cells of Cajal, from which GIST is believed to derive. Although used by surgical pathologists primarily in the differential diagnosis of intra-abdominal tumors, much interest in c-Kit as a specific therapeutic target has arisen since the introduction of imatinib.

**Table I**
c-Kit Immunohistochemical Staining in Desmoid Fibromatosis

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Dilution and Pretreatment</th>
<th>Positive Desmoids (n = 19)</th>
<th>Intensity of c-Kit Staining</th>
<th>Desmoids With c-Kit–Positive Mast Cells (n = 14)</th>
<th>GIST Control Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A4502, DAKO</td>
<td>1:50</td>
<td>12 (63)</td>
<td>5, +; 7, ++</td>
<td>13 (93)</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1:50 HIER</td>
<td>17 (89)</td>
<td>6, +, 11, ++</td>
<td>13 (93)</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1:150</td>
<td>1 (5)</td>
<td>1, +</td>
<td>9 (64)</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1:150 HIER</td>
<td>5 (26)</td>
<td>5, +</td>
<td>12 (86)</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1:250</td>
<td>1 (5)</td>
<td>1, +</td>
<td>12 (86)</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1:250 HIER</td>
<td>0 (0)</td>
<td>—</td>
<td>9 (64)</td>
<td>++</td>
</tr>
<tr>
<td>C-19, Santa Cruz</td>
<td>1:50</td>
<td>14 (74)</td>
<td>13, +; 1, ++</td>
<td>14 (100)</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1:50 HIER</td>
<td>7 (37)</td>
<td>7, +</td>
<td>14 (100)</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1:100</td>
<td>0 (0)</td>
<td>—</td>
<td>2 (14)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1:100 HIER</td>
<td>0 (0)</td>
<td>—</td>
<td>3 (21)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1:200</td>
<td>0 (0)</td>
<td>—</td>
<td>0 (0)</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>1:200 HIER</td>
<td>0 (0)</td>
<td>—</td>
<td>3 (21)</td>
<td>±</td>
</tr>
</tbody>
</table>

GIST, gastrointestinal stromal tumor; HIER, heat-induced epitope retrieval; ++, strongly positive; +, weakly positive; ±, weak to negative.

* Nineteen samples of desmoid fibromatosis (desmoids) from various sites were stained with 2 rabbit polyclonal, anti-c-Kit antibodies (A4502, DAKO, Carpinteria, CA, recognizes epitope 963-973; and C-19, Santa Cruz Biotechnology, Santa Cruz, CA, recognizes epitope 959-973) at the dilutions indicated. Numeric data are given as number (percentage), except in the “Intensity of c-Kit Staining” column, in which the number of cases is followed by the indicator of staining intensity.
Recently, a handful of immunohistochemical studies of c-Kit in desmoid have appeared with contradictory results.\(^6\)\(^,\)\(^8\) In a study designed to compare intra-abdominal desmoids with GISTs, Yantiss et al\(^8\) reported positive staining in 9 (75\%) of 12 desmoids. These investigators used the A4502 antibody at a dilution of 1:30 with HIER and reported as positive any tumor in which more than 5\% of the cells stained. By contrast, in a concurrent study, Miettinen et al\(^7\)
using the C-19 antibody at a dilution of 1:400 with Nae- thylenediaminetetraacetic acid as the HIER solution, found no staining in 7 desmoids. Miettinen,9 in a letter, compared the A4502 with the C-19 antibody. By using the aforementioned method for the C-19 antibody, none of 14 desmoids was positive. By contrast, all 14 were positive with the A4502 antibody at a dilution of 1:100 (with or without HIER), as were the majority of nodular fasciitis cases. Miettinen9 concluded that the A4502 antibody, unlike the C-19 antibody, shows extensive reactivity with fibroblasts and myofibroblasts and suggested that this may represent a cross-reaction. By contrast, Hornick and Fletcher8 recently reported a much lower incidence of staining with the A4502 antibody, with only 1 of 20 desmoids showing focal, weak staining. Their analysis was performed at a higher dilution than in the previous studies (1:250) and without HIER.

Because the published literature on this topic is inconsistent, most likely owing to the use of different methods, we sought to determine the frequency of c-Kit staining in desmoids by optimizing a technique that could be reproducible and easily performed in a hospital-based immunohistochemistry laboratory. In our study, we found that c-Kit staining in desmoids depended not only on the antibody used, but also on dilution and less on HIER in citrate buffer. We found that the A4502 antibody performed better than the C-19 antibody. It gave a crisper, more intense signal in the control samples with less nonspecific staining in the desmoids and performed well without the need for antigen retrieval.

When used at low titers (1:50), both antibodies stained substantial numbers of desmoids. However, nonspecific and
background staining became problematic at this dilution. At higher titers, most desmoids became nonreactive with either antibody. However, the A4502 antibody retained its strong diffuse reactivity in the external (GISTs) and internal (intraluminal mast cells and breast epithelium) control samples, while the C-19 antibody became less intense and less reliable at high titers. The A4502 antibody at the 1:250 dilution gave the best results, and its sensitivity did not seem to improve with HIER. Similarly, Hornick and Fletcher observed that antigen retrieval did not enhance c-Kit staining.

Although c-Kit is a specific target for imatinib, and, as we have shown, desmoid is best regarded as a c-Kit–negative tumor, it may be premature to conclude that imatinib has no role in its therapy. The reason is that desmoid may be driven by autocrine and paracrine activation of the platelet-derived growth factor receptor, which, like c-Kit, also is a tyrosine kinase receptor. Thus, the possibility that imatinib could have some effect on desmoid through this alternative pathway cannot be entirely excluded until it has been fully investigated.

Conclusions

As we strive for uniformity in immunohistochemical analysis, it is important to develop techniques that are reliable and reproducible and that can be performed easily in most immunohistochemistry laboratories. This is especially important for markers such as c-Kit, since very specific therapeutic decisions are made based on the results of this test. From review of the literature, there seems to be a general lack of uniformity among laboratories about choice of antibody and methods, leading to disparate conclusions about c-Kit staining in desmoids. The method that we found most efficacious and that we advocate is a simple test that can be fully run on an automated stainer and for which antigen retrieval is not needed. Of note, this is the same method for c-Kit staining used in a large clinical trial in GISTs, which lends additional support for its use. And, finally, from these results, we believe that desmoid is best regarded as a c-Kit–negative tumor and that imatinib most likely has no role in its treatment.

References
