KIT (CD117)-Positive Breast Cancers Are Infrequent and Lack KIT Gene Mutations

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ABSTRACT

Purpose: KIT (CD117) is a transmembrane tyrosine kinase representing a target for STI571 (Glivec) therapy. Some KIT-overexpressing solid tumors have responded favorably to STI571, potentially because of the presence of KIT-activating mutations.

Experimental Design: To investigate the epidemiology of KIT overexpression and mutations, we investigated a series of 1654 breast cancers. All tumors were analyzed by immunohistochemistry in a tissue microarray format.

Results: KIT expression was always present in normal breast epithelium. However, cancer analysis revealed the only 43 of 1654 (2.6%) tumors were KIT-positive. KIT expression was more frequent in medullary cancer (9 of 47 positive; 19.1%) than in any other histological tumor subtype (P < 0.001). KIT expression was significantly associated with high tumor grade (P < 0.0001) but unrelated to pT and pN categories or patient survival. Mutation analysis of exons 2, 8, 9, 11, 13, and 17 was negative in 10 KIT-positive tumors.

Conclusions: Overall, our data show that a high level of KIT expression occurs infrequently in breast cancer. KIT-positive breast cancers may not reflect “KIT up-regulation” because KIT is also expressed in normal breast epithelium. The lack of KIT mutations also argues against the therapeutic efficacy of STI571 in breast cancer.

INTRODUCTION

KIT (CD117) is a transmembrane tyrosine kinase acting as a type III receptor for mast cell growth factor. It plays an important role in the development of multiple cell types, including hematopoietic cells, germ cells, and melanocytes (1). KIT can also be detected in various tumor entities (2–6). KIT alterations in malignant tumors are of high interest because KIT is one of the targets of the tyrosine kinase inhibitor imatinib mesylate (STI571; Glivec). STI571 has initially been shown to be effective in the treatment of chronic myeloid leukemia, where it targets the BCR-ABL fusion protein (7). More recently, significant treatment responses were also seen in patients with advanced KIT-positive gastrointestinal stroma tumors (8) and dermatofibrosarcoma protuberans (9). It is hoped that other KIT-positive tumors may also benefit from STI571 therapy.

In the breast, KIT protein was found in normal breast epithelium, nonneoplastic breast diseases (10), and in 1–13% of cancers (10–12). Despite a relatively low prevalence of KIT expression, breast cancer may be an important candidate for STI571 therapy because of its high frequency. Several clinical trials are now investigating the effect of STI571 on KIT-positive malignancies of various origins (13–17). It has been suggested that the response rate may be particularly high in KIT-expressing tumors that also harbor activating KIT mutations (18–24). The rate of KIT mutations in breast cancer has never been studied. Collecting such information would require the analysis of a very large number of tumors if the prevalence of KIT positivity is as low as the 1% suggested by one recent study (10). In this project we took advantage of a preexisting tissue microarray (TMA) containing samples from >2000 breast cancer patients to identify a subset of KIT-positive breast cancers. These tumors were then sequenced to screen for mutations at multiple exons of the KIT gene.

MATERIALS AND METHODS

Breast Cancer TMA. A total of 2197 patients with a median age of 62 (range, 26–101) years were evaluated retrospectively. Raw survival data were either obtained from the cancer registry of Basel or collected from the patients’ attending physicians. The mean follow-up period was 68 months (range, 1–176 months). Formalin-fixed (buffered 4%), paraffin-embedded tumor material was available from the Institute of Pathology, University Hospital Basel; the Institute for Clinical Pathology in Basel; and the Triemli Hospital in Zürich. The use of these specimens and data in research were approved by the Ethics Committee of the Basel University Hospital. The pathological stage and nodal status were obtained from the primary pathology reports. All slides from all tumors were reviewed by one of two pathologists (J. T. and G. S.) to define the histological grade according to Elston and Ellis (BRE: Ref. 25) and indicate this fact.

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mor TMA blocks were transferred to an adhesive-coated slide system (Instrumedics Inc., Hackensack, NJ).

**Immunohistochemistry (IHC).** IHC was performed with the anti-CD117 antibody from DAKO (A4502). In a comparison of multiple antibodies, A4502 had previously yielded the highest specificity and the least background. A4502 was applied at a dilution of 1:300 at room temperature for 2.5 h, after 3 min of pressure cooking in 10 mM sodium citrate buffer (pH 6.0) for antigen retrieval. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide diluted in methanol for 30 min. A standard kit (Vector ABC) was used for visualization. For check for specificity, we performed preabsorption experiments with the antibody (CD117 peptide stock solution; PP1518: Neomarkers). This antigen matches the sequence of the epitope recognized by the DAKO polyclonal antibody. A gastrointestinal stromal tumor sample was used as a positive control. For each tissue sample, the percentage of positive cells was estimated and the staining intensity was recorded semiquantitatively as 1, 2, or 3+. For statistical analyses, the staining results were categorized into three groups. Tumors without any staining were considered negative. Tumors with 1+ staining and tumors with 2+ staining in <50% of cells were considered weakly positive. All remaining samples (2+ staining in 50% of cells and samples with 3+ staining) were considered strongly positive. Conventional “large sections” were analyzed by IHC from 36 KIT-positive and 28 KIT-negative tumors to further validate the IHC results obtained on TMAs.

**Sequence Analysis.** Ten breast cancers showing intense CD117 staining were selected for KIT mutation analysis. The formalin-fixed tissues were deparaffinized and the DNA was extracted according to the protocols provided by Qiagen (Basel, Switzerland). Exons 2, 8, 9, 11, 13, and 17 of the KIT gene were amplified by semi-nested PCR. All exons were sequenced directly using Big Dye Terminators Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). The primers designed for the PCR and the sequence reactions are listed in Table 1. Sequence products were analyzed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems).

**RESULTS**

**IHC.** The IHC staining was interpretable in 1654 (75.3%) of the 2197 arrayed tissue spots on the TMA. KIT staining was observed in the membranes of 43 (2.6%) of these tumors. Staining was considered weak in 29 (1.8%) and strong in 14 (0.8%) of 25 tumors that were scored positive on the TMA (Table 3; P < 0.0002). KIT staining was not seen in large sections of all 28 tumors that were scored positive on the TMA, but was detectable on 24 of 36 tumors that were scored positive on the TMA (Table 3; P < 0.001). KIT staining intensity on large sections was related to the KIT expression level detected on the TMA. In 5 of 11 cases, tumors that were considered strongly positive on the TMA were also scored strongly positive on large sections. However, only 4 of 25 tumors that were scored weakly positive on TMA sections were considered strongly positive on large sections (P < 0.0001). In 12 cases, the TMA analysis had suggested KIT positivity, which was subsequently not confirmed on large sections. A review of these cases suggested that in the majority of these samples, some weak cytoplasmic background staining had

**Table 1** Primer sequences for KIT sequence analysis

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward</th>
<th>Reverse 1</th>
<th>Reverse 2 (nested)</th>
<th>Sequence (5' → 3')</th>
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<td>Reverse</td>
<td>Reverse 2 (nested)</td>
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<td>ACT GCA GAA AGC CAA GTA TT</td>
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<td>Forward</td>
<td>Reverse</td>
<td>Reverse 2 (nested)</td>
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<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>GCT GAG GTT TTC CAG CAC TC</td>
</tr>
<tr>
<td>9</td>
<td>Forward</td>
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<td>Reverse 2 (nested)</td>
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<td></td>
<td>1</td>
<td></td>
<td>TCC TAG AGT AAG CCA GGG CTT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>AGC CAG GGC TTT TGT TTT CT</td>
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<td>Reverse 2 (nested)</td>
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<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>CCA GAG TGC TCT AAT GAC TG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>AGC CCC TGT TTC ATA CTG AC</td>
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<td>Reverse 2 (nested)</td>
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<td>1</td>
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<td>GCT TGA CAT CAG TTT GGC AG</td>
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<td></td>
<td></td>
<td>2</td>
<td></td>
<td>TGA CAT CAG TTT GCC AGT TG</td>
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<td>Reverse</td>
<td>Reverse 2 (nested)</td>
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<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>TCC TTA CTC ATG GTC GGA TC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>CAG GAC TGT CAA GCA GAG AA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>Reverse 2 (nested)</td>
<td>ACT GTC AGA GAG AGA ATG GG</td>
</tr>
</tbody>
</table>

4 P. Went, unpublished data.
been overinterpreted on the TMA section. However, in an attempt to find as many positive cases as possible on the TMA, questionable cases had been scored positive. The IHC analysis was noninformative in 543 (24.7%) of 2197 arrayed tumors because of missing tissue or a lack of tumor cells in the arrayed tissue sample.

**Mutation Analysis.** No alterations compared with the KIT genomic sequence (GenBank accession no. U63834; Ref. 27) were found in exons 2, 8, 9, 11, 13, and 17 in the set of 10 examined breast cancers.

**DISCUSSION**

We used a TMA to evaluate the KIT expression frequency in a series of 2197 breast cancers. In this method, minute tissue samples (0.6-mm diameter) of hundreds of tumors are analyzed on one glass microscope slide. It is obvious that focal gene alterations are not always detected in a TMA setting. However, this perceived disadvantage appears to have limited practical impact. Numerous studies have shown that associations between molecular alterations and clinical or pathological information can be readily detected in TMA analyses (28–34). Multiple other studies have reported a high level of concordance between TMA findings and results obtained on corresponding large sections (28, 31, 32, 35–47). In this project, a TMA was used for

### Table 2

<table>
<thead>
<tr>
<th>KIT expression</th>
<th>On array (n)</th>
<th>Analyzable (n)</th>
<th>Weak (%)</th>
<th>Strong (%)</th>
<th>P</th>
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<tbody>
<tr>
<td>All samples</td>
<td>2197</td>
<td>1654</td>
<td>1.8</td>
<td>0.8</td>
<td></td>
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<td>Histology</td>
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<td></td>
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<td></td>
<td></td>
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<td>Ductal carcinoma</td>
<td>1531</td>
<td>1183</td>
<td>1.5</td>
<td>0.6</td>
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<tr>
<td>Lobular carcinoma</td>
<td>311</td>
<td>205</td>
<td>0.5</td>
<td>0.0</td>
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<tr>
<td>Medullary carcinoma</td>
<td>57</td>
<td>47</td>
<td>10.6</td>
<td>8.5</td>
<td>&lt;0.0001*</td>
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<td>Mucinous carcinoma</td>
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<td>53</td>
<td>1.9</td>
<td>0.0</td>
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<td>42</td>
<td>2.4</td>
<td>0.0</td>
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<td>Cribriform carcinoma</td>
<td>64</td>
<td>47</td>
<td>2.1</td>
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<td>Papillary carcinoma</td>
<td>30</td>
<td>23</td>
<td>4.3</td>
<td>4.3</td>
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<tr>
<td>Other typesb</td>
<td>79</td>
<td>54</td>
<td>1.9</td>
<td>3.7</td>
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<td>Stage</td>
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<tr>
<td>pT0</td>
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<td>577</td>
<td>0.9</td>
<td>0.5</td>
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<tr>
<td>pT1</td>
<td>1015</td>
<td>779</td>
<td>2.3</td>
<td>0.8</td>
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<tr>
<td>pT2</td>
<td>124</td>
<td>92</td>
<td>4.3</td>
<td>3.3</td>
<td>NSc</td>
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<tr>
<td>pT3</td>
<td>242</td>
<td>199</td>
<td>0.5</td>
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<td>Nodal stage</td>
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<tr>
<td>pN0</td>
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<td>681</td>
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<td>0.7</td>
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<tr>
<td>pN1</td>
<td>783</td>
<td>590</td>
<td>2.4</td>
<td>1.0</td>
<td>NS</td>
</tr>
<tr>
<td>pN2</td>
<td>121</td>
<td>99</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
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<tr>
<td>BRE grade</td>
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<td></td>
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<td></td>
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<tr>
<td>1</td>
<td>539</td>
<td>384</td>
<td>0.3</td>
<td>0.8</td>
<td></td>
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<tr>
<td>2</td>
<td>839</td>
<td>629</td>
<td>1.1</td>
<td>0.0</td>
<td>&lt;0.0001</td>
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<tr>
<td>3</td>
<td>646</td>
<td>515</td>
<td>3.9</td>
<td>2.1</td>
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</tr>
</tbody>
</table>

*Medullary carcinoma vs. other histologies.

b Other types include adenoid-cystic carcinoma (n = 1), apocrine carcinoma (n = 15), atypical medullary carcinoma (n = 9), carcinosarcoma (n = 2), clear cell carcinoma (n = 14), histiocytic carcinoma (n = 1), lipid-rich carcinoma (n = 2), and histiocytic carcinoma (n = 2).

 NS, not significant.

**Fig. 1** Examples of KIT immunohistochemistry results for tissue microarray spots. A, normal breast tissue; B, invasive breast carcinoma showing strong KIT expression in the membrane; C, tissue spot with KIT-positive normal breast and KIT-negative tumor cells (arrowhead). The insets show magnifications of representative areas.

**Fig. 2** Survival analysis (Kaplan–Meier model) of KIT-positive (weak or strong immunostaining) versus KIT-negative cancers.
"rare event detection." Because of the low frequency of KIT expression in breast cancers, it was necessary to first analyze a very large number of tumors by IHC to identify KIT-positive cases for subsequent mutation analysis. Comparison of the results obtained with TMA and large section analysis indicated that the TMA technology is highly suited for identification of rare positive cases. Surprisingly, we found no cases that were false negative in the TMA analysis, but there were 12 cases that were considered positive on the TMA only. A review of these cases revealed that a weak cytoplasmic background staining had been overinterpreted as true positivity in the majority of these cases. However, a small number of false-positive cases was acceptable in our TMA analysis because in our screening mode we attempted to identify as many of the true-positive cases as possible. Because all large-section-positive cases were identified in our TMA setting, one could consider the possibility that TAMs made from tissues of living cancer patients can be used to select patients for future targeted therapy regimens (48).

Because there is no officially recommended method for IHC KIT testing, there is some variability of reported KIT positivity rates in breast cancer (1–13%; Refs, 10–12) as well as in other tumor entities (2, 4, 5, 49–56). We therefore made significant efforts to optimize our IHC KIT detection system. Seven commercially available KIT antibodies were evaluated in a previous study, which revealed considerable variability of their staining properties. Antibody A4502 (DAKO) was judged to be optimal in this study because it revealed the highest frequency of positivity in arrayed gastrointestinal stroma tumors and because reagents are available for preabsorption control experiments to assure maximum specificity of the system. Using this system, we found KIT expression in only 43 of 1654 interpretable tumors (2.6%). The concordant lack of detectable KIT expression in both large sections and arrayed tissue spots of 28 randomly selected KIT-negative tumors makes it unlikely that examination of more tissue samples per tumor would yield a substantially higher number of positive tumors. Our result is similar to the 1% positive cases recently reported by Tsuchiya et al. (10) in a large study involving 197 breast cancers. Another study had previously reported KIT positivity in 13% of breast cancers (12). This discrepancy can be explained by the use of different antibodies, staining conditions, scoring methods, and/or variations in preanalytical tissue processing.

Although KIT expression is obviously infrequent in breast cancer, it is still possible that a small fraction of breast cancer patients could potentially benefit from STI571 therapy. This may be particularly true in the case for medullary breast cancers, in which 19.1% positive cases were observed. However, because of the rather good prognosis of medullary cancers, only a few of these patients may need to be enrolled in one of the clinical trials open at present. The increased frequency of KIT positivity detected in papillary and apocrine cancers may be less meaningful because only one or two positive cases were observed in these rare tumor types. At present there is no evidence suggesting that KIT-positive breast cancers can benefit from STI571 therapy. Because the KIT protein is expressed in normal breast epithelium, it seems that KIT expression is down-regulated rather than up-regulated in most breast cancers in a small tumor subset. The lack of KIT mutations in our 10 tumors sequenced for exons 2, 8, 9, 11, 13, and 17 also does not provide evidence for KIT activation in breast cancer. It has been suggested that the response rate to STI571 may be dependent on the presence and type of KIT mutation in the tumor cells (18–24). If this is true, our mutation analysis data further argued against a possible response of KIT-positive breast cancers to STI571 therapy. However, other authors have reported some therapeutic effect in KIT-positive tumors expressing wild-type protein (57).

Taken together, our data show that immunohistochemically detectable KIT protein is infrequent in breast cancer. Although this may reflect preservation of "normal level KIT expression" in breast epithelium and no KIT mutations were found, studies are required to investigate the possible beneficial effects of imatinib mesylate in KIT-positive breast cancers.

REFERENCES


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