Imatinib Is Not a Potential Alternative Treatment for Uterine Leiomyosarcoma

We read with great interest the article written by Raspollini et al. The authors studied a series including 32 cases of uterine leiomyosarcomas, which showed a 50% rate of positive immunostaining for KIT. Based on these results, the authors suggested the possibility of carrying on an anti–tyrosine kinase treatment for this neoplasm. Our study aims to validate these results on a series including 18 uterine leiomyosarcoma cases, focusing on investigation of KIT signaling pathway activation. Previous approval of the Institutional Review Board was obtained. We first did a tissue microarray using representative areas of paraffin-embedded uterine leiomyosarcomas, including four 0.6 mm cores for each case. Two gastrointestinal stromal tumors (GIST) were used as a positive control. Immunostaining for KIT using two antibodies from different sources (DakoCytomation, Carpinteria, CA A4502, 1:400; BioGenex, San Ramon, CA T595, prediluted) used following manufacturers’ instructions, was negative in all our uterine leiomyosarcoma series, showing strong positivity in the mast cells infiltrating many samples of uterine leiomyosarcoma, which served as an internal control for the immunostaining. Accordingly, flow cytometric studies done on cell suspensions of the uterine leiomyosarcoma depicted in Fig. 1A showed a small cell KIT+ population with a characteristic mast cell immunophenotype (CD45+/FCRIgE+, data not shown; Fig. 1C). Other uterine leiomyosarcoma case showed lack of a KIT+ cell population (Fig. 1D). One case of GIST served as an external positive control, showing strong and extensive immunoreactivity for KIT as shown by immunohistochemistry (Fig. 1B) and flow cytometry (Fig. 1E).

Genomic DNA was extracted from paraffin-embedded tissues of the 18 uterine leiomyosarcomas, and DNA sequencing for exons 11 and 9 of c-kit was negative for mutations (data not shown). Moreover, DNA sequencing for exon 12 of PDGFRA, another tyrosine kinase receptor associated with the KIT signaling pathway, also showed lack of mutations. For a therapy for uterine leiomyosarcomas based on KIT receptor signaling blockade to be effective, constitutive phosphorylation of KIT should be present. To assess whether this was the case in our series of uterine leiomyosarcomas, we extracted proteins from frozen tissue corresponding to four cases of uterine leiomyosarcomas and one GIST, all of them included in the previously studied tissue microarray. Western blot, with an anti–c-KIT antibody (A4502, 1:400, DakoCytomation), showed a strongly positive band for the GIST and a weakly positive band in one uterine leiomyosarcoma, which we attributed to mast cell staining (Fig. 2). Nevertheless, Western blot for phospho–719Tyr-KIT (Cell Signaling, Inc., Beverly, MA) was positive only in the GIST but negative in all four uterine leiomyosarcomas (Fig. 2), indicating this particular residue, which is key for the KIT pathway activation (2), was not phosphorylated. Moreover, Western blot for anti–phosphotyrosine residues (Upstate Biotechnology, Lake Placid, NY) after immunoprecipitation with KIT showed the analogous results: a strongly positive
control band in the GIST and absence of band in uterine leiomyosarcoma cases, indicating the lack of any phosphorylated tyrosine residues in KIT protein (Fig. 3). KIT receptor overexpression in uterine leiomyosarcoma remains controversial (3–8). KIT is a tyrosine kinase receptor that has been involved in the molecular pathogenesis of several tumor types (GIST, seminoma, small cell lung carcinoma, endometrial carcinomas, ovarian epithelial carcinomas, etc.; ref. 9) and, more recently, of uterine leiomyosarcomas (1). KIT, however, needs to be phosphorylated to start its signaling cascade. We have shown that the uterine leiomyosarcomas included in this study show no phosphorylation of KIT, as shown by Western blot and immunoprecipitation in the absence of either kit or PDGFR mutations.

The antibody for total KIT we used in the Western blot/immunoprecipitation studies as well as one of those used in the immunohistochemical studies (DakoCytomation A4502) has been recently shown to be the most sensitive and specific antibody (less background staining) in a comparative study done with seven different KIT antibodies using tissue microarrays (10). Our study shows that validation of a new therapeutic target requires a combined technical approach. Our results also indicate that KIT is probably not involved in uterine leiomyosarcoma pathogenesis, and that KIT is not a very likely target for anti–tyrosine kinase drug therapy in uterine leiomyosarcoma.
Fig. 1. Expression of KIT receptor. A, representative immunohistochemical study of KIT receptor in paraffin-embedded tissue of a uterine leiomyosarcoma included in the tissue array (anti-CD117, DakoCytomation A4502) showing lack of KIT immunostaining in the tumor cells and a mast cell with a strong staining with KIT, which served as a positive internal control. Inset, typical plasma membrane staining pattern (×400; inset, ×1,000). B, a 0.6 mm core from a GIST used as a positive control for KIT immunostaining. We can observe spindle tumor cells with eosinophilic fibrillar cytoplasm and ovoid vesicular nuclei. The inset allows us to appreciate strong and extensive immunoreactivity with a plasma membrane/cytoplasm pattern. C–E, illustrative bivariate dot plot histograms of immunophenotypic expression of c-kit (CD117) on two uterine leiomyosarcomas (C and D) and a GIST (E), as analyzed by multiparameter flow cytometry. As illustrated in (E), high CD117 expression was observed in the GIST tumor, whereas CD117 was either absent or expressed in a minor proportion of all cells (<1%) of the two uterine leiomyosarcomas (LMS1 and LMS2, C and D, respectively). Interestingly, CD117+ cells from the GIST tumor were CD45 negative; those from the tumor displayed in (C) were CD45+/FCCR1gE1+, a phenotype highly suggestive of a mast cell origin (data not shown).

Fig. 2. Expression and functionality of KIT receptor. Western blotting was done using four frozen LMS samples previously included in the tissue array; a frozen sample from a GIST also included in the tissue array was used as a positive control. 4B42 is a Ewing tumor sample and LAN1 is a neuroblastoma cell line. Top, to assess phosphorylation of KIT, we incubated the membrane with an antibody for the phosphorylated form of KIT p-KIT 719Tyr. An intense band for the GIST was observed with no expression in any LMS. A673 and TC71, two Ewing tumor cell lines used as a positive control, were also positive. All LMS were negative. Middle, stripping and reincubation with anti-total KIT showed a strong 145 kDa band in the GIST and Ewing tumor cell lines, and a very faint one in LMS1. Bottom, h-actin was used as a loading control.

Fig. 3. Functionality of KIT receptor. Immunoprecipitation studies were done in the same samples used in Fig. 2. Western blotting using an antibody for phosphotyrosine residues showed a strong 145 kDa band only in the GIST sample, whereas no uterine leiomyosarcoma was positive. That means there is no constitutive activation of KIT pathway signaling in uterine leiomyosarcomas. TC-71 and SK-ES-1 are two Ewing tumor cell lines. 4B42 is a Ewing tumor sample.