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LETTER TO THE EDITOR

GIST with homozygous *KIT* exon 11 mutations

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Sir,

We found the article of Lasota *et al*[1] about gastrointestinal stromal tumors (GIST) with homozygous *KIT* exon 11 mutations of great interest. Indeed, most of the GISTs have heterozygous mutations, and only few studies have focused on homozygosity of *KIT* mutations in these tumors. The authors analyzed a series of 32 patients, and obtained data suggesting that most of these homozygous mutations result from a loss of *KIT* wild-type allele and duplication of the mutant allele. Could these complex mutations result from a mitotic recombination?

We would like to have more precision concerning the definition of homozygous deletion of *KIT*. Indeed, the authors said that mutations were detected by PCR amplification and direct sequencing of PCR products of tumor DNA. However, tumor DNA is always contaminated by variable

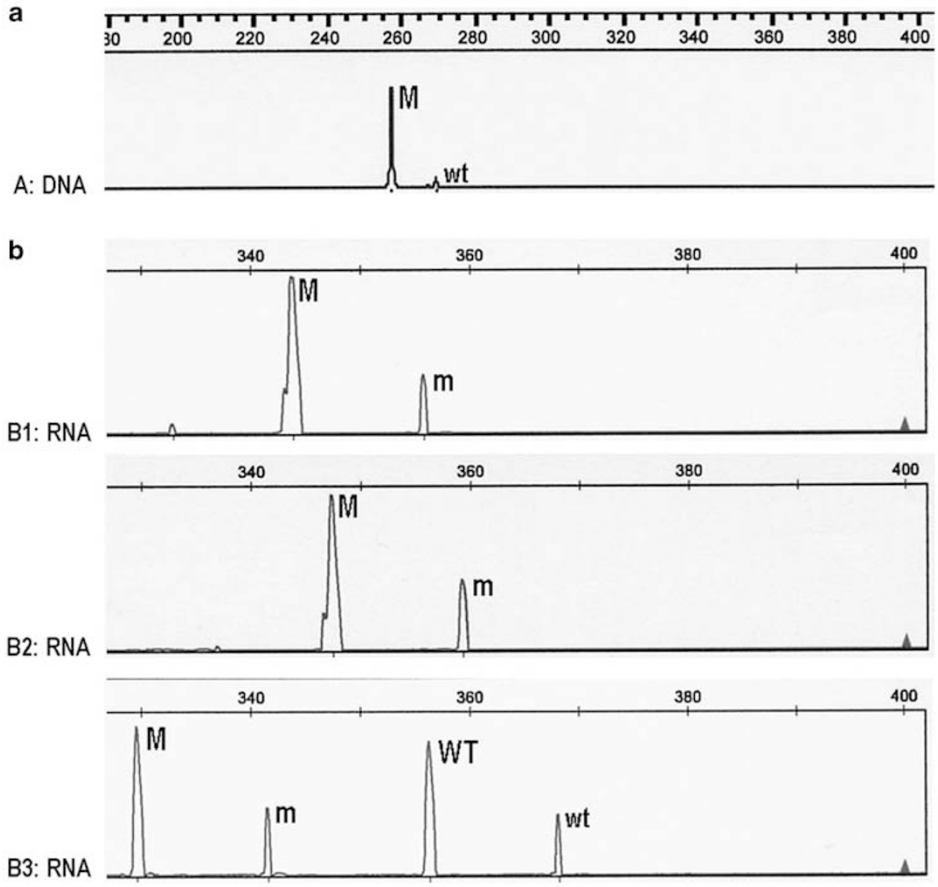

Figure 1 Detection and quantification of mutant alleles by LAPP. (**a**) Amplification of *KIT* exon 11 was performed on genomic DNA of a GIST as described previously.[2] The largest peak (M) corresponds to the mutant allele of *KIT*, while wild type (wt) is almost undetectable in this case with a homozygous mutation. (**b**) Amplification of *KIT* exon 9–12 was performed on cDNA obtained with RNA of three GISTs as described previously.[3] In all cases, the major (M) and minor (m) peaks correspond to the GNNK− and GNNK+ isoforms, respectively. Two cases with homozygous mutation (B1 and B2) only express the mutant isoforms of *KIT*, as compared with one case with a heterozygous mutations (B3), which expresses similar amounts of both alleles.

amounts of non-tumor cells and also, sequencing is not a quantitative method. Therefore, to better define homozygous deletions or insertions on tumor DNA, we suggest to perform a PCR with fluorescent probes, which allows to detect deletion and insertion mutant according to the length[2] of the products (Figure 1a). Detection of *KIT* and *PDGFRA* mutation in GISTs has been performed by several groups. Most authors first perform a screening of the mutations, followed by their identification with direct sequencing of PCR products. Among screening methods, DHPLC is the most frequently published and allows detection of deletions, insertions and substitutions, whereas length analysis of PCR products (LAPP) does not detect these latter mutations. Meanwhile, LAPP allows to quantify the fluorescence intensity of PCR products. Thus, it is possible to consider that a mutation is homozygous when the mutant/wild type ratio is higher than 1.5, as it is currently followed for loss of heterozygosity detection. Using this method, we found 26 cases with homozygous mutation in a series of 216 consecutive patients with *KIT* exon 11 mutation (12.0%), while the frequency was 36/732 (4.9%) in Lasota's series. The absence of detection of a homozygous mutation may also be related with the contamination of GIST cell DNA by the wild-type DNA from normal cells infiltrating the tumor. Therefore, a histological control is mandatory before extraction. In the rare cases of GIST with important infiltration by non-tumor cells, a microdissection of GIST cell is necessary. We also suggest to confirm homozygosity with a second PCR using a different set of probes, to eliminate a rare polymorphism inhibiting annealing of one of the first set of probes.

As the genetic causes of homozygosity, the biological consequences of homozygous *KIT* mutations in GISTs deserve to be studied. To address this question, we analyzed the transcripts of *KIT* in frozen samples of 27 GISTs with deletion or insertion in *KIT* exon 11, 16 of whom were published previously.[3] A homozygous *KIT* exon 11 mutation was detected in four cases (15%) on tumor DNA. Both wild-type and mutated alleles of *KIT* were present in similar amount in 21/22 cases with heterozygous mutations, whereas only the mutant allele was detectable in the four GISTs with homozygous *KIT* mutations (Figure 1b) and in one case with heterozygous mutations. Thus, our data show that GISTs with homozygous *KIT* mutations only express the mutant allele of KIT receptor. The mechanism implicated in homozygous expression of KIT mutant, but apparently heterozygous mutation at the DNA level, was not investigated. It might be related with a frameshift mutation in another exon of the second allele of *KIT*, as reported more recently.[4]

Homozygous *KIT* mutations may also have clinical consequences in GISTs. Indeed, Lasota's data suggest that GIST with homozygous *KIT* exon 11 mutations may have a poor prognosis as 26/29 of their cases had metastases or died of disease. In our large series of GISTs (mainly paraffin-embedded samples), we retrieved 26 patients with homozygous *KIT* exon 11 mutations, of whom 23 (88%) had recurrences or metastases. The three cases without metastasis had a follow-up of 9, 12 and 21 months, respectively. This frequency of 89% of malignant GISTs in both series is significantly higher than 44.7 and 25.7% published in unselected GISTs by our[5] and Miettinen's groups[6,7] respectively. Thus, whatever the method used for the detection, GISTs with homozygous mutation have a worse prognosis, and the prognostic value of homozygous mutation of *KIT* should be compared with tumor size, mitotic index and primary locus of tumor, in a larger series. The high malignancy of GISTs with homozygous *KIT* exon 11 mutations raises the question whether homozygous *KIT* exon 11 mutations have an intrinsic high oncogenic effect, or are a marker of late-stage tumors that have undergone multiple random genetic events. In Lasota's series, two patients had a homozygous mutation in recurrent but not in primary lesions, suggesting that it may be a secondary genetic event.

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