Re: Response of a KIT-Positive Extra-abdominal Fibromatosis to Imatinib Mesylate and KIT Genetic Analysis

We read with interest the correspondence by Goncalves et al. (1) because we have found the M541L KIT receptor mutation, in which the methionine at position 541 was replaced with a leucine, in many tumoral histotypes and healthy tissues. We have identified the A → C substitution in position 1621 of the c-Kit gene (National Center for Biotechnology Information accession number X06182) in three of 13 small-cell lung cancer specimens, six of 22 gastroesophageal X06182) in three of 13 small-cell lung cancer specimens, six of 22 gastro-intestinal stromal tumor specimens, and 11 of 31 chordoma specimens examined, as well as in one of seven normal lung specimens, one of four liver specimens, one of five skin specimens, one of five intestinal mucosa specimens, and one of two parotid gland tissue specimens.

We used site-directed mutagenesis to introduce the M541L substitution into a wild-type KIT-expressing vector and then used this vector to transiently transfect COS1 cells (monkey kidney fibroblasts, provided by American Type Culture Collection, Manassas, VA) so that we could assess whether this mutation played a role in KIT activation. Because immunoprecipitation and western blot experiments with total cell extracts and an anti-phosphorylated tyrosine antibody showed that the M541L KIT receptor was phosphorylated to a lower level than that of the positive control (KIT/Δ559) (Fig. 1, A), we concluded that the mutation is not activating. We also found that imatinib had no effect on the KIT phosphorylation status of the transfected cells.

These functional data were confirmed by molecular dynamic simulations involving our homology models of wild-type and M541L-mutant KIT in the active and inactive conformation (Fig. 1, B and C), which showed that residue 541 is located in the final part of the transmembrane helix, far from the imatinib-binding site. The corresponding side chains of the wild-type and mutant proteins are not involved in any important stabilizing interaction and point toward the solvent. Furthermore, the conservative methionine → leucine substitution did not lead to appreciable variation in the free-energy difference between the wild-type and M541L-mutant KITs or to an increase or decrease in the affinity of the receptors for ATP or imatinib.

Our experimental results and the frequency of the mutation in tumoral and normal tissues indicate that the M541L substitution is a polymorphism in the c-Kit gene, as previously demonstrated (2,3). For these reasons, we suggest that the sensitivity of fibromatosis to imatinib requires another explanation. Finally, it is worth mentioning that, because c-KIT exon 10 is not a mutation hot spot and, therefore, is not routinely screened, the frequency of this substitution is certainly underestimated.

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REFERENCES


NOTES

Editor’s note: The authors of Goncalves et al. declined to respond to this Correspondence.
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