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 gastrointestinal 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 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-mutated 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.

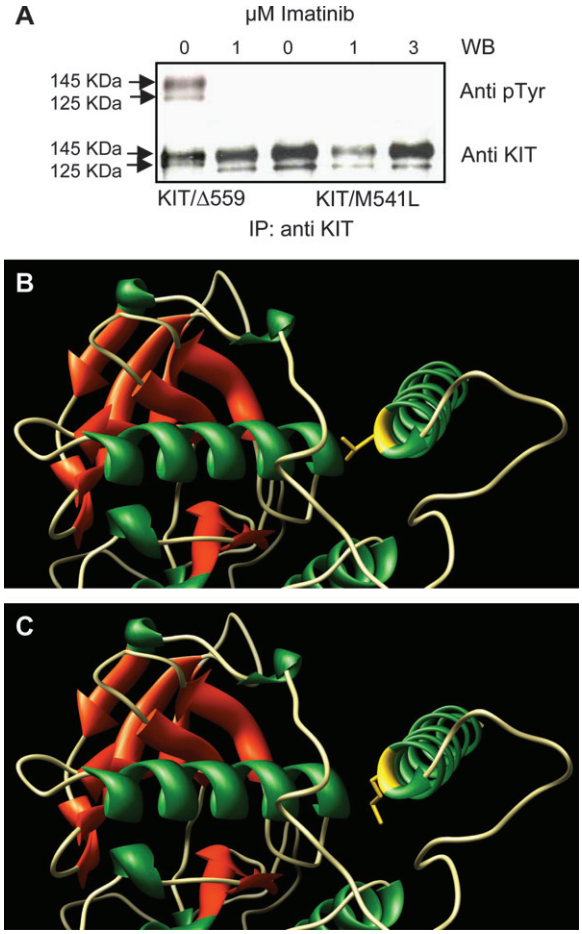

Fig. 1. Functional analysis of the KIT/M541L receptor that carries a methionine to leucine substitution at position 541. **A**) COS1 cells were transiently transfected with the c-Kit constructs (KIT/Δ559 or KIT/M541L) and then incubated with imatinib as indicated for 8 hours. The Δ559 cell line (carrying a mutation in KIT exon 11) was used as a positive control. For each sample, 1 mg of total protein extract was immunoprecipitated with anti-KIT antibody, subjected to polyacrylamide gel electrophoresis, and then subjected to western blot analysis with the indicated antibody. pTyr = phosphorylated tyrosine. **B** and **C**) Homology models of the wild-type (**B**) and M541L-mutant KIT (**C**) with the mutated residue shown in **gold**.

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

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Notes

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

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