



PDGFRA Activating Mutations in Gastrointestinal Stromal Tumors

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the apicoplast. The transit peptide is cleaved by a stromal processing peptidase (21), and the mature protein refolds with the assistance of the apicoplast-targeted GroEL homolog Cpn60 (5).

References and Notes

1. B. D. Bruce, *Biochim. Biophys. Acta* **1541**, 2 (2001).
2. The Arabidopsis Genome Initiative, *Nature* **408**, 796 (2000).
3. R. F. Waller *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 12352 (1998).
4. D. S. Roos *et al.*, *Curr. Opin. Microbiol.* **2**, 426 (1999).
5. M. J. Gardner *et al.*, *Nature* **419**, 498 (2002).
6. O. Emanuelsson, H. Nielsen, G. von Heijne, *Protein Sci.* **8**, 978 (1999).
7. P. Jarvis, J. Soll, *Biochim. Biophys. Acta* **1590**, 177 (2002).
8. R. A. Ivey 3rd, B. D. Bruce, *Cell Stress Chaperones* **5**, 62 (2000).
9. R. A. Ivey 3rd, C. Subramanian, B. D. Bruce, *Plant Physiol.* **122**, 1289 (2000).
10. D. V. Rial, A. K. Arakaki, E. A. Ceccarelli, *Eur. J. Biochem.* **267**, 6239 (2000).
11. G. von Heijne, K. Nishikawa, *FEBS Lett.* **278**, 1 (1991).
12. R. F. Waller, M. B. Reed, A. F. Cowman, G. I. McFadden, *EMBO J.* **19**, 1794 (2000).
13. G. G. van Dooren, S. D. Schwartzbach, T. Osafune, G. I. McFadden, *Biochim. Biophys. Acta* **1541**, 34 (2001).
14. S. A. Ralph, M. C. D'Ombrain, G. I. McFadden, *Drug Resist. Updates* **4**, 145 (2001).
15. J. Zuegge, S. Ralph, M. Schmuker, G. I. McFadden, G. Schneider, *Gene* **280**, 19 (2001).
16. H. Nielsen, J. Engelbrecht, S. Brunak, G. von Heijne, *Protein Eng.* **10**, 1 (1997).
17. Materials and methods are available as supporting material on Science Online.
18. T. S. Heard, H. Weiner, *J. Biol. Chem.* **273**, 29389 (1998).
19. L. Ni, T. S. Heard, H. Weiner, *J. Biol. Chem.* **274**, 12685 (1999).
20. J. C. Kissinger *et al.*, *Nature* **419**, 490 (2002).
21. G. G. van Dooren, V. Su, M. C. D'Ombrain, G. I. McFadden, *J. Biol. Chem.* **277**, 23612 (2002).
22. B. J. Foth *et al.*, data not shown.
23. S. Rudiger, L. Germeroth, J. Schneider-Mergener, B. Bukau, *EMBO J.* **16**, 1501 (1997).
24. S. Yung, T. R. Unnasch, N. Lang-Unnasch, *Mol. Biochem. Parasitol.* **118**, 11 (2001).
25. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
26. X. P. Zhang, E. Glaser, *Trends Plant Sci.* **7**, 14 (2002).
27. T. Komiya *et al.*, *EMBO J.* **17**, 3886 (1998).
28. D. Jackson-Constan, M. Akita, K. Keegstra, *Biochim. Biophys. Acta* **1541**, 102 (2001).
29. R. J. Wilson, *J. Mol. Biol.* **319**, 257 (2002).
30. _____ *et al.*, *J. Mol. Biol.* **261**, 155 (1996).
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PDGFRA Activating Mutations in Gastrointestinal Stromal Tumors

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Most gastrointestinal stromal tumors (GISTs) have activating mutations in the KIT receptor tyrosine kinase, and most patients with GISTs respond well to Gleevec, which inhibits KIT kinase activity. Here we show that ~35% (14 of 40) of GISTs lacking KIT mutations have intragenic activation mutations in the related receptor tyrosine kinase, platelet-derived growth factor receptor α (PDGFRA). Tumors expressing KIT or PDGFRA oncoproteins were indistinguishable with respect to activation of downstream signaling intermediates and cytogenetic changes associated with tumor progression. Thus, KIT and PDGFRA mutations appear to be alternative and mutually exclusive oncogenic mechanisms in GISTs.

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract and are particularly sensitive to Gleevec, a new cancer therapy (1–5). Gleevec inhibits the constitutively activated form of the KIT receptor tyrosine kinase, which is the critical transforming oncoprotein in more than 85% of GISTs. Although most GISTs have activating KIT mutations, a subset are KIT wild type (KIT-WT) (6, 7). Notably, a high level of total KIT protein expression, which is a defining feature of GISTs (2, 7, 8), is not characteristic of KIT-WT GISTs (fig. S1). However, cells of the interstitial cells of Cajal lineage— from which GISTs are thought to arise—express KIT strongly (2, 9). Therefore, activation of another oncoprotein in KIT-WT GISTs might be accompanied by KIT transcriptional down-regulation.

To explore alternative receptor tyrosine kinase (RTK) oncoproteins that might participate in GIST pathogenesis, we performed immunoprecipitations with polyclonal antisera (panRTK antisera) against peptides from regions of strong sequence conservation across the family of receptor tyrosine kinases. These panRTK antisera have been extensively validated in immunoprecipitations of ERBB2, NTRK3, ALK, and KIT kinase oncoproteins from lysates of frozen human tu-

mors (10). Phosphotyrosine immunostaining of panRTK immunoprecipitates from a KIT-WT GIST (GIST478) revealed presumptive phosphoproteins of about 150 and 170 kD, consistent with the size of immature and mature glycosylated PDGFRA, respectively (Fig. 1, A and B). We next showed that phosphorylated PDGFRA (phosphoPDGFRA) was strongly expressed and comigrated with the panRTK phosphoproteins and several of the predominant phosphorylated proteins from the GIST478 whole-cell lysate (Fig. 1, A and B). By contrast, KIT was not demonstrably phosphorylated. Therefore, phosphoPDGFRA appeared to be the predominant phosphoRTK in GIST478.

Mutually exclusive phosphoKIT and phosphoPDGFRA expression was demonstrated, respectively, in a GIST with a KIT juxtamembrane region mutation and another KIT-WT GIST (Fig. 1, C to E). We then confirmed differential phosphoPDGFRA expression in three KIT-WT and two KIT-mutant GISTs. PhosphoPDGFRA expression was restricted to the KIT-WT GISTs, where KIT expression was low to undetectable (Fig. 1, F to H). Likewise, PDGFRA expression was low to undetectable in ~70% of KIT-mutant GISTs (fig. S2). Confirmation of GIST diagnosis in KIT-WT GISTs was enabled by immunostaining for protein kinase C θ (fig. S3).

We identified PDGFRA activation loop (exon 18) mutations in the three KIT-WT GISTs that expressed phosphoPDGFRA (Fig. 1). Two of the KIT-WT GISTs had an identical PDGFRA missense mutation, leading to substitution of valine for the highly conserved aspartic acid at codon 842 (PDGFRA D842V) (11). The other KIT-WT GIST had an in-frame deletion, resulting in loss of PDGFRA amino acid residues 842 to 845 (DIMH). These PDGFRA mutations (Table 1) (figs. S4 to S6) are homologous to those responsible for KIT and FMS-

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related tyrosine kinase 3 (FLT3) ligand-independent kinase activation in human mast cell disorders, acute myeloid leukemia, and germ cell (seminoma) tumors (12–16).

We then determined the frequency of *PDGFRA* mutations in GIST formalin-fixed tissues, where *PDGFRA* phosphorylation status was not known. We evaluated *PDGFRA* genomic mutations in exons 10, 12, 14, and 18, which correspond to the *KIT* exons containing oncogenic mutations in many GISTs. We found *PDGFRA* mutations in 11 of 37 (29.7%) *KIT*-WT GISTs but not in 36 *KIT*-mutant GISTs. The somatic nature of the *PDGFRA* mutations was confirmed by genomic sequencing of non-neoplastic tissues, which were exclusively *PDGFRA* wild type, in four patients (17). The 11 *KIT*-WT GISTs with *PDGFRA* mutations included six additional tumors with D842V and one with an in-frame exon 18 deletion mutation (HDSN845-848P) overlapping the deleted residues 842 to 845 (DIMH) (Table 1) (fig. S6). The other four *PDGFRA* mutations involved the juxtamembrane region encoded by exon 12 (Table 1) (fig. S6) and would be expected to induce constitutive *PDGFRA* kinase activation. Overall, we detected *PDGFRA* mutations in 14 of 40 *KIT*-WT GISTs (35%) and in none of 36 *KIT*-mutant GISTs ($P < 0.0001$, Fisher's exact test). Thus, *KIT* and *PDGFRA* mutations appear to be mutually exclusive in GISTs.

We studied the biochemical consequences of somatic *PDGFRA* mutations by transient expression of wild-type and mutant *PDGFRA* cDNA constructs in Chinese hamster ovary (CHO) cells. Baseline tyrosine phosphorylation was weak for nonmutant *PDGFRA* and was substantially increased by ligand stimulation (Fig. 2). By contrast, baseline tyrosine phosphorylation was strong in all five of the tested *PDGFRA* mutants and was not increased by ligand stimulation (Fig. 2).

We next compared the signal transduction pathways activated in *PDGFRA*-mutant versus *KIT*-mutant GISTs. The *PDGFRA*-mutant GISTs showed uniform activation of signaling intermediates protein kinase B (AKT), mitogen-activated protein kinase (MAPK), and the STAT (signal transducers and activators of transcription) proteins Stat1 and Stat3;

Table 1. Summary of *PDGFRA* mutations in *KIT*-WT GISTs (11).

<i>PDGFRA</i> region	Mutation	Number of GISTs
Activation loop (exon 18)	D842V	8
	Del DIMH842-845	1
	Del HDSN845-848P	1
Juxtamembrane (exon 12)	V561D	1
	Ins ER561-562	1
	Del RVIES560-564	1
	Del SPDGHE566-571R	1

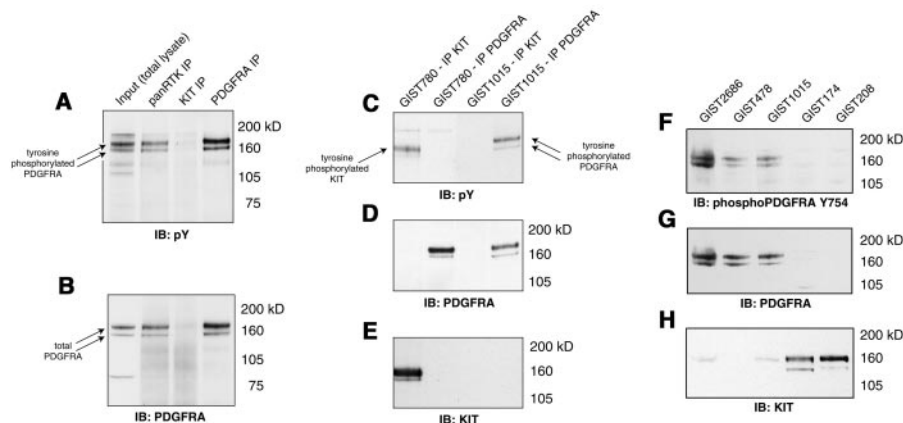


Fig. 1. Identification of phosphorylated receptor tyrosine kinases in *KIT*-mutant and *KIT*-WT GISTs. (A) Expression of phosphoRTKs and phosphoPDGFRA, but not phosphoKIT, in *KIT*-WT GIST478. Lysates were prepared from frozen tumor; immunoprecipitated with polyclonal antibodies to panRTK, KIT, and PDGFRA; and immunostained for phosphotyrosine (pY). A tyrosine-phosphorylated panRTK 150/170-kD doublet (lane 2) corresponds to two of the phosphoproteins in the total cell lysate (lane 1) and comigrates with the phosphorylated PDGFRA doublet (lane 4). There is no detectable phosphorylation of KIT (lane 3). IP, immunoprecipitate; IB, immunoblot. (B) *PDGFRA* immunostaining of the same blot confirms that the phosphorylated RTK (lane 2) is *PDGFRA*. (C) Tyrosine-phosphorylated KIT (145 kD) and *PDGFRA* (150/170 kD) are expressed in the *KIT*-mutant GIST780 and *KIT*-WT GIST1015, respectively. GIST780 does not express phosphoPDGFRA, and GIST1015 does not express phosphoKIT. (D) *PDGFRA* is detected in both *KIT*-mutant GIST780 and *KIT*-WT GIST1015. (E) KIT is detected in *KIT*-mutant GIST780 but not in *KIT*-WT GIST1015. (F to H) Immunostaining of whole-cell lysates demonstrates tyrosine-phosphorylated *PDGFRA* in *KIT*-WT GISTs (2686, 478, and 1015), whereas KIT is expressed only in *KIT*-mutant GISTs (174 and 208).

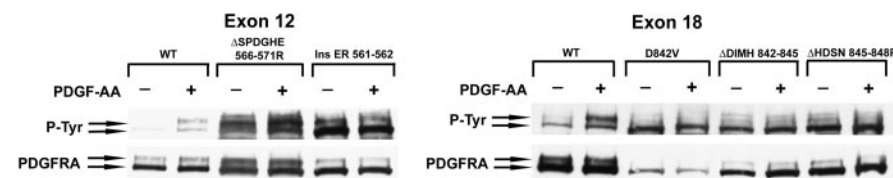


Fig. 2. *PDGFRA* mutations in GISTs result in constitutive activation of *PDGFRA* kinase. CHO cells were transiently transfected with expression vectors encoding cDNAs for wild-type (WT) or mutant *PDGFRA*. Two juxtamembrane (exon 12) and three activation loop (exon 18) mutations were tested for constitutive activation. Transfected cells were serum-starved overnight and treated with vehicle or ligand (recombinant human PDGF-AA) for 10 min. Whole-cell lysates were immunostained sequentially for phosphotyrosine and *PDGFRA*. Wild-type *PDGFRA* displays low-level phosphorylation that is up-regulated by ligand stimulation with PDGF-AA. In contrast, the mutant *PDGFRA* proteins display ligand-independent phosphorylation.

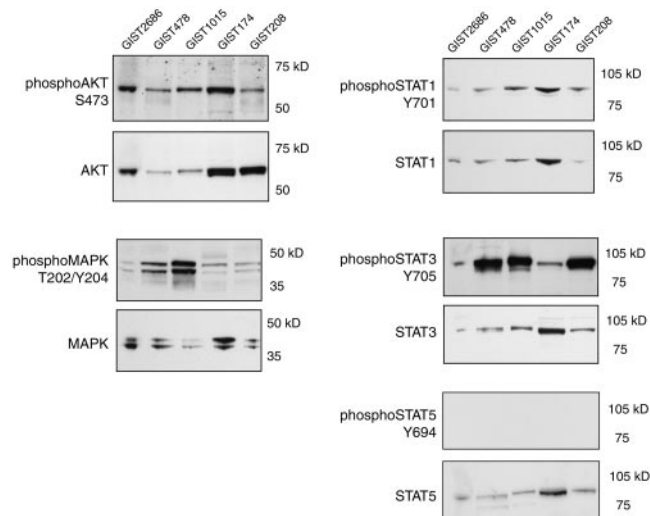


Fig. 3. Cell signaling profiles in *PDGFRA*-mutant (2686, 478, and 1015) and *KIT*-mutant GISTs (174 and 208). Whole-cell lysates were prepared from snap-frozen GISTs, and immunoblots were detected with antibodies to phosphorylated and total forms of AKT, MAPK, and STATs. All GISTs express phosphorylated AKT, MAPK, Stat1, and Stat3, whereas Stat5 is not tyrosine-phosphorylated.

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all of these are also activated in most *KIT*-mutant GISTs (Fig. 3) (18). The *PDGFRA*-mutant GISTs lacked expression of phosphoStat5 despite strong expression of total Stat5, which is also typical of *KIT*-mutant GISTs. We also compared the cytogenetic profiles of four *PDGFRA*-mutant GISTs and 52 *KIT*-mutant GISTs. *KIT* mutations are early events in GIST tumorigenesis, whereas cytogenetic aberrations occur later in disease progression (8). Most of these GISTs, regardless of *PDGFRA* or *KIT* mutation, featured noncomplex karyotypes with deletions of chromosome 1p and with monosomies of chromosomes 14 and 22 (table S1 and fig. S7). Hence, our studies suggest that the mechanisms of cytogenetic progression and oncoprotein-driven signal transduction are similar in GISTs expressing oncogenic forms of *PDGFRA* and *KIT*.

We conclude that activating mutations of *KIT* or *PDGFRA* are mutually exclusive oncogenic events in GISTs and that these mutations have similar biological consequences. Our data also highlight a crucial role for *PDGFRA* in the pathogenesis of a solid tumor. Notably, a translocation involving the *BCR* and *PDGFRA* genes has been described in *BCR-ABL*-negative chronic myelogenous leukemia and is predicted to result in dimerization and kinase activation of the fusion protein (19). *PDGFRA* is widely expressed in human tissues, so it will be important to determine whether *PDGFRA* mutations play a role in other human malignancies. Such tumors could be sensitive to Gleevec and other small-molecule drugs that inhibit *PDGFRA* kinase activity (20–22).

References and Notes

- M. Miettinen, J. Lasota, *Virchows Arch.* **438**, 1 (2001).
- S. Hirota *et al.*, *Science* **279**, 577 (1998).
- D. A. Tuveson *et al.*, *Oncogene* **20**, 5054 (2001).
- A. T. van Oosterom *et al.*, *Lancet* **358**, 1421 (2001).
- G. D. Demetri *et al.*, *N. Engl. J. Med.* **347**, 472 (2002).
- M. Taniguchi *et al.*, *Cancer Res.* **59**, 4297 (1999).
- B. P. Rubin *et al.*, *Cancer Res.* **61**, 8118 (2001).
- M. C. Heinrich, B. P. Rubin, B. J. Longley, J. A. Fletcher, *Hum. Pathol.* **33**, 484 (2002).
- J. D. Huizinga *et al.*, *Nature* **373**, 347 (1995).
- A. Oliveira, J. A. Fletcher, data not shown.
- Single-letter abbreviations for amino acid residues: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
- B. J. Longley, M. J. Reguera, Y. Ma, *Leuk. Res.* **25**, 571 (2001).
- H. Nagata *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 10560 (1995).
- Q. Tian, H. F. Frierson Jr., G. W. Krystal, C. A. Moskaluk, *Am. J. Pathol.* **154**, 1643 (1999).
- Y. Yamamoto *et al.*, *Blood* **97**, 2434 (2001).
- K. Spiekermann *et al.*, *Blood* **100**, 3423 (2002).
- C. L. Corless, M. C. Heinrich, data not shown.
- A. Duensing, J. A. Fletcher, data not shown.
- E. J. Baxter *et al.*, *Hum. Mol. Genet.* **11**, 1391 (2002).
- E. Buchdunger *et al.*, *J. Pharmacol. Exp. Ther.* **295**, 139 (2000).
- N. A. Lokker, C. M. Sullivan, S. J. Hollenbach, M. A. Israel, N. A. Giese, *Cancer Res.* **62**, 3729 (2002).
- L. Sun *et al.*, *J. Med. Chem.* **43**, 2655 (2000).
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Spongiform Degeneration in *mahoganoid* Mutant Mice

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mahoganoid is a mouse coat-color mutation whose pigmentary phenotype and genetic interactions resemble those of *Attractin* (*Atrn*). *Atrn* mutations also cause spongiform neurodegeneration. Here, we show that a null mutation for *mahoganoid* causes a similar age-dependent neuropathology that includes many features of prion diseases but without accumulation of protease-resistant prion protein. The gene mutated in *mahoganoid* encodes a RING-containing protein with E3 ubiquitin ligase activity in vitro. Similarities in phenotype, expression, and genetic interactions suggest that *mahoganoid* and *Atrn* genes are part of a conserved pathway for regulated protein turnover whose function is essential for neuronal viability.

Pigment-type switching in mice is a model system for several aspects of cell and animal physiology in which a paracrine ligand, Agouti protein, binds to the melanocortin-1 receptor (*Mc1r*) and *Attractin* (*Atrn*), causing melanocytes to produce yellow instead of black pigment (1–5). Mutations in *Agouti*, *Mc1r*, or *Atrn* cause the “classical” coat color mutations *nonagouti*, *extension*, or *mahogany*, respectively (6). Loss of function for *Agouti* or *Mc1r* affects only pigmentation, but loss of function for *Atrn* causes spongiform encephalopathy, hypomyelination, and body tremor (7–10).

mahoganoid is another coat-color mutation whose pigmentation is very similar to that of *Atrn* (formerly *mahogany*) (11–13). Both mutations lie in the same epistasis group and suppress obesity caused by ectopic brain expression of *Agouti* (14). To investigate whether the gene mutated in *mahoganoid* carries out a neuronal function similar to that of *Atrn*, we examined two alleles of *mahog-*

anoid that differ in their effects on coat color (fig. S1). Animals carrying the original allele, *md*, synthesize small amounts of yellow pigment on the flank and the ventrum and do not develop spongy degeneration up to 5 months of age (15, 16). However, animals carrying the *md^{nc}* allele (originally known as *nonagouti curly*) develop progressive spongiform changes, first apparent in the hippocampus CA3 region at 2 months of age and later extending to multiple regions of the brain (Fig. 1A and fig. S1). Vacuolation predominates in gray matter and is associated with neuronal loss but preservation of tissue architecture; the cerebral cortex, hippocampus, thalamus, brain stem, caudate-putamen, and cerebellum granule layer are the most consistently affected regions. These pathological features are nearly identical to those of *Atrn^{mg-3J}/Atrn^{mg-3J}* mutants, although the onset of neurodegeneration and its age-dependent progression in *md^{nc}/md^{nc}* animals are delayed compared to that of *Atrn^{mg-3J}/Atrn^{mg-3J}*.

By 11 to 12 months of age, many brain regions of *md^{nc}/md^{nc}* and *Atrn^{mg-3J}/Atrn^{mg-3J}* mice exhibit moderate to severe astrocytosis, the extent of which correlates with the degree of vacuolation and neuronal cell loss (Fig. 1B). In *md^{nc}/md^{nc}* mice at 10 months of age, some regions of the deep cortex and lateral thalamus exhibit mild astrocytosis before the formation of microscopically visible vacuoles. Electron microscopy showed that most vacuoles in the

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