

# Pediatric *KIT*-Wild-Type and Platelet-Derived Growth Factor Receptor $\alpha$ -Wild-Type Gastrointestinal Stromal Tumors Share *KIT* Activation but not Mechanisms of Genetic Progression with Adult Gastrointestinal Stromal Tumors

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## Abstract

Fewer than 15% of gastrointestinal stromal tumors (GIST) in pediatric patients harbor *KIT* or platelet-derived growth factor receptor  $\alpha$  (*PDGFRA*) mutations in contrast to a mutation rate of 80% in adult GISTs. However, some therapeutic inhibitors of *KIT* have efficacy in pediatric GIST, suggesting that *KIT* may, nevertheless, play an important role in oncogenesis. In adult GIST, characteristic cytogenetic changes occur during progression to malignancy. A better understanding of mechanisms of genetic progression and *KIT* and *PDGFRA* transforming roles in pediatric GIST might facilitate treatment advances. *KIT* and *PDGFRA* mutation analysis was done in 27 pediatric GISTs. The activation status of *KIT*, *PDGFRA*, and downstream signaling intermediates was defined, and chromosomal aberrations were determined by single nucleotide polymorphism assays. Mutations in *KIT* or *PDGFRA* were identified in 11% of pediatric GISTs. *KIT* and the signaling intermediates AKT and mitogen-activated protein kinase were activated in pediatric GISTs. In particular, most pediatric *KIT*-wild-type GISTs displayed levels of *KIT* activation similar to levels in adult *KIT*-mutant GISTs. Pediatric *KIT*-wild-type GISTs lacked the typical cytogenetic deletions seen in adult *KIT*-mutant GISTs. Notably, most pediatric *KIT*-wild-type GISTs progress to malignancy without acquiring large-scale chromosomal aberrations, which is a phenomenon not reported previously in malignant solid tumors. *KIT* activation levels in pediatric *KIT*-wild-type GISTs are comparable with those in *KIT*-mutant GISTs. Therapies that inhibit *KIT* activation, or crucial *KIT* signaling intermediates, should be explored in pediatric *KIT*-wild-type GIST. [Cancer Res 2007;67(19):9084-8]

## Introduction

Gastrointestinal stromal tumor (GIST) is a neoplasm of mesenchymal origin in the gastrointestinal tract of adults and children.

A key, early transforming event in 80% of adult GISTs is mutation, leading to ligand-independent activation of *KIT* or, less commonly, platelet-derived growth factor receptor  $\alpha$  (*PDGFRA*; ref. 1). In adult GIST, oncogenic *KIT* or *PDGFRA* mutations are likely sufficient for transformation, but progression from benign to malignant GIST is characterized by sequential acquisition of chromosomal deletions at 14q, 22q, 1p, and 9p (1). Whereas pediatric GIST expresses *KIT* at levels comparable with adult GIST, only 15% of the pediatric tumors harbor activating mutations in *KIT* or *PDGFRA* (2).

GIST in both pediatric and adult patients is resistant to standard chemotherapeutic agents. However, therapeutic *KIT* and *PDGFRA* inhibitors, such as imatinib and sunitinib, have prolonged survival in adult patients with metastatic GIST (3-5). Although case reports suggest only limited therapeutic benefit of imatinib in metastatic pediatric GIST (6-8), there is recent evidence that sunitinib, a more potent inhibitor of wild-type *KIT*, can slow progression in pediatric GIST (9). A rational approach to investigation and use of drugs that inhibit receptor tyrosine kinases (RTK) in pediatric GIST requires a better understanding of the *KIT* and *PDGFRA* transforming roles in these tumors. To this end, we report herein the *KIT* and *PDGFRA* genotype in 27 pediatric GISTs and correlate genotype results with the activation status of *KIT*, *PDGFRA*, and downstream signaling intermediates.

Cytogenetic aberrations in adult GIST are similar, irrespective of whether the tumor contains a *KIT* or *PDGFRA* oncogenic mutation (10). Therefore, the mechanisms of genetic progression seem to be the same in adult GISTs with various activating RTK mutations. However, it is unknown whether similar chromosomal aberrations predominate in pediatric GIST. Evaluations of pediatric GIST have shown only normal (diploid) karyotypes (11), but such findings, in malignant solid tumors generally, are apt to result from overgrowth of the neoplastic population by reactive cells, such as normal myofibroblasts, during the cytogenetic analysis (12). To determine whether chromosomal aberrations in pediatric GIST are different from those in adult GIST, we investigated genomic alterations in 15 pediatric GISTs using 10K single nucleotide polymorphism (SNP) arrays.

## Materials and Methods

**Patient materials.** Twenty-seven GISTs from patients <25 years at diagnosis were analyzed after Institutional Review Board (IRB) review and approval. All 27 cases were histologically reviewed and confirmed to be GIST by one of the authors (C.C.) and were tested for *KIT* and *PDGFRA* mutations. Fifteen cryopreserved tumor samples were available, and this subset was analyzed for *KIT* activation and *KIT* signaling by Western

**Note:** Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

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blotting and genomic changes by SNP assays. Normal tissue or peripheral blood from three of the patients and from three healthy control subjects was also analyzed by SNP assays after IRB approval.

**Reagents.** Polyclonal rabbit antibody to KIT was from DAKO. Polyclonal rabbit antibody to mitogen-activated protein kinase (MAPK) p42/44 was from Zymed Laboratories. Polyclonal rabbit antibodies to phosphorylated KIT Y721, phosphorylated AKT S473, total AKT, and phosphorylated MAPK T202/Y204 were from Cell Signaling. Polyclonal goat antibody to PKC $\theta$  was from Santa Cruz Biotechnology. Mouse monoclonal antibody to actin was from Sigma.

**Western blotting.** Whole cell lysates of cryopreserved tumors were prepared as previously described (13). The lysates were separated by gel electrophoresis using NuPAGE 4% to 12% Bis-Tris gels (Invitrogen) and blotted to nitrocellulose membranes. Immunostains were detected using enhanced chemiluminescence (ECL Amersham Pharmacia Biotech) and captured and quantified using a Fuji LAS1000-plus imaging system. A standard lane (GIST882 cell line lysate; ref. 14) was used to equalize the staining between gels.

**KIT and PDGFRA mutational analyses.** Genomic DNA was isolated from paraffin-embedded or cryopreserved tumor. *KIT* exons 9, 11, 13, and 17 and *PDGFRA* exons 12 and 18 were amplified by PCR and screened for mutations by denaturing high-performance liquid chromatography. Primers, PCR conditions, and sequencing methods have been described previously (1). cDNA sequencing of the entire *KIT* coding sequence was also done in four pediatric GISTs lacking *KIT* or *PDGFRA* genomic mutations. The SuperScript one-step reverse transcription-PCR system with Platinum Taq (Invitrogen) was used for cDNA synthesis and PCR. *KIT* cDNA was synthesized in eight overlapping segments. Primer sequences used for PCR and sequencing are given in Supplementary Table S1. Sequencing was done with an ABI 3730 xl sequencer after gel purification of the PCR products.

**SNP assay.** DNAs were isolated from cryopreserved tumor using a standard phenol-chloroform method. The DNAs were analyzed using an Affymetrix 10K SNP array at the Dana-Farber Cancer Institute Microarray Core Facility. DNA (250 ng) was digested with XbaI, and linkers were ligated to the restriction fragments to permit PCR amplification. The PCR products were then purified and fragmented by treatment with DNase I. The fragmented PCR products were labeled and hybridized to microarray chips. The positions and intensities of the fluorescent emissions were analyzed using dCHIPSNP software.<sup>9</sup> Array intensity was normalized to the array with median intensity. Median smoothing was used to infer copy number. Loss of heterozygosity (LOH) analysis was done using a Hidden Markov Model (15).

## Results and Discussion

**Patients.** Patients were 6 to 22 years at diagnosis with a mean age of 14 years. Twenty-three (85%) patients were female. Two patients had Carney Triad (cases 8 and 13). The age and gender distribution of the patients in this report are representative of previously published series of pediatric GIST (7, 16).

**KIT and PDGFRA mutation analyses.** Three of twenty-seven GISTs (11%) had *KIT* or *PDGFRA* mutations that included a homozygous *KIT* exon 11 mutation, resulting in deletion VV559-560 (case 1), a heterozygous *KIT* exon 9 mutation resulting in AY502-503 insertion (case 2), and a heterozygous *PDGFRA* exon 18 mutation resulting in D842V substitution. Patients with *KIT*-mutant GISTs were 17 and 22 years at diagnosis, and the patient with *PDGFRA*-mutant GIST was 14 years at diagnosis. To investigate the possibility that novel *KIT* mutations are present in pediatric GIST, cDNA sequencing of the entire *KIT* coding sequence was done. cDNA sequencing did not reveal mutations in four cases (cases 5, 6, 8, and 10) wherein the *KIT* and *PDGFRA* genomic mutation screening had been wild-type.

The *PDGFRA*-mutant GIST did not have sufficient tumor material for inclusion in the subset analyzed by Western blotting and SNP assays. However, it is known that, in Chinese hamster ovary cells, the D842V *PDGFRA* variant leads to constitutive activation of PDGFRA in the absence of ligand stimulation. The D842V *PDGFRA* mutation is present in over half of adult GISTs with *PDGFRA* mutations (10).

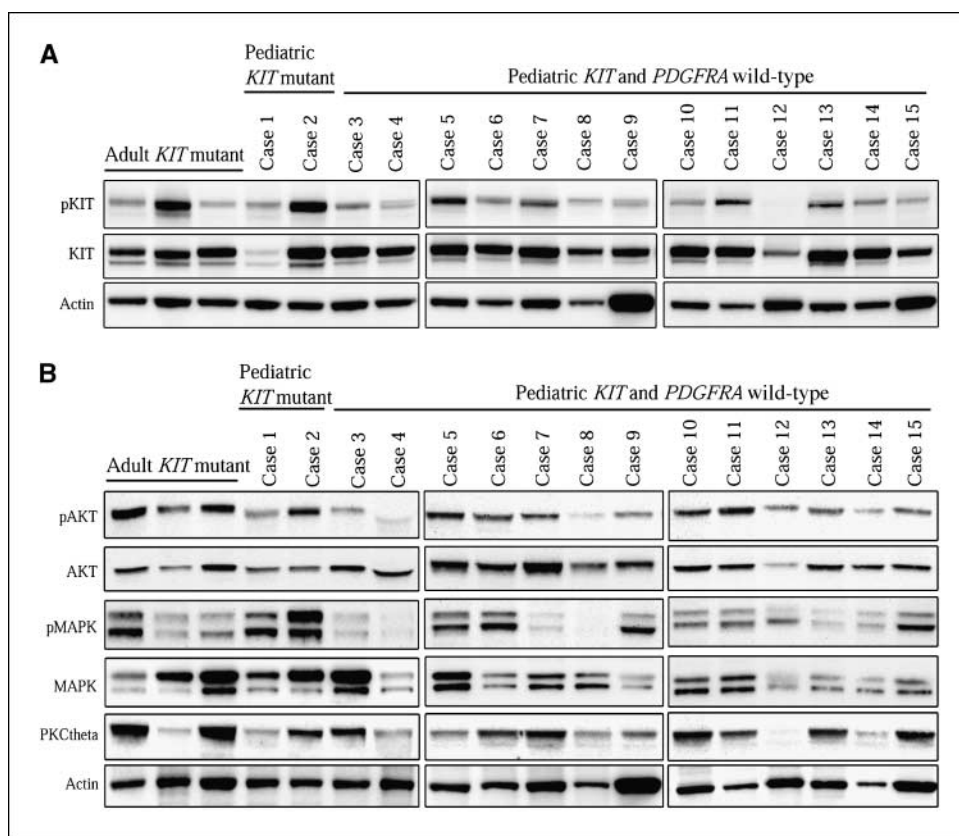
*KIT* and *PDGFRA* genotyping in these 27 cases, together with data published previously for 31 pediatric GISTs, provides strong evidence that oncogenic *KIT* and *PDGFRA* mutations are found in only 15% of pediatric GISTs (2, 8). By contrast, >80% of adult GISTs feature *KIT* or *PDGFRA* mutations, with *KIT* exon 11, *KIT* exon 9, and *PDGFRA* mutations found in 66%, 10%, and 7%, respectively (1). These findings also suggest that the distribution of *KIT* and *PDGFRA* mutations differ between pediatric and adult GISTs, with *KIT* exon 11, *KIT* exon 9, and *PDGFRA* mutations found in 5%, 9%, and 3% of pediatric GISTs, respectively.

**KIT and KIT signaling.** *KIT* and PKC $\theta$  expression are highly specific characteristics of GIST with the exception of *PDGFRA* mutant tumors, in which *KIT* expression is often nearly undetectable (13). All 15 cryopreserved pediatric GISTs variably expressed *KIT*, and 14 coexpressed PKC $\theta$ , confirming the diagnosis of GIST (Fig. 1). Twelve of thirteen *KIT*-wild-type pediatric GISTs expressed tyrosine phosphorylated *KIT*, consistent with a nonmutational mechanism of *KIT* activation (Fig. 1). In general, the level of *KIT* activation in pediatric *KIT*-wild-type GISTs was similar to that in adult and pediatric *KIT*-mutant GISTs. The AKT and MAPK downstream signaling intermediates were expressed and activated in all pediatric GISTs, except for case 8 which did not have MAPK activation. The level of total *KIT* expression was similar to adult *KIT*-mutant GISTs in all pediatric GISTs with the exception of slightly lower levels in one pediatric *KIT*-wild-type and one pediatric *KIT*-mutant case. None of the pediatric *KIT*-wild-type GISTs expressed PDGFRA (data not shown). The specificity of the phosphorylated *KIT* antibody used is underscored by the lack of phosphorylated *KIT* staining in case 12, which does express total *KIT*. The antibodies detecting phosphorylated downstream signaling intermediates have been validated in GIST cell lines treated with Imatinib (17).

*KIT* activation can serve as an initiating event in GIST oncogenesis. Early GISTs are often diagnosed incidentally and are characterized by small size and a low mitotic index. In adults, these early GISTs have activating *KIT* mutations. Furthermore, germline *KIT* mutations are associated with familial GIST syndromes. In adult *KIT*-mutant GIST, imatinib therapeutic responses correlate with *KIT* inactivation, indicating that *KIT* activation is essential in GIST progression (18). Likewise, recent clinical observations suggest that *KIT* activation might be important in pediatric GIST, even though oncogenic *KIT* mutations are uncommon. Namely, four of six pediatric patients with metastatic *KIT*-wild-type GIST had either disease stabilization or partial response during therapy with sunitinib, a potent inhibitor of wild-type *KIT*<sup>10</sup> (9). In the present study, we find that most pediatric *KIT*-wild-type GISTs have *KIT* activation at levels comparable with *KIT*-mutant GISTs. Together with the therapeutic responses to sunitinib, these findings suggest that *KIT*, although lacking mutations, nonetheless might provide an important transforming mechanism in pediatric GIST. Thus, therapies that inhibit *KIT* activation or crucial *KIT* signaling intermediates should be explored in pediatric *KIT*-wild-type GIST,

<sup>9</sup> <http://biosun1.harvard.edu/complab/dchip/snp>

<sup>10</sup> K.A. Janeway, personal communication.



**Figure 1.** Expression and activation of KIT (A) and KIT signaling intermediates (B) shown by immunoblotting in *KIT*-wild-type pediatric GIST ( $n = 13$ ), *KIT*-mutant pediatric GIST ( $n = 2$ ), and *KIT*-mutant adult GIST ( $n = 3$ ).

and potent inhibitors of wild-type KIT might be particularly effective in this disease.

There are several mechanisms by which KIT might be activated in *KIT*-wild-type pediatric GISTs. One possibility is an autocrine/paracrine loop involving overexpression of the KIT ligand stem cell factor. There is precedent for such a mechanism in dermatofibrosarcoma protuberans, wherein PDGFRB activation results from a genomic mutation, causing overexpression of PDGFB (19). Alternatively, KIT activation in *KIT*-wild-type pediatric GIST could be due to impaired degradation, as can result from mutation of a Cbl ubiquitin-protein ligase or the adaptor containing PH and SH2 domains protein (20). Or, unchecked KIT activation could result from a phosphatase mutation, such as has been reported in juvenile myelomonocytic leukemia (21). Finally, tyrosine kinase heterooligomerization could result in KIT cross-phosphorylation by another RTK family member. This type of heterooligomerization is well-described in the epidermal growth factor receptor family. In addition, forced interaction between KIT and FLT-3 has been shown to result in KIT activation and cell proliferation (22).

*In vitro* and *in vivo* data confirm that activation of MAPK and AKT is dependent upon KIT activation in *KIT*-mutant GIST. In GIST cell lines, treatment with the selective KIT inhibitor imatinib abolishes KIT, AKT, and MAPK activation. Furthermore, analysis of GIST biopsies show complete inhibition of KIT, AKT, and MAPK shortly after initiation of imatinib (18). The KIT-dependent nature of AKT and MAPK activation in most adult GISTs suggests that AKT and MAPK activation in pediatric wild-type GISTs might also be regulated by KIT. Alternatively, AKT and MAPK activation in some pediatric GISTs might not be entirely regulated by KIT or PDGFRA signaling. Such possibilities are underscored by our immunoblotting data, which suggest that signaling relationships vary in pediatric

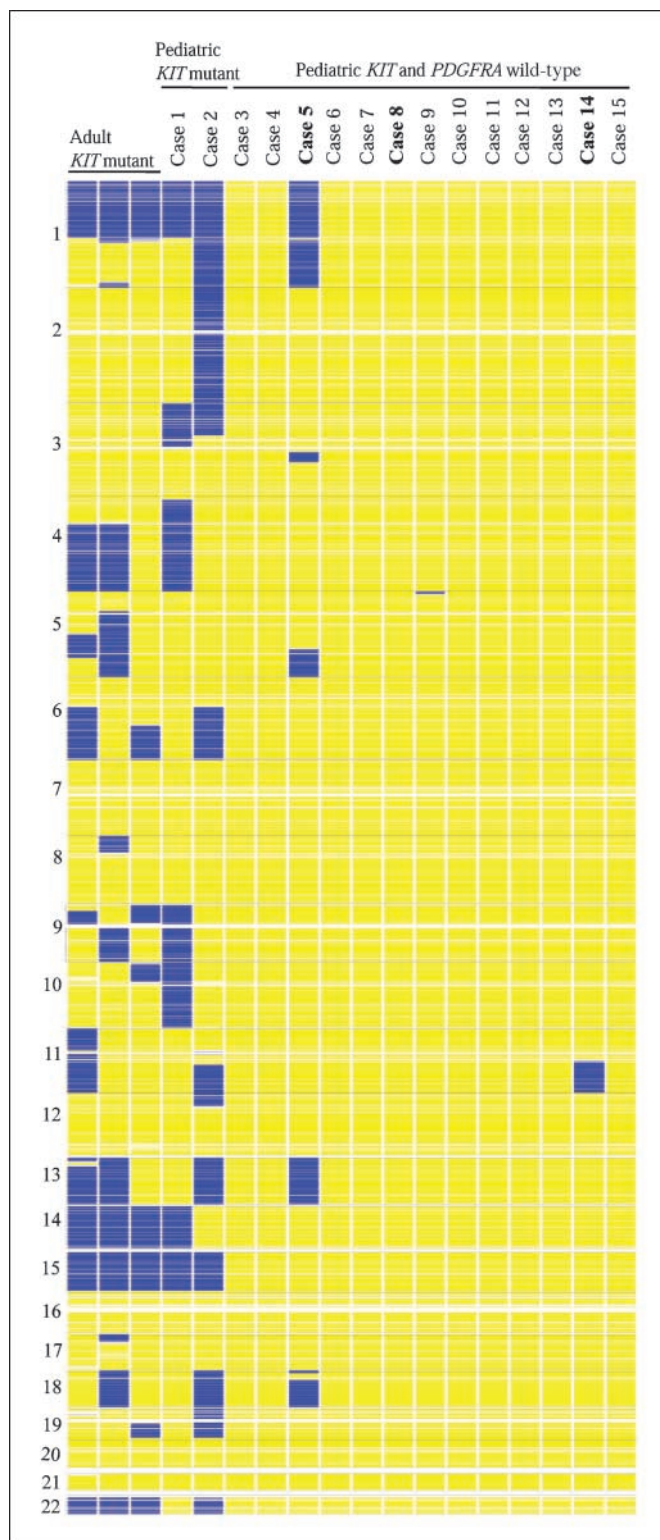
GISTs. Case 8, with a moderate level of KIT phosphorylation, had minimal AKT and no MAPK phosphorylation, whereas case 12, with no KIT phosphorylation, had a moderate level of AKT and MAPK phosphorylation. One explanation for this observation is the variable participation of additional upstream kinases. A subset of the *KIT*-wild-type GISTs reported herein were screened with a proteomic technique (10) for strongly activated RTKs. However, other than KIT, no strongly activated RTKs were identified.<sup>11</sup>

**SNP analysis.** SNP analyses of three adult *KIT*-mutant GISTs showed typical cytogenetic alterations, including LOH at 1p and LOH and decreased copy number at 14q and 22q (Fig. 2). Each of these adult *KIT*-mutant GISTs had additional regions of LOH and copy number change, spanning large numbers of SNPs. The two pediatric *KIT*-mutant GISTs had chromosomal changes typical of adult *KIT*-mutant GIST. Case 1 had loss of 1p, 9p, and 14q, whereas case 2 had loss of 1p and 22q. Both of these pediatric *KIT*-mutant cases, like the adult *KIT*-mutant cases, had multiple additional large chromosomal regions with LOH and copy number change. In contrast, the pediatric *KIT*-wild-type cases lacked the typical cytogenetic deletions (1p, 14q, and 22q) seen in *KIT*-mutant GISTs, and indeed had minimal areas of LOH or copy number change. Large chromosomal region(s) with LOH or copy number change were seen in only three *KIT*-wild-type pediatric GISTs. Case 9 had 5p LOH with apparent trisomy in this region, consistent with allelic duplication and retention of heterozygosity rather than true LOH. Case 14 had 11q LOH without associated copy number change, consistent with true LOH, followed by allelic duplication. Case 5 had multiple regions of apparent LOH, including 1p, 3q, 5q, and

<sup>11</sup> Unpublished data.

chromosomes 13 and 18. There was also 1p LOH but without a decrease in copy number.

Diploid karyotypes are seen frequently in benign solid tumors but not in malignant or metastatic solid tumors (12). In a study of



**Figure 2.** SNP LOH display shows few LOH regions in *KIT*-wild-type pediatric GISTs compared with *KIT*-mutant pediatric or adult GISTs. Cases in bold have companion normal DNAs. Blue, LOH; yellow, retained heterozygosity.

24 “benign” adult GISTs, characterized by <2 mitoses/hpf and lack of clinical recurrence, all had chromosomal aberrations, and the most common changes were loss of 1p, 14q, and 22q (23). Malignant adult primary GISTs and metastatic GISTs have still more cytogenetic changes with a mean of nine demonstrable aberrations in metastatic GISTs. In our group of pediatric *KIT*-wild-type GISTs lacking chromosomal aberrations, one GIST (case 12) was high risk (Fletcher classification) based on mitoses, two GISTs (cases 3 and 6) were metastatic, and two GISTs recurred after resection (cases 8 and 15). If genetic mechanisms of progression in pediatric GIST mirrored those in adult tumors, each of these high-grade pediatric GISTs would be expected to contain multiple large-scale chromosomal changes.

Notably, our present findings show that pediatric malignant GISTs are the first clinically aggressive solid tumor, in which cytogenetic aberrations, even when queried by high-resolution SNP assays, are undetectable in most cases. The normal genomic profiles in these malignant pediatric GISTs are representative of the neoplastic population based on pathologic examination of cryopreserved specimen regions, showing 10% or less nonneoplastic cells, from which DNAs for SNP assays were extracted (further details available in Supplementary Material). In addition, protein and DNA isolates were prepared from the exact same tumor aliquot in each case, and immunoblotting studies showed variable expression of the GIST-specific biomarkers *KIT* and *PKCθ* in all but one of these samples. Further, concurrent SNP analyses in the adult GISTs and pediatric *KIT*-wild-type GISTs (which, like pediatric *KIT*-wild-type GISTs, are composed primarily of neoplastic cells) showed numerous large-scale chromosomal deletions in all cases.

The methods used for analysis of the SNP data do not require matched normal DNA to identify large-scale genetic changes in heterozygosity or copy number. As a demonstration of this point, the cases in which SNP analysis identified the greatest extent of LOH and copy number change were those that lacked matched normal DNA (see Fig. 2), whereas one case (case 8) with matched normal DNA had no large-scale genetic changes. SNP (10K) analysis, without the use of matched normal controls, has been validated as a highly sensitive method for detecting regions of copy number change compared with genomic hybridization (CGH) or karyotyping (24).

Data from CGH array analyses and karyotypes (23, 25) of adult GIST strongly support the conclusion that chromosomal changes contribute to clinical progression. Similar chromosomal regions are affected in most GISTs, and the pattern of genetic changes is similar in *KIT*-mutant and *PDGFRA*-mutant GISTs (10). Finally, there is similarity between GIST and other oncogene-driven malignancies, such as chronic myelogenous leukemia, in which cytogenetic abnormalities, after the acquisition of an oncogene, are progressively acquired during disease progression.

The markedly different pattern of chromosomal aberrations in pediatric *KIT*-wild-type versus pediatric and adult *KIT*-mutant GIST suggests that, despite sharing *KIT* activation, these tumors likely have quite distinct mechanisms of genetic progression. In that sense, our findings show that pediatric and adult GISTs differ genetically and biologically. By CGH analysis, the genetic pattern of one pediatric GIST in a Carney Triad patient also differed from the pattern seen in adult tumors (2). Similarly, a cDNA profiling study has shown that pediatric GISTs cluster separately from adult GISTs (7). The paucity of chromosomal changes in most *KIT*-wild-type malignant pediatric GISTs suggests that substantial accumulation of additional genetic events does not occur or more likely occurs

via highly localized genetic alterations, such as intragenic point mutations or balanced cytogenetic rearrangements, which are undetected by conventional high-density SNP assays. It is also possible that GIST genetic progression in pediatric patients results, at least in part, from epigenetic phenomena, such as gene promoter methylation, which are undetected by the genomic evaluations used in this study.

In summary, this study shows that pediatric *KIT*-wild-type GISTs exhibit *KIT* activation at levels comparable with *KIT*-mutant pediatric and adult GISTs although they do not have *KIT* (or *PDGFRA*) mutations. In addition, genetic progression seems to be very different in *KIT*-mutant versus *KIT*-wild-type pediatric GISTs. *KIT*-mutant pediatric GISTs have large-scale chromosomal aberrations similar to those seen in *KIT*-mutant adult GISTs,

whereas *KIT*-wild-type pediatric GISTs have few large-scale chromosomal aberrations. Our findings suggest that targeted therapies for pediatric GIST should focus on inhibitors of *KIT* activation or signaling molecules downstream of *KIT* with an emphasis on those agents that strongly inhibit wild-type *KIT*.

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## References

- Corless CL, Fletcher JA, Heinrich MC. Biology of gastrointestinal stromal tumors. *J Clin Oncol* 2004;22:3813-25.
- Agaimy A, Pelz A, Corless C, et al. Epithelioid gastric stromal tumours of the antrum in young females with the Carney triad: A report of three new cases with mutational analysis and comparative genomic hybridization. *Oncol Rep* 2007. Ref Type: Generic.
- Blanke CD, Demetri GD, von Mehren M, et al. Long-term follow-up of a phase II randomized trial in advanced gastrointestinal stromal tumor (GIST) patients (pts) treated with imatinib mesylate. *J Clin Oncol* 2006;24:9528.
- Demetri GD, van Oosterom A, Blackstein M, et al. Phase 3, multicenter, randomized, double-blind, placebo-controlled trial of SU11248 in patients following failure of imatinib for metastatic GIST [abstract]. ASCO Annual Meeting 2005.
- Demetri GD, van Oosterom AT, Garrett CR, et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. *Lancet* 2006;368:1329-38.
- Durham MM, Gow KW, Shehata BM, Katzenstein HM, Lorenzo RL, Ricketts RR. Gastrointestinal stromal tumors arising from the stomach: a report of three children. *J Pediatr Surg* 2004;39:1495-9.
- Prakash S, Sarrao L, Socci N, et al. Gastrointestinal stromal tumors in children and young adults: a clinicopathologic, molecular, and genomic study of 15 cases and review of the literature. *J Pediatr Hematol Oncol* 2005;27:179-87.
- Kuroiwa M, Hiwatari M, Hirato J, et al. Advanced-stage gastrointestinal stromal tumor treated with imatinib in a 12-year-old girl with a unique mutation of PDGFRA. *J Pediatr Surg* 2005;40:1798-801.
- Janeway KA, Matthews DC, Butrynski JE, et al. Sunitinib treatment of pediatric metastatic GIST after failure of imatinib. *J Clin Oncol* 2006;24:9519.
- Heinrich MC, Corless CL, Duensing A, et al. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science* 2003;299:708-10.
- Li P, Wei J, West AB, Perle M, Greco MA, Yang GC. Epithelioid gastrointestinal stromal tumor of the stomach with liver metastases in a 12-year-old girl: aspiration cytology and molecular study. *Pediatr Dev Pathol* 2002;5:386-94.
- Fletcher JA, Kozakewich HP, Hoffer FA, et al. Diagnostic relevance of clonal cytogenetic aberrations in malignant soft-tissue tumors. *N Engl J Med* 1991;324:436-42.
- Duensing A, Medeiros F, McConarty B, et al. Mechanisms of oncogenic *KIT* signal transduction in primary gastrointestinal stromal tumors (GISTs). *Oncogene* 2004;23:3999-4006.
- Bauer S, Yu LK, Demetri GD, Fletcher JA. Heat shock protein 90 inhibition in imatinib-resistant gastrointestinal stromal tumor. *Cancer Res* 2006;66:9153-61.
- Lin M, Wei LJ, Sellers WR, Lieberfarb M, Wong WH, Li C. dChipSNP: significance curve and clustering of SNP-array-based loss-of-heterozygosity data. *Bioinformatics* 2004;20:1233-40.
- Miettinen M, Lasota J, Sobin LH. Gastrointestinal stromal tumors of the stomach in children and young adults: a clinicopathologic, immunohistochemical, and molecular genetic study of 44 cases with long-term follow-up and review of the literature. *Am J Surg Pathol* 2005;29:1373-81.
- Bauer S, Duensing A, Demetri GD, Fletcher JA. *KIT* oncogenic signaling mechanisms in imatinib-resistant gastrointestinal stromal tumor: PI3-kinase/AKT is a crucial survival pathway. *Oncogene* Epub 4 June, 2007; doi: 10.1038/sj.onc.1210558.
- Heinrich MC, Corless CL, Blanke CD, et al. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. *J Clin Oncol* 2006;24:4764-74.
- Simon MP, Pedeutour F, Sirvent N, et al. Deregulation of the platelet-derived growth factor B-chain gene via fusion with collagen gene COL1A1 in dermatofibrosarcoma protuberans and giant-cell fibroblastoma. *Nat Genet* 1997;15:95-8.
- Peschard P, Park M. Escape from Cbl-mediated downregulation: a recurrent theme for oncogenic deregulation of receptor tyrosine kinases. *Cancer Cell* 2003;3:519-23.
- Tartaglia M, Mehler EL, Goldberg R, et al. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. *Nat Genet* 2001;29:465-8.
- Otto KG, Jin L, Spencer DM, Blau CA. Cell proliferation through forced engagement of c-Kit and Flt-3. *Blood* 2001;97:3662-4.
- El-Rifai W, Sarlomo-Rikala M, Andersson LC, Knuutila S, Miettinen M. DNA sequence copy number changes in gastrointestinal stromal tumors: tumor progression and prognostic significance. *Cancer Res* 2000;60:3899-903.
- Carr J, Bown NP, Case MC, Hall AG, Lunec J, Tweddle DA. High-resolution analysis of allelic imbalance in neuroblastoma cell lines by single nucleotide polymorphism arrays. *Cancer Genet Cytogenet* 2007;172:127-38.
- Heinrich MC, Rubin BP, Longley BJ, Fletcher JA. Biology and genetic aspects of gastrointestinal stromal tumors: *KIT* activation and cytogenetic alterations. *Hum Pathol* 2002;33:484-95.