Cloning of the t(1;5)(q23;q33) in a Myeloproliferative Disorder associated with Eosinophilia: Involvement of PDGFRB and Response to Imatinib

Short Title: Imatinib in MPD with t(1;5) and novel PDGFRB fusion

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ABSTRACT:

Eosinophilia is common in myeloproliferative disorders (MPD) with abnormalities of chromosome band 5q31-33, including those that present with t(1;5)(q23;q33). With the development of rational drug therapy, characterization of the molecular targets for these translocations could guide treatment and impact on patient survival. We cloned the t(1;5)(q23;q33) and showed that it fuses PDGFRB to the coiled-coil domains of a novel partner protein, Myomegalin. Using two-color interphase FISH, we also demonstrated that the eosinophils are clonal in these disorders. Imatinib mesylate has recently been shown to be efficacious in MPDs with PDGFR activation. Therefore, following our molecular studies, we were able to redirect this patient’s treatment. Although she had refractory and progressive disease, once imatinib was started complete clinical and hematological remission, and major cytogenetic response was achieved. Given the therapeutic implications, our findings stress the need to aggressively investigate the molecular basis of these diseases, with emphasis on the PDGFR family.

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INTRODUCTION:

The cloning of genes affected by recurrent chromosomal translocations has furthered our understanding of the molecular basis of hematological malignancies, and allowed the development of molecular assays that have improved diagnosis and monitoring of therapy. It has also led to the development of rational targeted drug therapy, including the specific tyrosine kinase inhibitor, imatinib mesylate \(^1\) (Gleevec, Novartis).

Eosinophilia is common in several myeloproliferative disorders (MPD), notably chronic myeloid leukemia (CML) and the 8p11 myeloproliferative syndrome \(^2,3\). Less common causes of eosinophilia include MPDs associated with translocations involving chromosome band 5q31-33 \(^4\). Cloning of these translocations \(^4-9\) showed that they consistently target the PDGFRB gene. In all cases, partner genes contribute oligomerization domains that constitutively activate PDGFRB’s tyrosine kinase. Recently, cryptic deletions of chromosome 4q12 resulting in activation of PDGFRA were found in cases of hypereosinophilic syndrome (HES) \(^10\), suggesting that activation of the PDGFR receptor tyrosine kinase family is a common theme in this phenotypically similar collection of disorders.

Of the MPDs with eosinophilia, one unique sub-group presents with a t(1;5)(q23;q33) \(^11,12\). Here, we describe the molecular cloning of this translocation and the clinical/translational consequences of this finding.
CASE REPORT AND METHODS:

An 11-month-old girl presented with malaise, poor feeding and hepatosplenomegaly. The initial blood count was Hb 6.0g/dl, WBC 43.9 x 10^9/L with a neutrophilic left shift, marked eosinophilia, monocytosis and thrombocytopenia. After a bone marrow examination, the diagnosis of an MDS/MPD syndrome with eosinophilia was established. Karyotype revealed a t(1;5)(q23;q33) in 100% of the bone marrow metaphases. The patient was refractory to conventional chemotherapy including etoposide, cytarabine and interferon. At the start of imatinib, one year after the diagnosis, the patient had progressive disease, and a new cytogenetic study confirmed the presence of the t(1;5) in 100% of metaphases.

All of the laboratory investigations and treatment decisions were approved by the ethics committee of the patient’s institution of origin (Hospital AC Camargo, Brazil). Written consent was obtained from the patient’s guardians.

Molecular cloning: Southern blot and 5’ RACE PCR were performed as described previously. Appropriate PCR products were cloned and sequenced.

RT-PCR: Single step RT-PCR was used for detection of the PDE4DIP PDGFRB and reciprocal PDGFB-PDE4DI fusons. To define the specific PDE4DIP (Myomegalin) isoform fused to PDGFRB, a long-range nested RT-PCR was performed. All oligos sequences are available upon request. All relevant PCR products were sequenced.
**Western blot:** Protein was isolated from the patient’s peripheral blood cells at diagnosis and two months into imatinib treatment. Immunoblotting was performed as previously described\(^{13}\), with total AKT and phosho-AKT (S473) antibodies used as directed by the manufacturer (Cell Signaling, MA).

**Two-color fluorescence in-situ hybridization:** An eosinophil-enriched cell population was isolated from the patient’s peripheral blood as described previously\(^ {14}\). BAC clones mapping centromeric (CTC-307M15, CTB-46E9, and CTB 13H5) and telomeric (CTB-5M9, CTB-108B20, and CTB-171P15) to the PDGFRB locus were from ResGen (Carlsbad, CA). BAC DNAs were hybridized to the patient’s slides as described previously\(^{3}\). After counterstaining with DAPI, images were processed using an Olympus AX70 fluorescence microscope and Genus imaging software (Applied Imaging). To assess post-treatment samples for cytogenetic response, cells were spun onto slides, fixed, and hybridized to PDGFRB probes as described above. Cells were scored for the presence of PDGFRB rearrangements on the BioView Duet Imaging system (Rehovot, Israel).

**RESULTS AND DISCUSSION**

*PDGFRB* is fused to a novel gene, *PDE4DIP*, in the t(1;5)(q23;q33). With Southern blot analysis, we detected rearrangement of the PDGFRB gene in the t(1;5) DNA (Figure 1A). Subsequently, sequencing of several 5’ RACE-PCR clones consistently showed the same novel DNA sequence fused in-frame to PDGFRB exon 11.
Database search confirmed that these sequences mapped to chromosome 1q21-23 and corresponded to a novel gene, PDE4DIP (phosphodiesterase 4D interacting protein, myomegalin)\textsuperscript{15}. Southern blot analysis with a PDE4DIP probe confirmed its disruption in this translocation (data not shown).

The chimeric PDE4DIP-PDGFRB mRNA was readily detectable in the patient with the t(1;5)(q23;q33), but not in two normal controls (Figure 1B). The reciprocal PDGFRB-PDE4DIP fusion is not expressed (Figure 1B). Myomegalin is the protein encoded by PDE4DIP and it was characterized because of its binding to the phosphodiesterase PDE4D\textsuperscript{14}. Of relevance for this report, myomegalin encodes several putative oligomerization domains capable of activating PDGFRB. They include a leucine zipper (LZ) domain and several coiled-coil structures (Fig. 1C). In humans, there are at least two major isoforms of myomegalin (KIAA0454 and KIAA0477)\textsuperscript{15}, encoding unique N and C termini (Figure 1C). This feature is of significance because one of these isoforms encodes an N-terminal LZ domain. Surprisingly, we found that in our case PDGFRB is fused to the PDE4DIP isoform lacking the LZ domain (KIAA0477) (Figure 1C) indirectly implicating the coiled-coils domains in the constitutive activation of PDGFRB.

In summary, in this fusion protein the first 905 amino acids of myomegalin are joined in-frame to the transmembrane and tyrosine kinase domains of PDGFRB (Figure 1D). It is highly likely that deregulation of PDGFRB activity is the major pathogenic defect in this MPD.
In MPD with t(1;5)(q23;q33) the eosinophils are part of the malignant clone.

Eosinophilia is a common feature of several hematological malignancies. It is generally assumed that, when a clonal cytogenetic abnormality has been demonstrated in a MDS/MPD with eosinophilia, these cells are part of the neoplastic clone. However, this is not always true\textsuperscript{16-18}, and only rarely has the clonality of the eosinophils been clearly established\textsuperscript{19}. As seen in figure 2, eosinophils are readily identified based on the autofluorescent of eosinophilic granules, a feature that is unique among cells of the granulocytic series\textsuperscript{19-21}. These granules were pseudo-colorized white to allow for clear
visualization of the FISH signals. Using a two-colour interphase FISH assay with probes flanking the PDGFRB locus, we observed that this patient's eosinophils have rearrangements of PDGFRB and are therefore components of the malignant clone (Figure 2).
Figure 2

Normal control

Eosinophils in MPD with t(1;5)(q23;q33)
Excellent response to imatinib in MPD associated with t(1;5)(q23;q33) and PDGFRB disruption. After documenting the PDGFRB disruption in this patient, she received a trial of imatinib therapy. All clinical and hematological abnormalities rapidly resolved. The bone marrow was normal at 5 months. FISH of peripheral blood cells after seven months of therapy showed the t(1;5) in only 7.1% of the cells (split apart PDGFRB signals in 8 of 106 cells, versus 0 of 210 cells from a normal control). We also evaluated the effects of imatinib on known PDGFRB targets in vivo, comparing AKT phosphorylation in the patient’s primary cells pre- and post-treatment. We demonstrated that post-treatment, there was a marked decrease in the phosphorylation of AKT (Figure 1E). Since AKT is one of the well-defined targets of PDGFRB mitogenic and transforming effects in hematopoietic cells, via activation of the PI3K pathway, these studies are consistent with imatinib specifically targeting the deregulated PDGFRB in this patient’s MPD.

In summary, we have shown that the t(1;5)(q23;q33) targets PDGFRB and that complete remission can be achieved with imatinib in a heavily pre-treated patient with progressive disease. Of interest, although only a few cases of MPD with t(1;5)(q23;q33) have been described, this disease appears to be more common in infants; it also lacks the extreme male bias found in other cases MPD associated with PDGRB activation. Our results confirm and extend the clinical relevance of imatinib treatment in cases of MPD with eosinophilia.
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FIGURE LEGENDS:

Figure 1. \textit{PDGFRB} is fused to \textit{PDE4DIP} in an MPD associated with eosinophilia and t(1;5)(q23;q33). A) Southern blot analysis of the t(1;5) and control DNA with a \textit{Hind}III-\textit{XhoI} \textit{PDGFRB} genomic probe spanning exon 11. Rearranged bands are indicated by a star. Cloning of the genomic breakpoints for this fusion confirmed that the wild-type \textit{PDGFRB} and \textit{PDE4DIP-PDGFRB} \textit{Hind}III restriction fragments are of very similar size and therefore co-migrated in the Southern blot. At DNA level, these genes fusion occurs at IVS 12 -767 (\textit{PDE4DIP}) and IVS 10 +289 (\textit{PDGFRB}). B) Single step RT-PCR confirms the expression of the \textit{PDE4DIP-PDGFRB} fusion. The reciprocal fusion is not expressed. \textit{PDGFRB} RT-PCR confirms the integrity of the cDNAs. C) Isoform-specific nested RT-PCR showing that the \textit{PDE4DIP} isoform lacking the LZ domain (KIAA 0477) is fused to \textit{PDGFRB} in the t(1;5). Diagram of the primary protein structure of human myomegalin, putative oligomerization domains, the breakpoint in the t(1;5), and the location of the primers (horizontal arrows) used in the isoform-specific nested RT-PCR. D) Diagrammatic representation of the Myomegalin (MM)-PDGFRB protein fusion and contributing nucleotides, aminoacids and domains. E) Western blot analysis showing lower levels of phosphorylated AKT (upper panel) in the patient’s primary cells two months into imatinib treatment. Total AKT is shown in the lower panel. Densitometry of relevant bands showed a 60% reduction in the expression of phospho-AKT post-imatinib (right panel).
Figure 2. The eosinophils have a rearranged PDGFRB locus and are part of the neoplastic clone. We used a two-color interphase FISH assay in an eosinophil-enriched preparation to define the clonality of these cells. Differentially labeled BAC clones located centromeric (red) or telomeric (green) to the PDGFRB breakpoint region in the t(1;5) were used as probes. Schematic representation of chromosome 5, breakpoint and probes location is shown on the left side. In normal metaphases and interphases (upper panel) the red and green signals are juxtaposed (yellow) in both chromosomes 5. In the patient’s peripheral blood eosinophils (lower panel), one pair of signals is juxtaposed (normal chromosome 5), whereas the other signals are split, indicating rearrangement of the PDGFRB locus. The eosinophilic granules were pseudo-colorized in white due to their auto-fluorescence.
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