Kinase genes in *BCR-ABL1* negative and *JAK2*V617F negative chronic myeloproliferative neoplasms (CMPNs)

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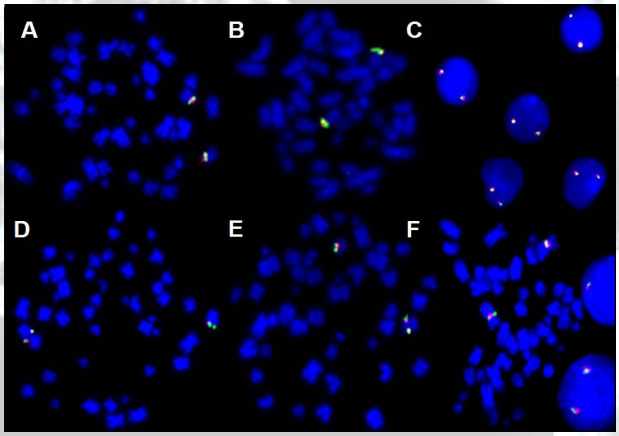
Introduction

BCR-ABL1 negative chronic myeloproliferative neoplasms (CMPNs) are a heterogeneous group of clonal haematological malignancies characterised by abnormal proliferation and survival of one or more myeloid lineage cells. Over the last years, some genetic alterations (translocations, amplifications or point mutations) have been described, most of them activating tyrosine kinases (TKs). However, there is still a significant proportion of patients in which the molecular lesion causing the disease remains to be discovered. The involvement of some TK genes and the success of the targeted anti-TK therapy point to these genes as good candidates for systematic screenings in these diseases

In this study, we used FISH to search putative cryptic rearrangements of all genes from the families III (*PDGFRA*, *PDGFRB*, *CSF1R*, *KIT* and *FLT3*) and IV (*FGFR1*, *FGFR2*, *FGFR3* and *FGFR4*) of receptor-TKs, as well as all genes from the families Jak (*JAK1*, *JAK2*, *JAK3* and *TYK2*), Abl (*ABL1* and *ABL2*) and Syk (*SYK* and *ZAP70*) of cytoplasmic-TKs. All of them code for proteins with tyrosine kinase activity and some of them have been found mutated in CMPNs and in other tumor types



FISH analysis with non-commercial flanking probes on nuclei and metaphases when available. All cases show a normal hybridization pattern with two fusion red-green signals, one for each chromosome. **A** *FGFR1* assay. **B** *FGFR2* assay. **C** *FGFR3* assay. **D** *FGFR4* assay. **E** *SYK* assay. **F** *ZAP70* assay.

Materials & methods

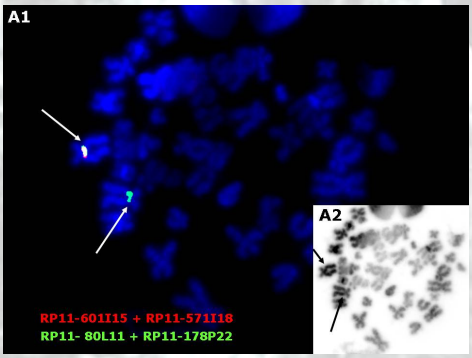
We obtained samples from 44 caucasian CMPN patients with normal karyotype (28 males and 16 females), four diagnosed with polycythaemia vera (PV), four with idiopathic myelofibrosis (IMF), 15 with essential thrombocythemia (ET) and 21 with atypical MPN (aCMPN-). *BCR-ABL1* fusion and V617F*JAK2* mutation were ruled out by conventional analyses (RT-PCR/FISH and ARMS-PCR respectively). Material for all tests was only available for 27 of the 44 patients

We designed two-colour FISH analyses with flanking probes using BAC clones according to the position showed by *UCSC Genome Browser* (http://genome.ucsc.edu) (Table). Clones were obtained from *BACPAC Resources Center* at *Children's Hospital Oakland Research Institute* (Oakland, CA) and *Clone Resources* at *The Welcome Trust Sanger Institute* (Hixton, UK)

Some of the TK genes selected for the analyses have been found fused with *ETV6* (12p13), so we included an analysis of this gene (with *Vysis LSI ETV6 Dual Color Breakapart Probe* from Abbott Molecular, Abbott Park, IL) in order to check if abnormalities in *ETV6* could unravel new TK fusions

GENE	5′ BACs	3′ BACs
PDGFRA 4q12	RP11-601I15 + RP11- 571I18	RP11-80L11 + RP11-178P22
PDGFRB 5q33	CTB-108B20 + RP11-1104C14	CTB-13H5 + RP11-69G19
CSF1R 5q33	CTB-108B20 + RP11-1104C14	CTB-46E9 + RP11-136E22
KIT 4q12	RP11-84L10 + RP11-1141K10	RP11-S86A2 + RP11-273B19
FLT3 13q12.2	RP11-502P18 + RP11-27H10	RP11-438F9 + RP11-153M24
FGFR1 8p12	RP11-118H9 + RP11-675F6	RP11-265K5 + RP11-90P5
FGFR2 10q26.12	RP11-255D5 + RP11-78A18	RP11-7P17 + RP11-466C13
FGFR3 4p16.3	RP11-42F9 + RP11-572O17	RP11-262P20 + RP11-1150B4
FGFR4 5q35.2	RP11- 606E24 + CTC-340P19	RP11- 99N22 + CTC-549A4
JAK1 1p31.3	RP11-960B3 + RP11-101O11	RP11-125L17 + RP11-947H9
JAK2 9p24.1	RP11-3H3 + RP11-23O2	RP11-60G18 + RP11-28A9
JAK3 19p13.11	RP11-767G23 + RP11-343E23	RP11-79E22 + RP11-63J1
TYK2 19p13.2	RP11-177J4 + RP11-266J17	RP11-365L4 + RP11-152C7
ABL1 9q34.11	RP11-57C19 + RP11-7M2	RP11-544A12 + RP11-643E14
ABL2 1q25.2	RP11-346D17 + RP11- 1054P1	RP11- 177A2 + RP11-595C2
SYK 9q22.2	RP11-1021I9 + RP11-80J10	RP11-652P2 + RP11-95G21
ZAP70 2q11.2	RP11-1082A11 + RP11-542D13	RP11-263L6 + RP11-973B20

A1. Metaphase FISH *PDGFRA* analysis with flanking probes. Although normal hybridization pattern would be two red-green fusion signals, we observed one red-green fusion signal from normal chr4 and an extra green signal from der(4) (white arrows). This pattern reveals a deletion of the 5´ upstream part of *PDGFRA*
A2. Light microscopy of the same metaphase as in A1



Results

Every probe was initially validated on normal samples to verify the correct hybridization and to establish a *cut-off* value of abnormal signals on interphase normal nuclei

For *PDGFRA*, we observed an abnormal FISH pattern with gene-flanking probes in an aCMPN patient (15081). Whereas the normal hybridization pattern would be two green-red fusion signals, this patient lost the red signal in 85% (170/200) of the nuclei and 8/10 of the metaphases analyzed. This pattern suggested a deletion of the 5´ part of the gene. An assay with a commercial FISH probe (QBiogene, Irvine, CA) to check this results, showed a pattern compatible with a *FIP1L1-PDGFRA* type of rearrangement, not detected by conventional RT-PCR

All other samples showed normal hybridization patterns for all the TK genes and *ETV6*

Conclusions

- FISH analysis of genes from TK families (*PDGFRA, PDGFRB, CSF1R, KIT, FLT3, FGFR1, FGFR2, FGFR3, FGFR4, JAK1*, *JAK2*, *JAK3, TYK2, ABL1, ABL2, SYK* and *ZAP70*) **did not show any new cryptic chromosomal rearrangement**
- Only in one patient a *FIP1L1-PDGFRA* fusion was detected that had been missed by conventional RT-PCR, confirming that **FISH analysis**, as other groups have shown, **is a good technique to detect the *FIP1L1-PDGFRA* gene fusion** when conventional RT-PCR fails due to the variability of breakpoints in *FIP1L1*
- Rearrangements affecting these genes are **very infrequent events** in BCR-ABL1 negative chronic myeloproliferative neoplasms (CMPNs)

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