C-CBL, CBL-B AND CBL-C ANALYSIS IN BCR-ABL1 NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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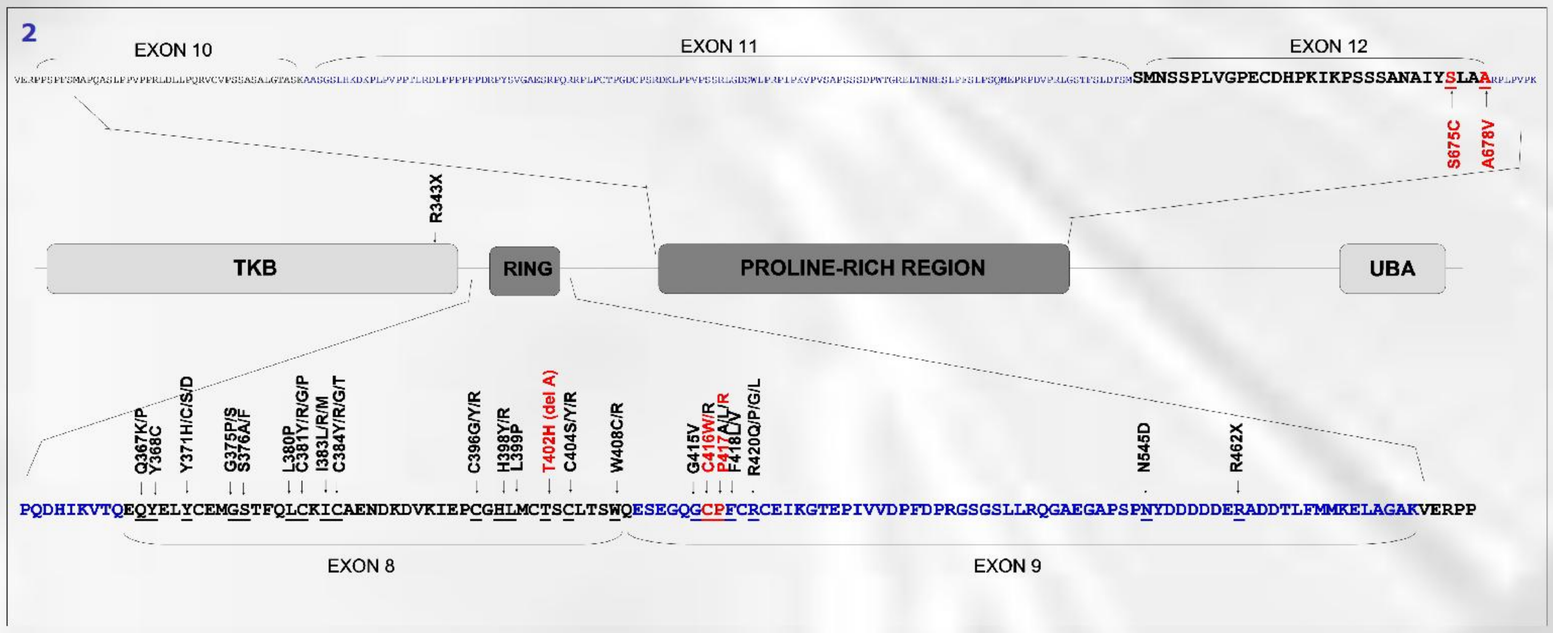
Background: *BCR-ABL1* negative chronic myeloproliferative neoplasms (CMPNs) are a heterogeneous group of clonal hematological malignancies. Some genetic aberrations have been described to cause these diseases, most of them activating tyrosine kinase (TK) genes. However, in last years, different groups have described mutations in other molecules involved in the same signalling pathways that could have important roles in the disease.

One of them is the CBL (*Casitas B-lineage Lymphoma*) family of E3-Ubiquitin-ligase proteins that are encoded by three genes: *C-CBL*, *CBL-B* and *CBL-C*. CBLs are involved in the negative regulation of several tyrosine-kinases, as EGFR, FGFR or SYK. Several groups have demonstrated that mutations in the *RING Finger* domain of C-CBL result in loss of ubiquitination and degradation of TKs (1-14). This effect contributes to deregulation of downstream targets of the signalling pathways in which they are involved. Most mutations found in this gene have been detected in AML patients, and some authors have been proposed *C-CBL* and *JAK2* mutations as exclusive events (12,14).

Methods: We have used dHPLC to detect sequence mutations on samples from 382 *BCR-ABL1* negative CMPN patients, 145 of them negative and 237 positive for the V617F*JAK2* mutation (see Figure 1). They were no 11qUPD selected. We included 20 samples from healthy individuals as controls. Firstly we analyzed the entire coding sequence of *C-CBL*, *CBL-B* and *CBL-C* in 44 of the V617F*JAK2* negative patients and in the control samples. For the remaining patients we only studied the exons coding for the *RING Finger* domain, as well as *C-CBL* exon 12 (*Proline-rich* region).

Results: We have found five non-described mutations in *C-CBL*, three of them located in the *RING Finger* domain (**T402H**, **C416W**, **P417R**), and two in the *Proline-Rich* region (**S675C** and **A678V**). T402H, P417R and S675C were found in V617F*JAK2* positive patients (S675C in two patients). In addition we could detect a non-described mutation (**R462W)** in the *RING Finger* domain of *CBL-B* in a V617F*JAK2* positive polycythemia vera patient. However, our experimental design does not allow to know if both mutations are present in the same clone or in different ones.



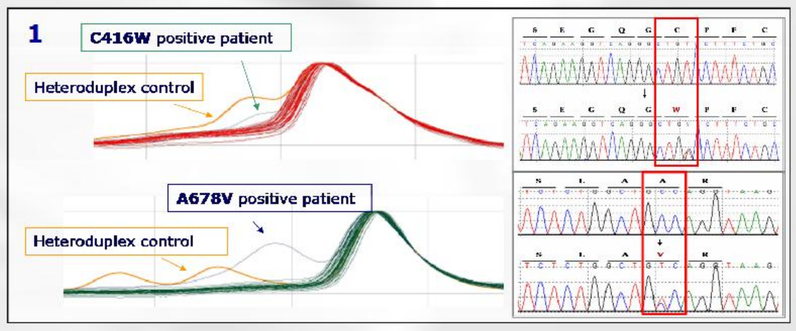


Figure 1. dHPLC elution profiles of *C-CBL* exons 9 (up) and 12 (down). Sequencing analysis showed that samples with an abnormal elution profile had **C416W** and **A678V** mutations, respectively.

Figure 2. Structure of C-CBL and position of the mutations described in the literature. The changes detected in our study are marked in red. Three of them were located in the *RING Finger* domain (T402H and P417R in an ET patient and a PMF patient both V617F*JAK2* positive, respectively, and C416W in an atypical CMPN V617F*JAK2* negative patient) and two in the *Proline Rich* region, (S675C in two V617F*JAK2* positive patients one with an ET and the other with PV).

Conclusions:

1) We have found that *C-CBL* is mutated in the 1.4% (5/382) of the CMPN patients unselected for chromosome 11qUPD

2) Mutations detected were located in the *RING Finger* domain, but also in the *Proline-rich* region of *C-CBL* in 3 of the 382 CMPNs patients. One of the *Proline-rich* region mutations (S675C) was detected in two cases (a PV and an ET).

3) Our results suggest that mutations in *CBL* genes and *JAK2* are not exclusive events: 1.7% (4/237) of V617F*JAK2* positive patients showed mutations in *C-CBL* and 1 of them in *CBL-B*

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