Lipocalin-2 is associated with modulation of disease phenotype in a patient with concurrent JAK2-V617F and BCR-ABL mutation

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Abstract

We investigated the role of lipocalin-2 (LCN-2) and its receptor (SLC22A17) in mediating clonal dominance in a patient with both BCR-ABL and JAK2-V617F mutations. LCN-2 mRNA showed a near 50-fold increase in expression, accompanied by downregulation of SLC22A17, coinciding with increase in BCR-ABL transcripts, loss of JAK2-V617F and change of clinical phenotype from polycythaemia vera to chronic myeloid leukaemia. These changes were reversed after commencing imatinib mesylate. Consistent with experimental studies, BCR-ABL+ cells express LCN-2 leading to suppression of BCR-ABL- cells and explain their eventual dominance when occurring together with JAK2-V617F.

Keywords;

Chronic Myeloid Leukemia, Myeloproliferative Disorders, BCR-ABL, JAK2-V617F, Liopcalin-2
The BCR-ABL fusion gene responsible for chronic myeloid leukaemia (CML) usually occurs mutually exclusive to the JAK2-V617F mutation associated with a proportion of myeloproliferative neoplasms (MPN). There have however been a number of case reports of both events occurring in the same patient although clonal dominance is often exhibited at any one time-point. In many of the reports, JAK2-V617F+ clones disappear with increase of BCR-ABL fusion transcripts and reappear on suppression of BCR-ABL by treatment with imatinib mesylate (IM). We observed the same in a patient we reported, who presented initially as polycythaemia vera (PV) and progressed to CML, leading us to postulate that occurrence of clonal dominance indicates the presence of two separate clones of BCR-ABL+ and JAK2-V617F+, initially mutually coexisting but eventually leading to the abolishment of JAK2-V617F cells, through as yet unexplained mechanisms.

Recently, it has been reported through a series of experiments using BCR-ABL transformed murine myeloid 32D cells that BCR-ABL+ cells express lipocalin 24p3, a secreted protein that induces apoptosis of cells containing the 24p3 cell surface receptor 24p3R, through a paracrine mechanism. It has also been demonstrated that BCR-ABL suppresses normal haematopoiesis through an active process involving secretion of 24p3 with self-repression of 24p3R. The human counterpart of 24p3 is NGAL (neutrophil gelatinase-associated lipocalin) encoded by the gene LCN2 (lipocalin-2) while the receptor, NGALR is encoded by the gene SLC22A17. We aimed to investigate whether this lipocalin may play a role in the dynamics of JAK2-V617F+ and BCR-ABL+ cells when they co-occur.

The clinical history of the patient has been reported previously. We retrieved archived serial samples stored as peripheral blood mononuclear cell lysates in Trizol, obtained from the patient over a period of 3 years; three (M-15 to M0) prior and 4 (M+6 to M+23) following commencement of IM therapy. Two of the samples (M-3, M0) were collected while the patient had JAK2-V617F mutant allele burden of less than 1%, as measured using the JAK2
MutaQuant™ assay (Ipsogen, Marseilles, France) but harboured high levels of BCR-ABL transcripts (Figure 1G). We also obtained samples from normal subjects, BCR-ABL+ CML patients on IM and JAK2-V617F+ MPN patients. Total RNA was extracted using Qiagen AllPrep DNA/RNA/Protein Mini Kit (Qiagen, Hilden, Germany) followed by reverse transcription to cDNA using the RT² First strand kit (SA Bioscience, Frederick, MD) according to manufacturer's instructions.

Real-time quantitative PCR for LCN2 and SLC22A17 gene expression was performed using TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA, USA) on a LightCycler 480 II (Roche, Mannheim, Germany) real time PCR instrument. All assays were run in triplicates in a total reaction volume of 10 µl. Both gene expressions were normalized to ACTB expression. LCN2 mRNA was expressed significantly higher in patients with JAK2-V617F+ MPN as compared to normal subjects although a difference was not observed with its receptor (Figs 1B, E). In the patient though, LCN2 was increased to supra-normal levels above that expressed even by JAK2-V617F+ cells, concomitant with escalation of BCR-ABL transcripts and change of clinical phenotype from PV to CML (Fig 1A, G).

SLC22A17 mRNA expression meanwhile showed a reciprocal relationship to LCN2 (Fig 1D). This pattern is consistent with experimental studies which show induction of 24p3 expression by BCR-ABL with concomitant down-regulation of 24p3R and abrogation of the effect on treatment with IM⁴. We also noted that LCN2 expression was abnormally low in the patient during other periods when BCR-ABL was expressed at relatively low levels. This phenomenon was also demonstrated among CML patients on IM who had not achieved major molecular remission (MMR) and harboured BCR-ABL/ABL (IS) transcript levels between 0.1 – 2% (Fig 1C).
The observations in our patient are consistent with experimental evidence from BCR-ABL transformed mouse cells, which indicate that BCR-ABL causes abnormal transcriptional activation of 24p3 while down-regulating expression of its receptor, thus escaping the pro-apoptotic effect of 24p3 while simultaneously inducing apoptosis of BCR-ABL-normal haematopoietic cells. This mechanism has been proposed to contribute to the proliferative advantage and tumourigenic activity of BCR-ABL. The misregulation is however reversed on treatment with IM. Our observations indicate that this also explains the clonal dominance that is often achieved by BCR-ABL+ cells when they co-occur with JAK2-V617F+ cells. A certain threshold of BCR-ABL+ cells may however be required before it can surmount the JAK2-V617F+ population as we observe that LCN2 expression is often unusually low in the presence of low BCR-ABL transcripts. This is also true among samples obtained from CML patients who have stable low BCR-ABL mRNA transcripts and do not achieve MMR. The repressed expression of LCN2 among this group of patients may partly explain how BCR-ABL+ cells continue to exist stably in them, as normal haematopoiesis cannot be adequately suppressed. A recent study further demonstrates that 24p3 may have regulatory roles in haematopoiesis and certain haematopoietic cell populations derived from mice lacking 24p3 develop resistance to apoptosis.

In addition, similar mechanisms involving LCN2 are also likely to operate in JAK2-V617F+ MPN as LCN2 mRNA expression was significantly increased in this group of patients. Experiments with mouse cells indicate that 24p3, the mouse equivalent to LCN2 are stimulated via the JAK/STAT pathway which results in phosphorylation and activation of STAT5, leading to transcriptional activation of 24p3. The JAK2-V617F mutation also contributes to JAK/STAT activation and likely induces LCN2 expression. A key difference may however exist between JAK2-V617F+ cells and BCR-ABL+ cells, in that JAK2-V617F may be unable to down-regulate the expression of the lipocalin receptor, as shown by the
continued increase of SLC22A17 in our patient upon return of the JAK2-V617F population. BCR-ABL represses 24p3R through the Ras/MAPK pathway, distinct from its effect on 24p3 transcription through the JAK/STAT pathway. Activation of Ras by JAK2-V617F is however not as intense as BCR-ABL, which may explain the differences in receptor expression.

One of the limitations of our observations is that all gene expression assays were performed from peripheral blood mononuclear cells which do not truly reflect the milieu of the marrow. JAK2-V617F, especially when present at low levels, require the presence of homodimeric receptors such as erythropoietin receptors and potentially heterodimeric receptors, for efficient JAK/STAT signalling and this may not be reflected in the cell populations that we studied. Nevertheless, studies on this patient provide clinical evidence for the role of BCR-ABL mediated misregulation of LCN2 in the progression of CML, and possibly JAK2-V617F+ MPN. The observations also support several important experimental evidence derived from mouse cell lines.

**Authorship and Disclosures**

VSN analysed, interpreted data and wrote the manuscript. CHA performed and analysed all gene expression assays. PCB analysed clinical data and provided clinical input. The authors declare no conflicts of interests.
References


Figure 1

Trends in mRNA expression in serial samples obtained from a patient with both *BCR-ABL* and *JAK2-V617F* together with patient and normal controls (n=39). Gene expression was measured by real-time quantitative PCR and values obtained for lipocalin-2 (LCN-2) and SLC22A17 were normalised to ACTB as the control gene, while SOCS3 expression was normalised to RPL. Normalisation was calculated using the $2^{-\Delta C_T}$ formula. (A) LCN-2 expression in serial samples from the patient. (B) Distribution of LCN-2 expression among *JAK2-V617F*+ patients and normal subjects. (C) Distribution of LCN-2 expression among patients with chronic myeloid leukaemia (CML) while on imatinib mesylate (IM). Patients were grouped according to their level of BCR-ABL transcripts as a percentage ratio to ABL as the control gene, and values corrected to an International Standard (IS). (D) SLC22A17 expression in serial samples from the patient. (E) Distribution of SLC22A17 expression among *JAK2-V617F*+ patients and normal subjects. (F) Distribution of SLC22A17 expression among patients with CML while on IM. (G) BCR-ABL/ABL (IS) transcript levels and *JAK2-V617F* mutant allele burden in serial samples obtained from the patient. Patient samples are denoted by the months prior to and after commencing IM, where M0 was obtained just before IM treatment was started, as indicated by the arrowhead.