The pro-metastasis tyrosine phosphatase, PRL-3 (PTP4A3), is a novel mediator of oncogenic function of BCR-ABL in human chronic myeloid leukemia

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Abstract

Background: Resistance to tyrosine kinase inhibitors (TKIs) remains a challenge in management of patients with chronic myeloid leukemia (CML). A better understanding of the BCR-ABL signalling network may lead to better therapy.

Findings: Here we report the discovery of a novel downstream target of BCR-ABL signalling, PRL-3 (PTP4A3), an oncogenic tyrosine phosphatase. Analysis of CML cancer cell lines and CML patient samples reveals the upregulation of PRL-3. Inhibition of BCR-ABL signalling either by Imatinib or by RNAi silencing BCR-ABL reduces PRL-3 and increases cleavage of PARP. In contrast, the amount of PRL-3 protein remains constant or even increased in response to Imatinib treatment in drug resistant cells expressing P210 T315I. Finally, analysis with specific shRNA shows PRL-3 involvement in the proliferation and self-renewal of CML cells.

Conclusions: These data support a role for PRL-3 in BCR-ABL signalling and CML biology and may be a potential therapeutic target downstream of BCR-ABL in TKI resistant mutant cells.

Keywords: Chronic myeloid leukemia (CML), Protein-tyrosine phosphatase of regenerating liver 3 (PRL-3), PTP4A3, BCR-ABL, Imatinib, Tyrosine kinase inhibitor (TKI)

Findings

Chronic myeloid leukemia (CML) is a hematopoietic stem cell malignancy with a hallmark cytogenetic abnormality, i.e., the BCR-ABL fusion oncogene, resulting from the reciprocal translocation of chromosomes 9 and 22 [also known as Philadelphia (Ph) chromosome] [1]. CML is the best and most successful disease model for tyrosine kinase inhibitor (TKI) therapy [2,3]. Unfortunately, acquired resistance can develop during the course of treatment. Effective therapies that can overcome resistance still remain a challenge for the clinical management of CML [2,4]. The mechanism of BCR-ABL induced transformation and signaling transduction networks have been intensively characterized over the decades [5-7]. However, new discoveries related to the BCR-ABL signaling pathway and mechanisms of TKI resistance continues to emerge, leading to a better understanding of disease progression and development of novel therapy [8-10].

Protein-tyrosine phosphatase of regenerating liver 3 (PRL-3, encoded by protein tyrosine phosphatase type IVA 3, PTP4A3) belongs to class I cysteine-based protein tyrosine phosphatases (PTPs) with dual-specificity [11-13]. PRL-3 has been identified as a critical player in cancer cell metastasis, invasion, migration, and tumor angiogenesis [11,14-16]. The association between elevated PRL-3 and the development of various human cancers has been validated in a wide range of solid tumors [11,14,15] and multiple myeloma [17].

We recently discovered that poly(rC) binding protein 1 (PCPB1, also known as heterogenous nuclear
ribonucleoprotein E1, hnRNP-E1) inhibited PRL-3 protein through binding 5'-UTR (untranslated region) of PRL-3 mRNA [18] and showed that PRL-3, acting as a downstream target of the internal tandem duplication (ITD) of fms-like tyrosine kinase (FLT3) signaling, was implicated in FLT3 inhibitor therapy in acute myeloid leukemia (AML) [19]. Furthermore, PRL-3 also has been demonstrated as an independent prognostic parameter for poor overall survival (OS) and event-free survival (EFS) in AML [20]. Importantly, targeting intracellular PRL-3 protein suppressed cancer growth [21]. In the present study, we hypothesize that PRL-3 might be involved in leukemogenesis of human CML.

Overexpression of PRL-3 in CML cell lines and primary patient samples

A search of the Gene Expression Atlas (http://www.ebi.ac.uk/gxa/gene/ ENSG00000184489) showed that the expression level of PRL-3 was highest in CML among 950 human cancer cell lines covering 32 different types of cancers (Dataset code: E-MTAB-37), suggesting a potential role of PRL-3 in CML pathogenesis (Figure 1A). To further confirm PRL-3 expression, we examined PRL-3 protein levels in a panel of CML cell lines and primary CML BM samples. By immunoblot analysis, (Additional file 1) we observed strong PRL-3 protein expression in two human CML cell lines (K562 and KCL-22, Figure 1B), murine hematopoietic cells expressing WT and mutant BCR-ABL constructs (P210 WT, P210 T315I, P210 M351T and P210 H396R, Figure 1B middle), and primary BM samples from CML patients (Figure 1B right). It is worth noting that PRL-3 is either not expressed or minimally expressed in bone marrow cells from 3 normal controls (NC) or parental BaF3 cells (Figure 1B) [19]. Altogether, our data obtained from Western blot analysis of CML cell lines and primary CML samples, as well as the analysis of a publicly available gene expression dataset demonstrated over-expression of PRL-3 in CML.

Imatinib suppressed PRL-3 through inhibition of STAT pathway

Imatinib blocks the binding of ATP to the BCR-ABL tyrosine kinase [22,23], and is currently used as the first-line treatment for CML [2,4]. To establish a connection between BCR-ABL signalling and PRL-3 expression, we treated human CML cell lines, K562 and KCL-22 cells with Imatinib and assessed the expression of PRL-3. Western blot analysis demonstrated that Imatinib dose-dependently decreased p-CrkL (a surrogate marker of BCR-ABL kinase activity), p-STAT3, p-STAT5, as well as PRL-3 (Figure 2A). Consistent with the effective inhibition of oncogenic BCR-ABL signalling, cleaved-PARP, a hallmark of apoptosis was increased as a response to the Imatinib treatment (Figure 2A). We next tested whether Imatinib could induce PRL-3 protein down-regulation in BaF3 murine hematopoietic cells engineered to express either wild-type, or the Imatinib resistant T315I mutant P210 BCR-ABL. As expected, the expression of p-CrkL, p-STAT3 and PRL-3 was down-regulated in a dose-dependent manner in the imatinib sensitive P210 WT
cells. In contrast, BCR-ABL activity in P210 T315I cells was resistant to Imatinib even at high doses (10 μM) as indicated by no change in p-CrkL. In this resistant cell line, PRL-3 was not downregulated but rather its level increased at higher doses of Imatinib (Figure 2B). Surprisingly, p-STAT5 expression was almost completely abolished in both P210 WT and p210 T315I cells upon exposure to Imatinib (Figure 2B). On the other hand, the down-regulation of PRL-3 correlated with the inhibition of STAT3. Consistent with inhibition of BCR-ABL, increased PARP cleavage fragment was observed in cells sensitive to Imatinib (K652, P210 WT), but not in resistant cells (P210 T315I) (Figure 2B). In addition to the STAT pathways, PI3K/AKT and MAPK/ERK signalling pathways were also downstream of BCR-ABL signalling and may contribute to the transformation of CML cells [7]. However, inhibition of these two pathways did not correlate with the down-regulation of PRL-3 protein in the P210 and K562 cells (Additional file 2). Taken together, these data suggest PRL-3 is downstream of BCR-ABL mainly through the STAT pathway in CML.

Silencing BCR-ABL fusion gene or STAT3 decreased PRL-3 expression
To further confirm that the downregulation of PRL-3 is not due to off-target effect of Imatinib, we used small
interfering RNA (siRNA) to specifically knock down the BCR-ABL fusion gene as reported by Scherr et al. [24]. qRT-PCR analysis confirmed the expression of PRL-3 gene was decreased approximate 80% in b3a2_1 siRNA transfected K562 cells, in parallel with reduction of BCR-ABL fusion genes (Figure 3A) confirming that PRL-3 is downstream of BCR-ABL signalling. To assess the specific role of STAT3 in upstream regulation of PRL-3, we decreased STAT3 mRNA by using siRNA in a Nuclofection device. qRT-PCR showed that the expression of PRL-3 was reduced in STAT3 siRNA expressing K562 cells compared with the control (NC) siRNA expressing K562 cells (Figure 3B). These results support the BCR-ABL and STAT3 are specific upstream regulators of PRL-3 signalling.

PRL-3 is involved in CML proliferation, self-renewal, tumorigenic capacity and drug response

To assess the functional effect of PRL-3 in CML, we knocked down PRL-3 using short-hairpin RNA (shRNA). RT-PCR analysis showed that shRNA-PRL-3 transduced K562 cells (K562-shP) demonstrated significantly reduced PRL-3 mRNA levels as compared to shRNA-scramble control transduced cells (K562-shC) (Figure 4Ai). K562-shP cells proliferated as much as 2-fold slower than K562-shC at day 8 (p < 0.001) (Figure 4Aii). K562-shP cells showed significantly impaired colony generating capacity, an indicator of self-renewal capacity, by 3-fold compared to K562-shC (p < 0.001) (Figure 3Aiii). We next evaluated the oncogenic role of PRL-3 in CML in vivo. Three million of K562-shC and K562-shP cells were
subcutaneously inoculated into the right and left side of NOD/SCID mice, respectively (3 mice total). After 3 weeks, only K562-shC cells developed tumors at average size 800 mm$^3$ (Figure 4Aiv, right side of the animals, indicated by black circles) and average tumor weight was 0.967 ± 0.21 g (Figure 4Aiv). These results indicate a critical role for PRL-3 in CML cell expansion, and self-renewal in vitro and in vivo. To examine if PRL-3 could be a potential therapeutic target downstream of BCR-ABL especially in TKI resistant cells with BCR-ABL mutations, we utilized RNAi to knockdown PRL-3 expression in P210 T315I cells. Downregulation of mouse PRL-3 (mPRL-3) was confirmed by RT-PCR (Figure 4B, left panel) and qRT-PCR (Figure 4B, middle panel). Indeed, while Imatinib had no effect on P210 T315I cells, mPRL-3 silencing led to significant cell death in these cells (Figure 4B, right panel).

In summary, the present study demonstrates that PRL-3 is upregulated in human CML cell lines, BCR-ABL transformed cell lines and primary CML patient samples. Interestingly, in a previous study, high expression of PRL-3 has been associated with aggressive phenotype of BCR-ABL positive acute lymphoblastic leukemia (ALL) [25]. This finding, together with our results highlight that PRL-3 is a novel downstream target of the BCR-ABL signalling pathway, and may be a novel mediator of BCR-ABL oncogenic functions such as cell survival and self-renewal. Suppression of PRL-3 could provide potential opportunity for further improving anti-CML therapy, especially in tumors with Imatinib or TKI resistant BCR-ABL mutants.
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