Brief communication

Dasatinib inhibits leukaemic cell survival by decreasing PRH/Hhex phosphorylation resulting in increased repression of VEGF signalling genes

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The PRH/Hhex transcription factor represses multiple genes in the VEGF signalling pathway (VSP) to inhibit myeloid cell survival. Protein kinase CK2 phosphorylates PRH and counteracts the inhibitory effect of this protein on cell survival by blocking the repression of VSP genes. Here we show that the BCR-ABL/Src kinase inhibitor dasatinib decreases PRH phosphorylation and increases PRH-dependent repression of Vegf and Vegfr-1. Moreover in the absence of PRH, dasatinib does not inhibit cell survival as effectively as in PRH expressing cells. Thus the re-establishment of gene control by PRH is in part responsible for the therapeutic effects of dasatinib.

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1. Introduction

Protein kinase CK2 is a constitutive stress-responsive kinase that promotes cell survival and is strongly implicated in tumourigenes- 
sis. CK2 activity is elevated in several cancer types including Acute Myeloid Leukaemia (AML) and Chronic Myeloid Leukaemia (CML) [1,2]. CML cells express the oncogenic p210 BCR-ABL fusion protein, a constitutively active tyrosine kinase that stimulates multiple growth promoting signalling pathways. BCR-ABL and Src family kinases interact with each other and both have been shown to increase CK2 activity [3,4]. The CML therapeutic imatinib and its derivative dasatinib inhibit the tyrosine kinase activity of BCR-ABL resulting in leukaemic cell death. Dasatinib is a dual ABL–Src kinase inhibitor that exhibits a more potent but less selective inhibition of BCR-ABL than imatinib and is commonly used in treatment of imatinib resistant CML. Although imatinib and dasatinib are well-characterised treatments for CML the downstream targets for their action are not fully delineated.

The Proline-Rich Homeodomain (PRH/Hhex) protein regulates many processes in embryonic development and in the adult (reviewed [5]). In the haematopoietic system PRH is expressed in myeloid lineages where it functions as a negative regulator of cell growth. Loss of PRH function in myeloid cells contributes to the development of AML subtypes [5]. PRH regulates myeloid survival through the direct transcriptional repression of multiple genes encoding components of the VEGF signalling pathway (VSP) including Vegf, Vegfr-1, Vegfr-2, and neuropillin-1 [6,7]. Phosphorylation of PRH by CK2 inhibits the DNA binding activity of this protein [7] and CK2 antagonises PRH-dependent growth inhibition by altering the stability and activity of PRH (manuscript in revision). Here we show that an important effect of dasatinib is to decrease phosphorylation of PRH resulting in increased PRH repression activity at VSP genes and decreased cell survival.

2. Materials and methods

Cell culture, quantitative PCR for Vegf and Vegfr-1 mRNA, PRH knockdown with shRNA PRH or shRNA GFP (control) were performed as described previously [6]. Western blotting was performed with mouse antibodies which preferentially recognise hypophosphorylated PRH and rabbit antibodies that preferentially recognise pPRH [7]. Antibody for phosphoXRCC1 was a kind gift from Dr. Grant Stewart (University of Birmingham). Antibody for pSrc was a kind gift from Dr. Yotis Senis (University of Birmingham). Antibody for pStat5 was obtained from New England Biolabs.

3. Results

Inhibition of BCR-ABL results in the inhibition of CK2 activity in BCR-ABL-dependent lymphoma cells [4]. K562 cells are a CML cell line that express BCR-ABL and we hypothesised that the inhibition of BCR-ABL in these cells might result in decreased CK2 activity leading to decreased phosphorylation of PRH and consequent reduced cell survival. To test this we generated PRH knockdown cells using shRNA for PRH or shRNA GFP as control (Fig. 1A) as
Control treated
phosphorylation
dasatinib
kinases. PRH
unrelated
phosphorylation
reduction this
PRH
Importantly,
PRH-mediated
loss (Fig. 1D)
have obtained
expression.
The survival of
cells over-expressing
VEGFR-1, VEGFR-2
VEGF is able to
counteract the
inhibitory effects
of dasatinib on
cell survival. The
survival of cells
over-expressing
VEGFR-1, VEGFR-2
and VEGF is not
significantly
inhibited by
dasatinib whereas
the survival of control
cells is strongly
inhibited under
the same
conditions (Fig. 3A).
Fig. 3B shows that
as expected
VEGF mRNA
levels are indeed
elevated in this
experiment. This
confirms that
the effects of
dasatinib on
cells are largely
mediated by the
inhibition of
VEGF gene
expression.

4. Discussion

Blast crisis CML cells have constitutively high BCR-ABL tyrosine kinase activity, elevated VEGF levels that promote angiogenesis and elevated CK2 activity. Imatinib and dasatinib inhibit the tyrosine kinase activity of BCR-ABL and in the case of dasatinib also target other kinases such as the Src family tyrosine kinases, csk and
Dasatinib, PRH, PCR, mRNA.

signalling

EphA2, resulting in cell death. Inhibition of these kinases leads to the down-regulation of a variety of signalling pathways including the PI3Kinase pathway and this results in decreased cell survival. We have shown that dasatinib treatment brings about a reduction in PRH phosphorylation. Significantly, the inhibition of K562 cell survival and the decrease in VSP gene expression brought about by dasatinib treatment are largely dependent on the presence of PRH (Fig. 3C). Although dasatinib inhibits multiple kinases, in the absence of PRH even high concentrations of dasatinib have little effect on cell survival. We conclude that the re-establishment of VSP gene regulation by PRH is responsible in large part for the effects of these inhibitors on K562 cells. In agreement with this conclusion de-regulated VSP gene expression is associated with CML [8]. We infer that the elevation of CK2 activity by BCR-ABL signalling [4] very likely results in elevated levels of PRH inactivation in CML. Although CK2 is a pleiotropic kinase CK2 inhibitors are being assessed for the treatment of BCR-ABL transformed ALL and multiple AML where CK2 activity has been found to be elevated [1]. Our results suggest a molecular rationale for the use of CK2 inhibitors in conjunction with BCR-ABL inhibitors in the treatment of primary CML. Experiments in primary cells are required to further investigate the importance of PRH phosphorylation and determine whether the restoration of PRH activity through inhibition of CK2 may be particularly of value in imatinib or dasatinib resistant CML.

Conflict of interest statement

The authors confirm that there is no conflict of interest.

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