Src kinase signaling in leukaemia

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Abstract

Role of Src kinases in acute lymphoblastic leukaemia has been recently demonstrated in leukaemia mouse model. Retained activation of Src kinases by the BCR-ABL oncoprotein in leukaemic cells following inhibition of BCR-ABL kinase activity by imatinib indicates that Src activation by BCR-ABL is independent of BCR-ABL kinase activity and provides an explanation for reduced effectiveness of the BCR-ABL kinase activity inhibitors in Philadelphia chromosome-positive acute lymphoblastic leukaemia. Simultaneous inhibition of kinase activity of both BCR-ABL and Src kinases results in long-term survival of mice with acute lymphoblastic leukaemia. Leukaemic stem cells exist in acute lymphoblastic leukaemia, and complete eradication of this group of cells would provide a curative therapy for this disease.

Keywords

Src kinases; acute lymphoblastic leukaemia (ALL); BCR-ABL oncogene; signaling pathway; tyrosine kinase inhibitor; leukaemic stem cell

1. Introduction

The human Philadelphia (Ph) chromosome arises from a translocation between chromosomes 9 and 22, and results in formation of the chimeric and constitutively activated BCR-ABL tyrosine kinase. Ph[+] leukaemias include chronic myeloid leukaemia (CML) and B-cell acute lymphoblastic leukaemia (B-ALL). CML often begins with a chronic phase and can progress to a terminal blastic phase, in which either acute myeloid or acute B-lymphoid leukemia develops. Some Ph[+] leukemia patients have B-ALL as their initial clinical appearance. The BCR-ABL tyrosine kinase inhibitor imatinib mesylate (Gleevec) is the standard of care for Ph[+] leukemia. Imatinib induces a remarkable hematologic response in chronic phase CML patients (Druker et al., 2001). However, imatinib does not completely eliminate BCR-ABL-expressing leukemic cells (Graham et al., 2002;Marley, Deininger, Davidson, Goldman, & Gordon, 2000), and patients develop drug resistance (Gorre et al., 2001). Imatinib prolongs survival of mice with BCR-ABL-induced CML (Hu et al., 2004;Wolff & Ilaria, 2001), but does not cure the disease (Hu et al., 2004). Regardless, current therapeutic efforts have focused on targeting BCR-ABL kinase activity using kinase inhibitors. It is generally believed that shutting down the kinase activity of BCR-ABL will completely inhibit its functions, leading to inactivation of its downstream signaling pathways. However, our obtained evidence suggests this is not the case. We find that Src kinases remain active following imatinib inhibition of BCR-ABL kinase activity in leukaemic cells (Hu et al., 2006). In addition, signaling molecules/

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pathways utilized by BCR-ABL to induce these leukaemias are not identical, although these two distinct diseases can be caused by the same BCR-ABL oncogene. This idea is proposed based on our recent finding that some Src kinases are required for proliferation of BCR-ABL-expressing B-lymphoid cells but not myeloid cells in mice (Hu et al., 2004). This finding is of important clinical implication, because it suggests that different therapeutic strategies are needed for treating chronic phase CML and B-ALL in patients. In this review, I will briefly summarize our current understanding of the signaling pathways that are activated by BCR-ABL, with an emphasis on the activation of Src kinases in leukaemia development in mice. This review will also emphasize our idea that sole inhibition of BCR-ABL kinase activity by kinase inhibitors is insufficient to shut down all BCR-ABL downstream signaling pathways, as BCR-ABL also activates, independently of its kinase activity, Src kinases and perhaps other signaling pathways. It should be pointed out that results discussed in this article are obtained mainly from studies in mice, and that my focus is on activation of Src kinase by BCR-ABL kinase other than other kinases or mechanisms.

2. BCR-ABL signaling and Src kinases

BCR-ABL activates multiple signaling pathways, including Ras, MAPK, STAT, JNK/SAPK, PI-3 kinase, NF-kB and c-MYC (Sawyers, 1997). BCR-ABL functions also link to apoptotic pathways (Amarante-Mendes et al., 1998;Dubrez et al., 1998;Goetz, van der Kuip, Maya, Oren, & Aulitzky, 2001;Honda & Hirai, 2001;Jonuleit et al., 2000;Majewski et al., 1999;McGahon et al., 1995;Neshat, Raitano, Wang, Reed, & Sawyers, 2000;Parada et al., 2001;Sanchez-Garcia & Martin-Zanca, 1997;Skorski et al., 1997;Skorski et al., 1996) and Src family kinases (Danhauser-Riedl, Warmuth, Druker, Emmerich, & Hallek, 1996;Lionberger, Wilson, & Smithgall, 2000;Warmuth et al., 1997). Src family kinases are a group of structurally related non-receptor protein tyrosine kinases. In mouse, there are at least eight family members (Blk, Fgr, Fyn, Hck, Lck, Lyn, c-Src, and Yes), and these kinases contain four Src homology domains but diverge in the unique domain (Lowell & Soriano, 1996). BCR-ABL interects with Src kinases. BCR-ABL coimmunoprecipitates with and activates Lyn and Hck in myeloid cell lines (Danhauser-Riedl et al., 1996;Warmuth et al., 1997). Hck binds directly to BCR-ABL through its three SH domains and distal portion of the C-terminal tail, and is required for transformation of the myeloid leukaemia cell line to IL-3 independency by BCR-ABL (Lionberger et al., 2000). Src kinases may be also linked to BCR-ABL function indirectly through other signaling molecules or pathways such as protein tyrosine phosphatases (Bruecher-Encke, Griffin, Neel, & Lorenz, 2001;Tauchi et al., 1994). In particular, Lyn and SHP-1 (SH2-containing tyrosine phosphotase-1) are functionally related (Daigle, Yousefi, Colonna, Green, & Simon, 2002;Gardai et al., 2002), and Lyn could regulate BCR-ABL function through SHP-1. Src family kinases are also functionally linked to Btk (Bruton's tyrosine kinase)(Park et al., 1996;Rawlings et al., 1996), and we have observed that Btk is involved in BCR-ABL leukemogenesis (unpublished data).

There are many indirect evidences that indicate an association between Src kinases and hematologic malignancies, as summarized previously (Li, 2005). (Burnett et al., 1991;Ernould, Ferry, Barret, Genton, & Boutin, 1994;Fischer et al., 1989;Lynch et al., 1993;Myers et al., 1995;O'Connor, Torigoe, Reed, & Santoli, 1992;Tilbrook et al., 2001;Torigoe, O'Connor, Santoli, & Reed, 1992;Uckun et al., 1995;Waki et al., 1994;Willman et al., 1991;Yamaguchi et al., 1997). Direct evidence for the roles of Src kinases in leukaemogenesis is inadequate; recently, we provided convincing evidence that Src kinases are required for proliferation of leukaemic cells in mice with ALL (Hu et al., 2004;Hu et al., 2006).

Critical role of Src kinases in proliferation of BCR-ABL-transformed B-lymphoid cells but not myeloid cells in mice

It is unclear whether specific oncogenes use cell type-specific signaling networks to induce particular types of cancer (Deininger, 2004). If this were true, it would help us to determine the choices of available therapeutic strategies and to develop new and effective therapies. Using gene knockout mice, we have found that three Src kinases (Lyn, Hck, and Fgr) are required for proliferation of BCR-ABL-expressing pre-B cells but not myeloid progenitor cells (Hu et al., 2004), although these Src kinases are activated by BCR-ABL in these myeloid cells. This finding is important, as it suggests that Src kinases are better targets for treating ALL but not chronic phase CML, although possible roles for these kinases in regulation of CML stem cells and the transition from chronic phase CML to lymphoid blast crisis. It should be pointed out that our study does not exclude a role for other Src family kinases (rather than Lyn, Hck, and Fgr) in proliferation of BCR-ABL-tranformed myeloid cells in CML mice. It is possible that cell type-specific signaling represents a common mechanism in cancer development. If so, identification of the unique signaling network involved in each type of cancer is critical for developing effective cancer therapies.

Activation of Src kinases by BCR-ABL is not dependent on its kinase activity and inhibition of Src kinases is critical to ALL therapy in mice

It is generally believed that the kinase activity of BCR-ABL is responsible for activation of all its downstream signaling pathways; thus, BCR-ABL kinase activity inhibitors should completely inhibit BCR-ABL functions and cure the disease. We find that Src kinases remain active following imatinib inhibition of BCR-ABL kinase activity in leukaemic cells (Hu et al., 2006). When we treated mice with ALL induced by BCR-ABL-T315I that is resistant to inhibition of BCR-ABL kinase activity by both imatinib (Gorre et al., 2001;Roumiantsev et al., 2002;Warmuth et al., 2003) and dasatinib (Shah et al., 2004), we observed that imatinib (which only inhibits BCR-ABL) had no therapeutic effect, whereas dasatinib (which inhibits both BCR-ABL and Src kinases) significantly prolonged survival of the mice (Hu et al., 2006). We have tested genetically whether Src kinases play a role in CML transition to lymphoid blast crisis using a serial transplantation assay (Pear et al., 1998). Mice were transplanted with BCR-ABL transduced bone marrow (BM) cells from either wild type or $Lyn^{-/-}Hck^{-/-}Fgr^{-/-}$ mice to induce CML, and BM cells from the CML mice were subsequently transferred into recipient mice. Strikingly, mice receiving wild type CML BM cells developed ALL, whereas none of the mice receiving $Lyn^{-/-}Hck^{-/-}Fgr^{-/-}$ CML BM cells developed this disease. These results indicate that CML transition to lymphoid blast crisis requires Src kinases. CML progression is associated with additional genetic changes including mutations in the tumor suppressor genes INK4[a], pRB, and p53 (Feinstein et al., 1991;Sill, Goldman, & Cross, 1995;Towatari, Adachi, Kato, & Saito, 1991). Arf gene loss enhances oncogenicity of and limits imatinib response to BCR-ABL-induced ALL in mice (Williams, Roussel, & Sherr, 2006). A potential genetic interaction between Src kinases and those tumor suppressors needs to be investigated. Together, kinase activity-independent activation of Src kinases by BCR-ABL provides a new idea to help our understanding of signaling mechanisms involved in leukemia development.

Pro-B leukemic cells function as ALL stem cells, and inhibition of Src kinases may help prevent them from developing into ALL

All mice treated with dasatinib survived long time as long as the treatment continues (Hu et al., 2006), indicating that inhibition of Src kinases is critical to the treatment of ALL. We asked whether dasatinib could completely eradicate leukaemic cells in ALL mice and cure the mice.

A small percentage of BCR-ABL-expressing cells (<1%) remained in peripheral blood of these mice, even after three months of dasatinib treatment. After treatment was stopped, BCR-ABL-expressing cells grew, but dropped again to less than 1% after the treatment resumed (Hu et al., 2006). However, these cells persisted in BM of the treated ALL mice, and were capable of transferring the same disease to secondary recipient mice (unpublished data). These results indicate that these residual leukaemic cells contain ALL stem cells and that continuous administration of dasatinib could prevent these residual cells from developing into fatal ALL, although this compound at the dose used did not completely kill these residual cells. These residual leukaemic cells are B220$^+$/CD43$^+$ pro-B cells and function as ALL stem cells after acquiring self-renewal capacity. Although inhibition of Src kinases by dasatinib did not completely eradicate B-ALL stem cells, preventing these cells from developing into lethal ALL suggests that Src inhibition may at least have a cytostatic effect on these stem cells. Our identification and isolation of ALL stem cells in mice provides a valuable system for studying biology of these stem cells and for developing new therapies to target these cells.

Conclusion

It becomes more and more clear that imatinib may not cure Ph$^+$ leukemia due to the development of clinical drug resistance. Recently, three BCR-ABL kinase activity inhibitors, dasatinib (Shah et al., 2004), AP23464 (O'Hare et al., 2004), and AMN107 (Weisberg et al., 2005), have been shown to inhibit almost all imatinib-resistant BCR-ABL mutants, with an exception of the BCR-ABL-T315I mutant. The development of new BCR-ABL kinase activity inhibitors that are effective on identified and emerging drug-resistant BCR-ABL mutants has been a major focus in treatment of Ph$^+$ leukemia, because it is believed that inhibition of BCR-ABL kinase activity would completely suppress BCR-ABL functions. We have obtained opposing evidence that imatinib-inhibited BCR-ABL can still activates Src kinases, which play a critical role in the development of BCR-ABL-induced ALL. This Src pathway would help leukaemic cells to survive treatment with BCR-ABL kinase activity inhibitors and eventually allow resistant BCR-ABL-T315I cells to grow out. The kinase activity-independent activation of Src kinases by BCR-ABL explains why Ph$^+$ B-ALL is less sensitive than chronic phase CML to imatinib therapy and suggests that sole inhibition of BCR-ABL kinase activity by kinase inhibitors will not cure Ph$^+$ leukaemia. Although the next generation of BCR-ABL kinase inhibitors aims at increasing drug potency or overriding imatinib resistance caused by kinase domain point mutations including BCR-ABL-T315I, to achieve a durable therapeutic effect in patients with Ph$^+$ B-ALL and lymphoid blast crisis, Src kinases must be targeted. Dasatinib does not completely eradicate leukaemic stem cells in ALL mice, but targeting Src kinases helps achieve long-term control of the disease. Curative drug therapy of ALL would require targeting not only BCR-ABL kinase activity and Src-dependent pathways but also pro-B leukemic stem cells (Fig. 1). It will be critical to assess whether BCR-ABL-expressing pro-B cells serve as stem cells in patients with Ph$^+$ B-ALL or lymphoid blast crisis CML. Moreover, identification of unknown pathways in leukaemic stem cells will be critical for developing curative therapies for Ph$^+$ leukaemia.

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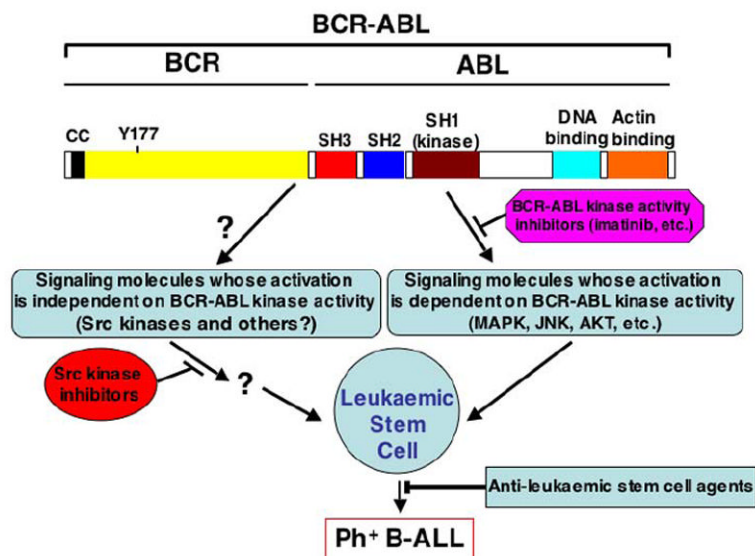
Fig 1. Roles of BCR-ABL kinase activity-independent Src pathway and leukaemic stem cells in ALL therapy

Because the activation of Src kinases is independent of BCR-ABL kinase activity, inhibition of BCR-ABL kinase activity by imatinib does not reduce BCR-ABL-stimulated Src activation. Inhibition of Src kinases may have a cytostatic effect on ALL stem cells, but these cells also survive through other unknown mechanisms. Therefore, simultaneous inhibition of functions of both BCR-ABL (by imatinib) and Src kinases (by an Src kinase inhibitor) and leukaemic stem cells (by an anti-stem cell agent to be developed) is needed for curative therapy of Ph[+] B-ALL. cc: coiled-coil domain; Y177: tyrosine 177; SH: Src homology domain.