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Extra View

Heat Shock Protein 90

A Potential Therapeutic Target in Leukemic Progenitor and Stem Cells Harboring Mutant BCR-ABL Resistant to Kinase Inhibitors

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heat shock protein 90, BCR-ABL, Ph+ leukemia, leukemic stem cells, imatinib resistance

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ABSTRACT

Development of drug resistance has become a major obstacle for tyrosine kinase inhibitors (TKIs) in the treatment of Philadelphia chromosome-positive (Ph+) chronic myelogenous leukemia (CML) and other cancers. The BCR-ABL-T315I mutant does not respond to clinically available TKIs, although some newly developed anti-BCR-ABL-T315I TKIs are now being tested in patients. TKIs transiently inhibit kinase activity of BCR-ABL, but do not reduce the level of the BCR-ABL protein. Elimination of mutant BCR-ABL protein would provide a new therapeutic strategy for treating Ph+ leukemia. We recently showed that inhibition of heat shock protein 90 (Hsp90) by a novel Hsp90 inhibitor, IPI-504, causes BCR-ABL protein degradation, decreased numbers of leukemia stem cells, and prolonged survival of mice with CML induced by BCR-ABL-T315I. Here we discuss further the mechanisms and effectiveness of Hsp90 inhibition in suppression of survival and proliferation of leukemic progenitor and stem cells in CML mice, and the potential of this anti-Hsp90 strategy in treating CML patients, including those who have developed resistance to TKIs.

INTRODUCTION

The human Philadelphia chromosome arises from a translocation between chromosomes 9 and 22 [t(9;22)(q34;q11)],[1] and the resulting chimeric gene, the BCR-ABL oncogene, encodes a constitutively activated, oncogenic tyrosine kinase. Philadelphia chromosome-positive leukemia (Ph+) leukemia includes two diseases: chronic myeloid leukemia (CML) and B-cell acute lymphoblastic leukemia (B-ALL). Imatinib mesylate is a BCR-ABL tyrosine kinase inhibitor (TKI), and induces a complete hematologic and cytogenetic response in the majority of chronic phase CML patients.[2] However, some patients develop drug resistance mainly due to emergence of kinase domain mutations.[3-9] While newly developed BCR-ABL TKIs[10-12] have inhibitory activities against most imatinib-resistant BCR-ABL mutants, they are ineffective against the BCR-ABL-T315I mutant.[13,14] Furthermore, BCR-ABL-induced ALL responds poorly to available TKIs.[13,14] These TKIs inhibit BCR-ABL kinase activity without causing degradation of the BCR-ABL protein. Heat shock proteins are highly conserved, constitutively expressed molecular chaperones that facilitate folding of their client proteins. Heat shock protein 90 (Hsp90) has many client proteins including BCR-ABL, and affects the stability of these proteins.[15-19] Interestingly, an in vitro assay showed that when BCR-ABL becomes mutated, its dependence on Hsp90 for stability increases.[18] In in vivo studies using leukemia mouse model, we showed that inhibition of Hsp90 more significantly attenuates leukemia induced by BCR-ABL with kinase domain mutations than by wild type BCR-ABL in mice.[20] Here we discuss further the strategies for treating Ph+ leukemia through Hsp90 inhibition.

Hsp90 INHIBITORS AND THEIR THERAPEUTIC EFFECT ON LEUKEMIA MICE

The first small-molecule Hsp90 inhibitor made available, the benzoquinone ansamycin 17-allylamino-17-desmethoxygeldanamycin (17-AAG),[15] has been shown to have anti-tumor activities.[21-23] The recently developed Hsp90 inhibitor IPI-504 is the hydroquinone hydrochloride derivative of 17-AAG and is a potent water-soluble inhibitor of Hsp90.[24] We recently showed that IPI-504 induced disassociation of the mutant BCR-ABL-T315I from Hsp90 in 32D myeloid cells, subsequently causing BCR-ABL

protein degradation[20] through a proteasome, as demonstrated using a proteasome inhibitor, PS-341,[25,26] which restored IPI-504-mediated depletion of the BCR-ABL protein.[20]

Because the stability of BCR-ABL was shown to be more dependent on Hsp90 in vitro when it carries resistance-conferring mutations,[27] we wanted to test the dependence of mutant BCR-ABL on Hsp90 in vivo. Therefore, we evaluated the therapeutic effect of Hsp90 inhibition by IPI-504 in our drug-resistant mouse models of leukemia induced by wild type BCR-ABL and by the BCR-ABL-T315I mutant, which is resistant to available BCR-ABL kinase inhibitors.[13,14] Treatment with IPI-504 alone significantly prolonged the survival of mice with wild type BCR-ABL-induced CML, but even more markedly prolonged the survival of mice with BCR-ABL-T315I-induced CML.[20] This result is consistent with previously reported results using the different Hsp90 inhibitor 17-AAG in SCID mice receiving a BCR-ABL-expressing cell line.[18] Importantly, the markedly prolonged survival of the IPI-504-treated mice with BCR-ABL-T315I-induced CML correlates with the more significant in vivo degradation of the mutant BCR-ABL than of wild type BCR-ABL.

Chronic phase CML progresses to a terminal blastic phase involving acute myeloid or acute lymphoid leukemia (ALL).[28] Some Ph+ leukemia patients have ALL as their first clinical appearance. Because ALL does not respond well to available BCR-ABL kinase inhibitors,[13,14] we tested the effect of Hsp90 inhibition on B-ALL development using our B-ALL mouse model.[29,30] In this model, malignant lymphoid cells express pre-/pro-B cell surface markers (B220 and CD19), and the disease phenotypically resembles de novo Ph+ ALL and lymphoid blast crisis of CML.[29,31] Similar to the effect seen in CML, IPI-504 treatment significantly prolonged survival of mice with ALL induced by BCR-ABL-T315I.

FUNCTIONAL RELATIONSHIP BETWEEN Hsp90 AND Hsp70 IN SURVIVAL AND PROLIFERATION OF BCR-ABL-EXPRESSING CELLS

What is the mechanism by which inhibition of Hsp90 results in degradation of its client proteins such as BCR-ABL? This mechanism appears to involve linkage of Hsp90 inhibition to induction of another heat shock protein, Hsp70, although the role of Hsp70 in BCR-ABL degradation and reduced cell proliferation induced by Hsp90 inhibition is controversial. On the one hand, it has been shown that the Hsp90 antagonists geldanamycin and 17-AAG alter chaperone association of Hsp90 with BCR-ABL and promote binding of BCR-ABL to Hsp70, leading to degradation of BCR-ABL by proteasome.[17,27,32,33] On the other hand, some studies have shown that Hsp70 actually facilitates BCR-ABL-mediated resistance to apoptosis,[34,35] which would in theory result in an increase of the stability of the BCR-ABL protein. In addition, imatinib has been shown to decrease the level of Hsp70 in BCR-ABL-expressing HL60 cells,[36] supporting the anti-apoptotic role of Hsp70 in BCR-ABL-stimulated cell growth. We studied further the relationship between Hsp70 inhibition and stability of the BCR-ABL protein. If Hsp70 cooperates with Hsp90 to facilitate degradation of BCR-ABL protein, inhibition of Hsp70 should increase level of BCR-ABL protein in cells. To test this hypothesis, we treated BCR-ABL-expressing 32D myeloid cells with an Hsp70 inhibitor, KNK437.[37] Inhibition of Hsp70 by KNK437 did not prevent BCR-ABL degradation caused by inhibition of Hsp90 by IPI-504 (Fig. 1). This result does not support a positive role of Hsp70 in Hsp90-mediated degradation of BCR-ABL. However, inhibition of Hsp70 by KNK347 did not synergistically increase IPI-504-induced apoptosis of BCR-ABL-expressing 32D myeloid cells either (data not shown). Functional relationship between Hsp70 and Hsp90 needs to be studied further.

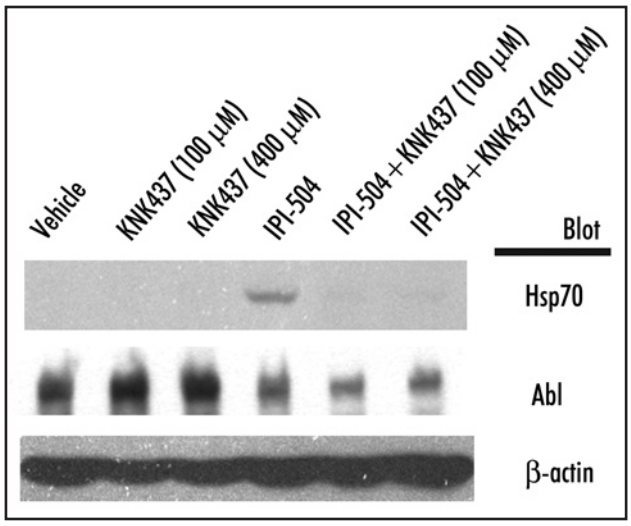

Figure 1. Inhibition of Hsp70 by KNK437 does not prevent BCR-ABL degradation caused by inhibition of Hsp90 by IPI-504. BCR-ABL-expressing 32D cells were treated with KNK437 (100 or 400 μM) or IPI-504 (2 μM) alone or both for 24 hours. Protein lysates were analyzed by Western blotting using antibodies indicated. Inhibition of Hsp70 by KNK437 did not prevent BCR-ABL degradation caused by inhibition of Hsp90 by IPI-504.

Hsp90 INHIBITION REDUCES SURVIVAL OF LEUKEMIC STEM CELLS IN CML MICE

While imatinib induces complete hematologic and cytogenetic remission in the majority of chronic phase CML patients,[38] molecular remission (negativity by RT-PCR) is difficult to achieve in these patients. For example, only 39% of imatinib-treated chronic phase CML patients showed a major molecular response (greater than or equal to 3-log reduction of BCR-ABL mRNA) after 18 months, with only 4% showing a complete molecular response.[39] This is likely due to the inability of imatinib to eradicate a small number of leukemic cells in CML patients, and these cells may function as CML stem cells that cause ultimate disease relapse. Similarly, although imatinib prolongs survival of mice with BCR-ABL-induced CML,[30,40] the disease continues to progress,[30] likely due to incomplete eradication of leukemia stem cells that have been identified as BCR-ABL-expressing hematopoietic stem cells (HSCs).[41] These cells are $Lin^{-}c\text{-}Kit^{+}Sca\text{-}1^{+}$ and can be cultured in conditions that support survival and growth of HSCs.[42,43] In experiments using these culture conditions, we found that imatinib treatment did not lower the percentage and number of leukemia stem cells, whereas inhibtion of Hsp90 by IPI-504 had a strong inhibitory effect on these stem cells.[20] Inhibition of leukemic stem cells by IPI-504 also occurs in CML mice harboring the BCR-ABL-T315I mutant.[20] In contrast, potent BCR-ABL kinase inhibitors did not have as significant an inhibitory effect on CML stem cells.[41]

The underlying mechanism for inhibition of leukemic stem cells by IPI-504 is unknown. However, it is reasonable to believe that inhibition of Hsp90 with IPI-504 not only causes degradation of BCR-ABL but also affects the stability of other proteins that play roles in maintaining survival of leukemic stem cells. These proteins may cooperate with BCR-ABL to regulate survival or self-renewal of leukemic stem cells, and targeting them alone without simultaneously inhibiting BCR-ABL would not have a strong inhibitory effect, consistent with our observation that survival of normal HSCs in mice was not significantly affected by inhibition of Hsp90 with IPI-504.[20] As we showed previously, targeting BCR-ABL alone with imatinib was also insufficient in eradicating leukemic stem cells in CML mice.[41] These results suggest that simultaneous targeting of both BCR-ABL and other Hsp90 client proteins would inhibit leukemic stem cells. If this is the case, we should ask why the simultaneous inhibition of both BCR-ABL and other Hsp90 client proteins by IPI-504 did not cure CML mice, although it resulted in the markedly prolonged survival of the mice.[20] One explanation is that in vivo BCR-ABL degradation induced by Hsp90 inhibition was not complete,[20] and that the residual level of BCR-ABL protein in leukemic cells may have been sufficient to stimulate growth of the cells although at a significantly decreased rate, reflected by the prolonged survival of CML mice treated with Hsp90 inhibitors.[18,20] In this regard, development of more potent Hsp90 inhibitors will be necessary to further evaluate the potential of Hsp90 inhibition in treating Ph+ leukemia. It is also possible that additional pathways in leukemic stem cells must be targeted to eradicate these cells. The identification of CML stem cells in mice[41] provides a powerful system for studying the molecular mechanisms responsible for regulating survival and self-renewal of leukemic stem cells.

PREFERENTIAL INHIBITION OF BCR-ABL-T315I-EXPRESSING CELLS IN CML MICE PROVIDES A NEW THERAPEUTIC STRATEGY

Because mutant BCR-ABL proteins are more sensitive than wild type BCR-ABL to degradation induced by Hsp90 inhibition,[18,20] inhibition of Hsp90 may preferentially prevent emergence of BCR-ABL-T315I-expressing clones over wild type BCR-ABL clones in mice. This hypothesis was tested by transplanting mixed wild type BCR-ABL- and BCR-ABL-T315I-expressing bone marrow cells into the same recipient mouse using the cell surface markers Ly5.1 and Ly5.2 to distinguish the two populations of leukemic cells. Mice were treated with IPI-504, and eventually the BCR-ABL-T315I-expressing cells became the minor population,[20] indicating that inhibition of Hsp90 preferentially suppresses BCR-ABL-T315I-expressing leukemic clones over the wild type BCR-ABL-expressing clones. These results suggest that the combined use of IPI-504 and imatinib in CML patients would be a viable strategy for preventing emergence of imatinib-resistant clones through inhibition of Hsp90 and for suppressing imatinib-sensitive clones though inhibition of BCR-ABL in CML patients. In fact, this idea is supported by our study in CML mice.[20] In this study, mouse bone marrow cells were transduced with BCR-ABL-T315I and wild type BCR-ABL, respectively, and equal numbers of the transduced cells were mixed and transplanted into recipient mice. Each recipient mouse received both types of transduced cells. Mice treated with the combination of IPI-504 and imatinib survived significantly longer than those treated with IPI-504 or imatinib alone. Preferential inhibition of the BCR-ABLT315I-expressing cells in CML mice provides a new therapeutic strategy for the treatment of Ph+ leukemia (Fig. 2).

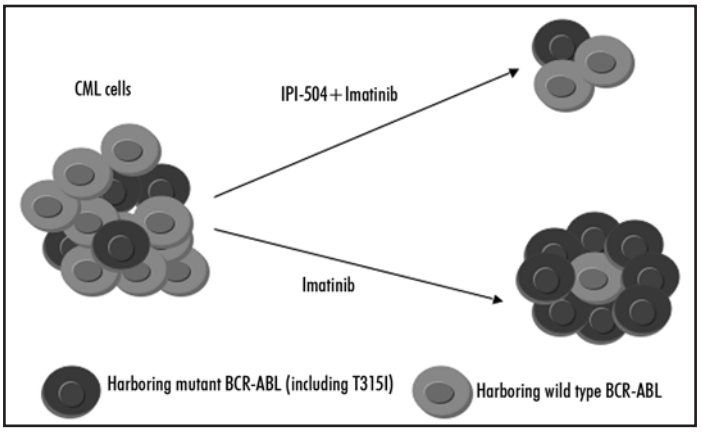

Figure 2. Combination therapy of CML using Hsp90 and BCR-ABL kinase inhibitors. After imatinib-resistant mutations of BCR-ABL occur in leukemic cells, two types of cells may exist in a patient: cells harboring mutant BCR-ABL (such as BCR-ABL-T315I) and cells harboring wild type BCR-ABL. Treatment with a BCR-ABL kinase inhibitor alone (such as imatinib) would lead to selective growth of leukemic cells harboring mutant BCR-ABL, although leukemic cells harboring wild type BCR-ABL were suppressed. In contrast, treatment with both Hsp90 and BCR-ABL kinase inhibitors (such as IPI-504 and imatinib) would inhibit growth of both types of leukemic cells, with a much stronger inhibition of leukemic cells harboring mutant BCR-ABL. This combination therapy provides a novel therapy for Ph+ leukemis.

SUMMARY AND REMAINING ISSUES

The major mechanisms of resistance to TKIs in CML patients are insensitivity of leukemic stem cells to inhibition by these inhibitors and the emergence of BCR-ABL kinase domain mutations. While the resistance of stem cells is poorly understood, BCR-ABL kinase domain mutations have been very well characterized. Most of these BCR-ABL mutants respond to potent kinase inhibitors,[10,12] but these drugs are ineffective in treating Ph+ leukemia patients with the BCR-ABL-T315I mutation. For example, patients known to have the BCR-ABL-T315I mutation prior to therapy had no objective response to dasatinib treatment.[14] Even with the significant effort in developing new BCR-ABL kinase inhibitors that effectively inhibit imatinib-resistant BCR-ABL mutants, a critical question to ask is whether inhibition of BCR-ABL kinase activity alone is sufficient to shut down all functions of BCR-ABL. We showed previously that Src kinases activated by BCR-ABL remained active when BCR-ABL kinase activity was inhibited by imatinib and that Src kinases play a crucial role in the development of BCR-ABL-induced ALL in mice.[30,41] This finding is in opposition to a general belief that shutting down of the kinase activity of BCR-ABL by imatinib will completely inhibit its functions, leading to inactivation of its downstream signaling pathways and cure of the disease. Besides Src kinases, we have identified more signaling molecules that play roles in BCR-ABL leukemogenesis, and whose activation does not depend on BCR-ABL kinase activities (data not shown). In addition, BCR-ABL kinase domain mutations cause insensitivity of leukemic cells to imatinib. However, these BCR-ABL mutants in some CML patients are insufficient to promote growth of leukemic cells,[44] implying a requirement of additional events (besides the presence of mutant

BCR-ABL) in CML development. Because these putative additional events cooperate with BCR-ABL in leukemogenesis, identification of BCR-ABL mutations in CML patients prior to treatment is still necessary. Two recent studies have shown the successful detection of BCR-ABL mutations in human CML cells using effective screening methods.[44,45] Furthermore, BCR-ABL kinase inhibitors only transiently inhibit its kinase activity without causing a reduction of the BCR-ABL protein level; an alternative treatment strategy is to cause degradation of the aberrant BCR-ABL protein (regardless of its mutational status) rather than rely solely on the inhibition of BCR-ABL kinase activity, as proposed by Blagosklonny's group based on their elegant study using BCR-ABL-expressing cell lines.[36] We have demonstrated in mice that direct inhibition of the Hsp90 protein represents an alternative and effective treatment strategy that attenuates BCR-ABL-induced leukemia by causing degradation of the wild type and mutant BCR-ABL proteins.[20] The impressive therapeutic effect of Hsp90 inhibition on Ph+ leukemia in mice suggests that inhibition of other targets linked to Hsp90 function might also be effective in treating the disease. For example, histone deacetylatase (HDAC) inhibitors that induce acetylation and inhibition of Hsp90 should also be effective.[46-49] The mechanisms for resistance of leukemic stem cells to BCR-ABL kinase inhibitors remain unknown; thus, identification and characterization of the pathways affected by Hsp90 inhibition in leukemic stem cells will provide useful information for developing novel therapies against leukemic stem cells. It is likely that a pathway distinct from but cooperative with BCR-ABL is involved in suppression of survival of leukemic stem cells by the Hsp90 inhibitor IPI-504, and that this non-BCR-ABL signaling pathway is driven by an unknown Hsp90 client protein to maintain survival of leukemic stem cells. Furthermore, this putative Hsp90-dependent pathway might be less critical for maintenance of survival of normal hemaotpoietic stem cells than of leukemic stem cells. Future study of this putative non-BCR-ABL pathway in leukemic stem cells has significant potential to lead to development of more effective therapies.

The inhibitory effect of IPI-504 on BCR-ABL-T315I-expressing cells emphasizes the potential effectiveness of Hsp90 inhibitors as therapy for patients with CML as well as those with blast crisis or with Ph+ ALL resistant to BCR-ABL kinase inhibitors. The simultaneous use of Hsp90 and BCR-ABL kinase inhibitors in chronic phase CML patients might prevent the development of imatinib-resistant clones while inhibiting growth of imatinib-sensitive leukemic cells through inhibition of BCR-ABL kinase activity. In addition, early use of IPI-504 to suppress initial B-ALL clones may help prevent the transition of CML to advanced B-ALL caused by the BCR-ABL-T315I mutation. A critical question remaining, however, is why mutant BCR-ABL is more sensitive than wild type BCR-ABL to Hsp90 inhibition. Answers to this question could provide valuable insights into how BCR-ABL and other Hsp90 client proteins function and how to more efficiently target these proteins for cancer therapies. In addition, the mechanisms by which Hsp90 affects stability of BCR-ABL merit further study. Such mechanisms include the role of Hsp70 in degradation of BCR-ABL upon Hsp90 inhibition, as previous studies are contradictory with respect to whether Hsp70 is anti-apoptotic[34,35] or apoptotic.[17,27,32,33] In sum, IPI-504 represents a novel therapeutic approach to treating CML and Ph+ ALL patients, and future clinical trials will help to evaluate its potential for treating cancer that has become resistant to therapy with tyrosine kinase inhibitors such as imatinib. Time will tell whether inhibition of Hsp90 is a useful strategy for treating Ph+ leukemia.

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