ZAP-70 is a novel conditional heat shock protein 90 (Hsp90) client: inhibition of Hsp90 leads to ZAP-70 degradation, apoptosis, and impaired signaling in chronic lymphocytic leukemia

Januario E. Castro, Carlos E. Prada, Olivier Loria, Adeela Kamal, Liguang Chen, Francis J. Burrows, and Thomas J. Kipps

The zeta-associated protein of 70 kDa (ZAP-70) is expressed in patients with aggressive chronic lymphocytic leukemia (CLL). We found that ZAP-70+ CLL cells expressed activated heat-shock protein 90 (Hsp90) with high binding affinity for Hsp90 inhibitors, such as 17-allyl-amino-demethoxy-geldanamycin (17-AAG), whereas normal lymphocytes or ZAP-70+ CLL cells expressed nonactivated Hsp90. Activated Hsp90 bound and stabilized ZAP-70, which behaved like an Hsp90 client protein only in CLL cells.

Treatment with Hsp90 inhibitors such as 17-AAG and 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) induced ZAP-70 degradation and apoptosis in CLL cells but not in T cells, and also impaired B-cell receptor signaling in leukemia cells. Transduction of ZAP-70+ CLL cells with an adenovirus encoding ZAP-70 activated Hsp90 and specifically rendered the leukemia cells sensitive to 17-AAG. These data indicate that Hsp90 is necessary for ZAP-70 expression and activity; that ZAP-70 is unique among Hsp90 clients, in that its chaperone-dependency is conditional on the cell type in which it is expressed; and also that ZAP-70 is required for cell survival and signaling in CLL. Additionally, ZAP-70 expression in CLL cells confers markedly heightened sensitivity to 17-AAG or 17-DMAG, suggesting that these or other Hsp90 inhibitors could be valuable therapeutically in patients with aggressive CLL.

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Materials and methods

Cells and reagents

Peripheral blood mononuclear cells (PBMCs) from patients with CLL were obtained from the CLL Research Consortium (CRC) tissue bank. PBMCs were cultured in complete medium at a density of 1 × 10^6 cells/mL. A.K. and F.J.B. are employed by Conforma Therapeutics Corporation, whose product was studied in the present work.

From the Moores University of California San Diego (UCSD) Cancer Center, University of California; the Conforma Therapeutics Corporation; and the Chronic Lymphocytic Leukemia Research Consortium (CRC), San Diego, CA.


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A.K. and F.J.B. are employed by Conforma Therapeutics Corporation, whose product was studied in the present work.

J.E.C. and C.E.P. contributed equally to this work.

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were isolated by density gradient centrifugation over Histopaque 1077 as described.15 These samples had more than 95% CD19+/CD5+ cells by flow cytometry. ZAP-70 expression and IgVH gene mutational status were assessed as previously described.16 Cells were incubated in RPMI media at 37°C with 5% CO₂. The MCF-7 breast cancer cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA). In some experiments the cells were treated with 2-Fluoro-Ara-A (gift from Drs Reed and Kitada; Burnham Institute, La Jolla, CA), 17-DMAG (InvivoGen, San Diego, CA), 17-AAG, or EC116 (17-AAG analog; Conforma Therapeutics, San Diego, CA). The biotin–geldanamycin (GM) probe was prepared by displacing the 17-methoxy of GM with a biotinyl-linked amine as described.17 Cell samples were incubated also in media with dimethyl sulfoxide (DMSO; 1%) as a control.

Antibodies used were as follows: Hsp90 (SPA-835; recognizes Hsp90α and Hsp90β) and immunoprecipitates free and complexed Hsp90, Hsp90α (SPA-830; recognizes Hsp90α and Hsp90β) and immunoprecipitates uncomplexed Hsp90; Stressgen Biotechnologies, Victoria, BC, Canada). In some experiments the cells were treated with 2-Fluoro-Ara-A (gift from D. Toft), ZAP-70 nonconjugated and Alexa 488 (Caltag Laboratories, Burlingame, CA), p725/6 (clone 4D10.1; Upstate Biotechnology, Lake Placid, NY), 4G10 (Upstate Biotechnology, Lake Placid, NY), Hsp70 (Stressgen Biotechnologies), CD3-PE (BD-Pharmingen, San Diego, CA), IkB-α, and IKK-α (BD-Pharmingen, San Diego, CA).

Hsp90 binding assays

Purified native protein (Stressgen Biotechnologies) or cell lysates in lysis buffer (20 mM HEPES [N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid], pH 7.3, 1 mM EDTA [ethylenediaminetetraacetic acid], 5 mM MgCl₂, 100 mM KCl) were incubated with or without 17-AAG for 30 minutes at 4°C, and then incubated with biotin-GM linked to BioMag streptavidin magnetic beads (Qiagen, Valencia, CA) for 1 hour at 4°C. Tubes were placed on a magnetic rack, and the unbound supernatant removed. The magnetic beads were washed in lysis buffer and heated for 5 minutes at 95°C in sodium dodecyl sulfate–polycrylamide gel electrophoresis (SDS-PAGE) sample buffer. Samples were analyzed on SDS protein gels, and immunoblots were performed using the indicated antibodies. We assessed the band intensities in the immunoblots using the Bio-rad Fluor-S MultiImager (BioRad, Hercules, CA), and calculated the percentage inhibition of binding of Hsp90 to the biotin-GM. The reported IC₅₀ is the concentration of the 17-methoxy of GM with a biotinyl-linked amine as described.17 Cell samples were incubated also in media with dimethyl sulfoxide (DMSO; 1%) as a control.

Immunoblot and coimmunoprecipitation

Lysates were prepared as described in “Hsp90 binding assays.” Protein-A Sepharose beads (Zymed Laboratories, South San Francisco, CA) were preblocked with 5% bovine serum albumin (BSA). The cell lysates were precleared by incubating with 50 μL Protein-A Sepharose beads (50% slurry). To 100 μL of the precleared cell lysate, either no antibody or antibodies to Hsp90, ZAP-70, p23, Hop, or p725/6 were added, and incubated by rotating for 1 hour at 4°C. Precleared beads (50 μL; 50% slurry) were then added and incubated by rotating for 1 hour at 4°C. Bound beads were centrifuged at 3000g and unbound samples were collected. Beads were washed thrice in lysis buffer and then once with 50 μL Tris, pH 6.8. We added SDS-sample buffer and then incubated the samples for 5 minutes at 95°C. Bound and unbound samples were analyzed by SDS-PAGE and immunoblot analyses using the indicated antibodies.

Protein lysates for immunoblot studies were prepared using RIPA buffer with protease inhibitors (10 μg/mL aprotinin, 10 μg/mL leupeptin, 10 μg/mL pepstatin, and 1 mM phenylmethylsulfonyl fluoride), and in some cases phosphatase inhibitors (1 mM Na-Vanadate and 10 mM β-glycerophosphat). Membranes were probed with antibodies as indicated. Detection was accomplished by chemiluminescence using horseradish peroxidase (HRP)–conjugated antibodies followed by development with ECL Plus (Amersham-Biosciences, Piscataway, NJ) and autoradiography with Super RX film (Fuji, Tokyo, Japan).

Flow cytometry and apoptosis detection

Fluorochrome-conjugated monoclonal antibodies were used for flow cytometry as described.15 The samples were processed using a FACScalibur (Becton Dickinson, Franklin Lakes, NJ) and the data were analyzed using FloJo 3.6 software (Stanford University-Tree Star, San Francisco, CA). Apoptotic and viable cells were discriminated via flow cytometry with 3,3’-dihexyloxacarbocyanine iodide (DiOC₆). Molecular Probes, Eugene, OR) and propidium iodide (PI, Sigma), as described.16 Using this method, viable cells exclude propidium iodide (PI) and stain brightly positive for DiOC₆.

Adenovirus transduction of CLL cells

We cloned the cDNA of ZAP-70 extracted from normal T cells (provided by A. Weiss)17 into the cytomegalovirus (CMV) promoter and polyadenylation signal of pcDNA3. This construct was then subcloned into the shuttle vector MCS(SK)pXCI2 as described before.18 This shuttle vector was cotransfected with pMJ17 into 293 cells using the calcium phosphate method. Isolated adenovirus plaques were harvested individually and used to infect 293 cells. High titer adenovirus preparations were obtained, as described.19 CLL cells were infected with either Ad-ZAP-70 or a control adenovirus vector (Ad-LacZ) for 48 hours at 37°C using a multiplicity of infection (MOI) of 1000.

Results

Hsp90 inhibitors induce apoptosis in CLL cells that express adverse prognostic markers

We treated primary leukemia cells (n = 25) with 17-AAG and examined them for drug-induced apoptosis at 48 hours (Table 1). Leukemia cells that used unmutated IgVH genes were significantly more sensitive to 17-AAG than were CLL cells that expressed mutated IgVH genes (Figure 1A; P < .001). Furthermore, there was a significant association between the level of apoptosis induced at 48 hours by 17-AAG and the level of ZAP-70 expressed by each of the leukemia-cell samples (r² = 0.9022; Figure 1B). 17-DMAG, which is another Hsp90 inhibitor, induced a similar proapoptotic activity in CLL cells. However, a synthetic analog of 17-AAG lacking the capacity to inhibit Hsp90 (EC116) did not induce significant apoptosis (Figure 1C-D).

ZAP-70 physically associates with activated Hsp90 in CLL cells

Protein lysates from primary leukemia cells were immunoprecipitated using different specific monoclonal antibodies, as shown in Figure 2A. CLL cells were defined as ZAP-70+ if more than 20% of the stained cells were positive by flow cytometry, as previously described.19 Controls included purified human B and T cells from healthy volunteers and MCF-7, a breast cancer cell line that expresses large amounts of activated Hsp90.11 All samples expressed similar levels of Hsp90. ZAP-70+ CLL and MCF-7 cells expressed Hsp90 that was complexed with the cochaperones Hop and p23. Immunoprecipitation with an antibody that recognizes the uncomplexed form of Hsp90 (Hsp90*), however, revealed that ZAP-70+ CLL samples, as well as T and B cells of healthy donors, expressed the uncomplexed (nonactive) form of Hsp90. These results were consistently reproduced in larger numbers of CLL samples (n = 16; Figure 2B). Interestingly, the ZAP-70 expressed in CLL cells, but not in normal T cells, coimmunoprecipitated with Hsp90, suggesting that this tyrosine kinase is a conditional Hsp90 client protein, as its client status varies depending on the cell in which it is expressed.
We performed competitive binding assays using a biotinylated GM (biotin-GM) probe to investigate whether the differential expression of active Hsp90 in CLL samples correlated with their binding affinity to 17-AAG (Figure 3A-B). Addition of 17-AAG to cell lysates inhibited the binding of Hsp90 to biotin-GM in a dose-dependent fashion, with ZAP-70/H11001 CLL cell lysates experiencing an IC50 of 31 nM (n = 3; SEM). In contrast, the IC50 was 300 nM for lysates from ZAP-70/H11002 CLL cells or normal T or B cells. Purified native Hsp90 protein binding was inhibited with an IC50 of 600 nM, as reported11 (Figure 3A-B).

Inhibition of Hsp90 induces degradation of ZAP-70 in CLL cells but not in T cells

We monitored the expression levels of ZAP-70 in leukemia cells treated in vitro with 17-AAG, 17-DMAG, and a control synthetic analog (EC116). After 24 hours, 17-AAG and 17-DMAG caused a specific dose-dependent reduction of leukemia-cell ZAP-70 expression (inhibitory concentration of 50% [IC50] = 60 nM). This effect was not observed in the samples treated with EC116 or media containing DMSO (Figure 4A-B). Down-regulation of ZAP-70 after incubation with Hsp90 inhibitors correlated with cleavage of poly ADP-ribose polymerase (PARP-1), indicating leukemia-cell apoptosis. It also correlated with the up-regulation of Hsp70, a chaperone protein that is induced by inhibition of active Hsp9020 (Figure 4B). Of note, treatment with 17-AAG did not affect ZAP-70 expression levels in T cells from patients with CLL or healthy donors, even at high concentrations (300 nM; Figure 4C; and data not shown).

In addition, we observed that treatment of CLL cells with Fludara (2-Fluoro-Ara-A) did not induce down-regulation of ZAP-70 despite promoting apoptosis in leukemia cells. This suggests that down-regulation of ZAP-70 after treatment with

Table 1. Characteristics of patient samples used in this study

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ND indicates no data.

Figure 1. Hsp90 inhibitors induce apoptosis in CLL cells that express adverse prognostic markers. (A) Cell apoptosis was measured in CLL cells (n = 25) after in vitro treatment with 17-AAG at 100 nM during 48 hours. IgVH gene mutation was assessed by gene sequencing. Sequences with less than 98% homology to the corresponding germ-line IgVH sequence were considered mutated. (B) Correlation by linear regression of the level of expression of ZAP-70 and apoptosis induced by 17-AAG (100 nM) after 48 hours of incubation in CLL samples. (C, D) Cell death was assessed in ZAP-70⁺ CLL cells after treatment with 17-AAG, 17-DMAG, and EC116 using 100 nM at different time points and also during 48 hours using increasing concentrations.
of p72Syk, which was detected in all samples. Stimulation of monoclonal antibody. We found that 17-AAG did not alter the level whereas Hsp90 from ZAP-70 binding affinity for 17-AAG with an IC50 of 31 nM (SEM of p72Syk and activation of nuclear factor kappa B (NF-

Inhibition of Hsp90 blocks B-cell receptor (BCR) signaling in ZAP-70+ CLL cells

We treated ZAP-70+ CLL cells with 17-AAG and monitored their capacity to respond to ligation of the BCR with F(ab')2 anti-μ monoclonal antibody. We found that 17-AAG did not alter the level of p72Syk11, which was detected in all samples. Stimulation of control-treated samples with anti-μ induced both phosphorylation of p72Syk and activation of nuclear factor kappa B (NF-κB), as assessed by degradation of IkB-α (Figure 6). However, prior treatment with 17-AAG for 24 hours rendered such leukemia cells inert to stimulation with anti-μ antibody.

Transduction of CLL cells to express ZAP-70 activates Hsp90 and induces sensitivity to 17-AAG–mediated apoptosis

We transduced ZAP-70− CLL cells with an adenovirus vector encoding ZAP-70 (Ad-ZAP-70) or β-galactosidase (Ad-LacZ) as a control. After 48 hours, Ad-ZAP-70–transduced CLL cells expressed ZAP-70 at levels that were comparable to that of ZAP-70+ CLL cells (Figure 7A). Transduction of CLL cells with Ad-ZAP-70, but not Ad-LacZ, induced formation of Hop and p23 multichaperone complexes, indicating a change in the conformation of Hsp90 from the latent state to the activated complexed form (Figure 7B). In addition, the de novo expression of ZAP-70 sensitized the cells to 17-AAG, which now could induce apoptosis in ZAP-70− transduced CLL cells even at low drug concentrations (Figure 7C).

Discussion

In the present study we show that ZAP-70 is an Hsp90 client protein in CLL cells but not in T cells and that ZAP-70 degradation via inhibition of Hsp90 leads to impaired signaling after BCR ligation and leukemia-cell apoptosis.

We found that CLL cells from different patients vary in their susceptibility to apoptosis induced by treatment with Hsp90 inhibitors. We investigated the basis for this and found a statistically significant correlation between the relative sensitivity to 17-AAG and expression of unmutated IgVH genes and ZAP-70. Moreover, there was a significant association between the level of apoptosis induced by 17-AAG and the level of ZAP-70 expression in the leukemia-cell samples.

Consistent with this notion, we found that ZAP-70+ CLL cells expressed Hsp90 in multichaperone complexes with high binding affinity for 17-AAG. Conversely, ZAP-70+ CLL cells as well as normal T and B lymphocytes expressed the inactive, uncomplexed form of Hsp90. This is in accordance with our previous data showing that the molecular basis for the selective antitumor activity of 17-AAG and other ansamycins is related to the presence of an increased binding affinity to these compounds in tumor tissues compared with normal cells.11 The increased affinity appears to be due to cochaperone-induced changes in the ATP binding site of Hsp90, because tumor Hsp90 is present entirely in multichaperone complexes with high ATPase activity, whereas Hsp90 from normal tissues is in a latent, apparently uncomplexed state.11 We have also observed that the degree of Hsp90 activation correlated with expression of certain client
kinases associated with poor prognosis, most notably HER-2 in breast cancer.11 Here, we provide evidence indicating that Hsp90 activation and sensitivity to 17-AAG in early-stage CLL cells correlate with poor prognostic factors such as unmutated IgVH genes and expression of ZAP-70 kinase.

Our studies demonstrated that ZAP-70 associates with activated Hsp90 in CLL cells and that treatment with Hsp90 inhibitors, such as 17-AAG or 17-DMAG, induced specific degradation of this kinase. Consistent with this, we did not observe degradation of p72\textsuperscript{Syk} in 17-AAG–treated CLL cells, despite the high degree of similarity of this protein kinase to ZAP-70. Also, treatment with 2-Fluoro-Ara-A did not induce changes in ZAP-70 expression despite inducing apoptosis in CLL cells. This indicates that down-regulation of ZAP-70 mediated by Hsp90 inhibitors is a specific effect that is not due to proteolysis induced by apoptosis per se.

Together, these data indicate that ZAP-70 is itself an Hsp90 client protein susceptible to specific degradation induced by Hsp90 inhibitors. To our knowledge, this is the first time that such association has been reported. Interestingly, the requirement for Hsp90 chaperoning support by ZAP-70 was limited to CLL cells and was not observed in T cells where this kinase is normally expressed.17 In this particular sense, ZAP-70 is unique among the proteins with an Hsp90-association dependence due to inappropriate expression in a different mammalian cell, there are parallels in other systems. Viruses must translate their proteins in the foreign environment of the host cell, and several essential proteins of such human pathogens are Hsp90 clients, including the hepatitis B virus (HBV) reverse transcriptase and hepatitis C virus (HCV) protease.21,22 Similarly, the BSA

Figure 5. Degradation of ZAP-70 mediated by Hsp90 inhibitors is specific and is not induced by cytotoxic chemotherapy in vitro. ZAP-70\textsuperscript{+} and ZAP-70\textsuperscript{-} CLL cells (n = 10) were treated with 17-AAG (100 nM) and 2-Fluoro-Ara-A (2.5 \mu M) for 48 hours. Flow cytometry was used to assess apoptosis (top panels) and ZAP-70 expression (bottom panels) on each sample. Control samples were incubated with media-DMSO as indicated. Error bars indicate the mean value for each group. *P values were calculated using a one-way analysis of variance (ANOVA) with Bonferroni posttest analysis.

Figure 6. Inhibition of Hsp90 impairs B-cell receptor (BCR) signaling in ZAP-70\textsuperscript{+} and ZAP-70\textsuperscript{-} CLL cells. ZAP-70\textsuperscript{+} and ZAP-70\textsuperscript{-} cells were preincubated with 17-AAG for 24 and 72 hours and then treated for 10 minutes with F(ab')\textsubscript{2} anti-\(\alpha\)-Ig antibodies to induce cellular activation. Protein lysates were immunoprecipitated (IP) with specific anti-p72\textsuperscript{Syk} antibody and assessed by Western blot (WB) for tyrosine phosphorylation using 4G10 antibody and p72\textsuperscript{Syk} expression. Protein lysates from the same samples were evaluated by Western blot using the indicated antibodies. Activation of NF-\(\alpha\)B was assessed by degradation of IC\(\alpha\)B-\(\alpha\). These results were reproduced more than 3 times.
Figure 7. Transduction of CLL cells to express ZAP-70 activates Hsp90 and induces sensitivity to 17-AAG–mediated apoptosis. (A) ZAP-70– leukemia cells were transduced with adenovirus expressing ZAP-70 (Ad-ZAP-70; solid histogram) and β-galactosidase (Ad-LacZ; bold line histogram). Cells were kept in media as a control (dashed line histogram). After 48 hours in culture Ad-ZAP-70– but not Ad-LacZ–transduced cells expressed the ZAP-70 transgene (46% expression with a mean fluorescence intensity ratio [MFIR] of 2.8). (B) ZAP-70+ CLL cells were transduced with adenovirus and after 48 hours they were harvested and lysed. Immunoprecipitations and immunoblots were performed with the antibodies indicated. The bound fractions represent the immunoprecipitated proteins and the unbound samples represent the soluble protein lysate after immunoprecipitation. (C) After 48 hours of transduction with adenovirus, the leukemia cells were treated in vitro with 17-AAG (100 nM) for additional 48 hours and then assessed for apoptosis by flow cytometry using DIOC6 and PI. After treatment with 17-AAG, CLL cells transduced with Ad-ZAP-70 had a significantly higher level of apoptosis compared with nontransduced or Ad-LacZ–transduced cells; P = .001. ■ indicates control, untreated with 17-AAG, □ treated with 17-AAG.

protein attains client status when it is inappropriately expressed in the HeLa-cell cytosol, presumably because it adopts an aberrant conformation.23

We did not observe activated Hsp90. 17-AAG–mediated ZAP-70 degradation, or apoptosis in normal T cells or T cells derived from patients with CLL, even when higher concentrations of 17-AAG were used. In addition, Hsp90 from T cells had a 10-fold-lower binding affinity for 17-AAG than did Hsp90 from ZAP-70+ CLL cells. As such, 17-AAG and its derivatives may selectively target CLL cells at concentrations that do not affect T cells. These results are similar to those recently reported for geldanamycin,24 which also appears selectively nontoxic for T cells and targetable in CLL.25

This may be because such leukemia-cell samples were from patients with advanced-stage disease, in which CLL cells may have incurred secondary genetic changes that could increase their sensitivity to geldanamycin, as has been noted for other leukemias.25

Treatment of ZAP-70+ CLL cells with 17-AAG also resulted in loss of BCR signaling. We have reported previously that expression of ZAP-70 in CLL allows for more effective IgM signaling in CLL cells, a feature that could contribute to the relatively aggressive clinical behavior associated with CLL cells that express unmutated IgVH genes.26 This enhanced signaling is associated with higher levels of phosphorylated p72Syk, B-cell linker protein (BLNK), and phospholipase-Cγ, and greater Ca2+ flux.26 Therefore, our findings lend further support to the emerging physiologic role of ZAP-70 in CLL cells. The signaling events after BCR ligation might not be specifically due to inhibition of ZAP-70, as other kinases that participate in BCR signaling also may be influenced by Hsp90 inhibition.27-29 Nevertheless, we found that 17-AAG did not induce degradation of p72Syk or IKK-α, suggesting that the loss of BCR signaling most likely is due to degradation of ZAP-70 resulting from inhibition of Hsp90. Reconstitution of the ZAP-70/Hsp90/cochaperone complex in vitro or the use of RNA interference techniques targeting ZAP-70 might provide additional support to this notion.

ZAP-70+ CLL cells underwent apoptosis upon treatment with low concentrations of 17-AAG or 17-DMAG. This effect was time- and dose-dependent and correlated closely with the level of ZAP-70 expression. Conceivably, other proteins, including some that support leukemia-cell survival, are also degraded upon inhibition of Hsp90, thereby contributing to the effects seen in treated CLL cells. Indeed, some proteins identified as Hsp90 client proteins are known survival-signaling kinases, such as IGF-1R, Akt, Raf-1, and IKK.5,8,11,30 However, transduction of ZAP-70+ CLL cells with an adenovirus encoding ZAP-70, but not with a control adenovirus vector, activated Hsp90 and specifically rendered the leukemia cells sensitive to apoptosis induced by 17-AAG. This indicates that the expression of wild-type ZAP-70 in CLL cells was sufficient to induce activation of Hsp90. Also, our experiments suggest that ZAP-70+ CLL cells are dependent on ZAP-70 for their survival and that degradation of this kinase, as observed after treatment with Hsp90 inhibitors, effectively impairs not only BCR signaling, but also cell survival.

Taken together, our data suggest that differences in clinical prognosis and cancer progression in CLL may be linked to the activity of Hsp90. Inappropriate ZAP-70 expression in malignant cells of the B lineage increases their malignant potential, indicating that ZAP-70 may represent an adaptive change in the somatic evolution of CLL in vivo. Provocative recent findings indicate that Hsp90 may play a buffering role in Darwinian evolution rescuing potentially misfolded mutant or aberrantly expressed proteins such that they do not become lethal to the cell.31,32 By analogy, activation of Hsp90 might protect aggressive CLL cells by stabilizing ZAP-70, thus allowing this kinase to support tumor-cell survival and/or proliferation. By the same token, our data indicate that ZAP-70 expression in CLL cells confers heightened sensitivity to 17-AAG or 17-DMAG, indicating that Hsp90 inhibitors might have substantial therapeutic activity, particularly in patients with aggressive, ZAP-70+ disease.

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References


ZAP-70 is a novel conditional heat shock protein 90 (Hsp90) client: inhibition of Hsp90 leads to ZAP-70 degradation, apoptosis, and impaired signaling in chronic lymphocytic leukemia

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