inside blood

25 OCTOBER 2012 | VOLUME 120, NUMBER 17

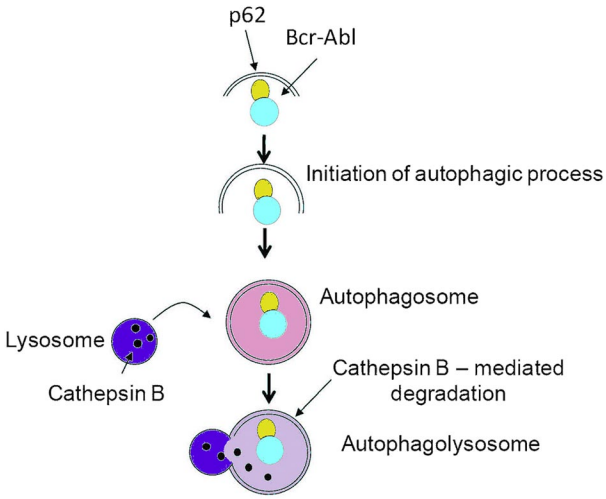
MYELOID NEOPLASIA

Comment on Goussetis et al, page 3555

BCR-ABL/p62/SQSTM1: a cannibal embrace

Patrick Auberger INSERM

In this issue of *Blood*, Goussetis et al identify autophagy as a new pathway for the degradation of the oncoprotein BCR-ABL. They show that the therapeutic drug arsenic trioxide (AS_2O_3) targets BCR-ABL for autophagic degradation via a p62/SQSTM1-dependent mechanism that is critical for the antileukemic effect of the drug.[1]



p62/SQSTM1-dependent autophagic degradation of BCR-ABL in CML. Proposed model for the As_2O_3-dependent interaction of p62/SQSMT1 with BCR-ABL and subsequent autophagic degradation of BCR-ABL. The As_2O_3 treatment of CML cells triggers the interaction of p62 with BCR-ABL and the subsequent CTSB-mediated autophagic degradation of the fusion protein that is responsible for the antileukemic effect of the drug. The inhibition of autophagy by different means (pharmacologic agents or siRNA-mediated inhibition of Atg7, p62/SQSTM1, or CTSB) impairs BCR-ABL degradation and the effect of AS_2O_3, demonstrating the crucial role of autophagy in the effect of As_2O_3. Adapted from Figure 6 in the article by Goussetis et al that begins on page 3555.

Chronic myelogenous leukemia (CML) is characterized by the t(9;22) chromosomal translocation that juxtaposes the Breakpoint Cluster Region (*BCR*) gene to the Abelson (*ABL*) gene. The corresponding fusion protein BCR-ABL is endowed with a constitutive tyrosine kinase activity that is responsible for the occurrence of the disease. CML is paradigmatic in that it was the first leukemia to benefit from a targeted therapy with tyrosine kinase inhibitors (TKIs). TKIs have been shown to induce both apoptosis and autophagy in CML cells and the modulation of autophagy has recently emerged as a promising therapeutic option in CML and other leukemias.

Autophagy is a lysosomal catabolic process that is responsible for the turnover and elimination of damaged organelles and macromolecules. This process is used consistently by cells to promote their survival under adverse conditions such as stress signals and nutrient deprivation. Of note, autophagy can promote either cell survival or death, depending on the circumstances, making this process a promising target for therapeutic intervention. Indeed, autophagy participates in numerous biologic processes, such as cellular homeostasis, proliferation, differentiation, and cell death, all events that are potentially linked to leukemia progression and transformation.[2]

Using both pharmacologic and siRNA approaches, Goussetis et al describe the autophagic degradation of BCR-ABL by the lysosomal cysteine protease cathepsin B (CTSB) in CML cells treated with As_2O_3.[1] They provide compelling evidence that p62/SQSTM1, a cargo protein involved in the targeting and elimination of ubiquitinated protein, is critical for the antileukemic effect of the drug. Clearly, these findings represent an important advance in our understanding of the regulation and function of BCR-ABL.

To date, several routes for the degradation of BCR-ABL have been reported including the proteasome,[3] caspase-mediated degradation,[4] CTSB-dependent cleavage consecutive to lysosomal membrane permeabilization,[4] and autophagy.[2] There is also evidence in the literature that autophagy is critical for the degradation of the PML-RARA (promyelocytic leukemia–retinoic acid receptor A) fusion protein.[5,6] In both reports, the degradation of PML-RARA was associated with an increased antileukemic effect of As_2O_3, or all trans-retinoic acid (ATRA).

The results reported by Goussetis et al are important in several regards. First, this study identifies for the first time p62/SQSTM1 as a critical player in the autophagic degradation of the BCR-ABL fusion protein (see figure). p62/SQSTM1 is a versatile protein that exerts both pro and anti-tumor functions.[7] Importantly, the study by Goussetis et al clearly identifies p62/SQTM1 with antileukemic properties in CML. In future experiments, it will be interesting to determine whether the interaction of p62/SQSTM1 with BCR-ABL is a direct one. Indeed, BCR-ABL has been shown to be ubiquitinated and p62/SQSTM1

is known to carry a ubiquitin-binding domain that could promote the direct interaction of both proteins.[7] Second, from a mechanistic point of view, it will be important to decipher how As_2O_3 initiates the interaction of p62/SQSTM1 with BCR-ABL. Along this line, the phytoalexin resveratrol has been shown to promote the interaction of p62/SQSTM1 with microtubule-associated protein light chain 3 (LC3), thus favoring autophagy.[8]

The study by Goussetis et al paves the way for the possibility of new therapeutic intervention in CML. TKIs that target BCR-ABL are currently the leading compounds for patients suffering CML, leading to complete remission in a majority of cases. However, although they inhibit the tyrosine kinase activity of BCR-ABL, TKIs failed to eliminate the so-called leukemic initiating cells (LICs) that are critically involved in the reinitiation of the disease in a non-negligible proportion of the treated patients. Conversely to TKIs, As_2O_3, ATRA, or resveratrol, all drugs that are susceptible to target LICs could represent new therapeutic options in the treatment of CML. This should be achieved using combination of TKIs with the above-mentioned compounds.

Furthermore, BCR-ABL promotes the activation of the mammalian target of rapamycin (mTOR) pathway and as such acts as a potent inhibitor of autophagy, either directly or via inhibition of the adenosine monophosphate kinase. As_2O_3 and resveratrol are both capable to inhibit the mTOR pathway and to trigger CTSB-dependent BCR-ABL degradation.[8,9] In the future, we must build on these dual effects of As_2O_3 for a better approach to CML treatment.

In conclusion, it is clear that the therapeutic modulation of autophagy represents a new avenue for the treatment of leukemia. The discovery that different clinically well-characterized therapeutic drugs trigger their effects via the autophagic degradation of oncogenic fusion proteins is of outstanding importance in oncohematology. Accordingly, the article by Goussetis et al sheds new light on the regulation and function of BCR-ABL, representing the premises for the use of these drugs in combination with TKIs in chronic phase CML and as a single therapy in TKI-resistant patients.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Goussetis DJ, Gounaris E, Wu EJ, et al. Autophagic degradation of the BCR-ABL oncoprotein and generation of antileukemic responses by arsenic trioxide. *Blood.* 2012; 120(17):3555-3562.
2. Helgason GV, Karvela M, Holyoake TL. Kill one bird with two stones: potential efficacy of BCR-ABL and autophagy inhibition in CML. *Blood.* 2011;118(8):2035-2043.
3. Tsukahara F, Maru Y. Bag1 directly routes immature BCR-ABL for proteasomal degradation. *Blood.* 2010; 116(18):3582-3592.
4. Puissant A, Colosetti P, Robert G, Cassuto JP, Raynaud S, Auberger P. Cathepsin B release after imatinib-mediated lysosomal membrane permeabilization triggers BCR-ABL cleavage and elimination of chronic myelogenous leukemia cells. *Leukemia.* 2010;24(1):115-124.
5. Nasr R, Guillemin MC, Ferhi O, et al. Eradication of acute promyelocytic leukemia-initiating cells through PML-RARA degradation. *Nat Med.* 2008;14(12):1333-1342.
6. Isakson P, Bjoras M, Boe SO, Simonsen A. Autophagy contributes to therapy-induced degradation of the PML/RARA oncoprotein. *Blood.* 2010;116(13):2324-2331.
7. Puissant A, Fenouille N, Auberger P. When autophagy meets cancer through p62/SQSTM1. *Am J Cancer Res.* 2012;2(4):397-413.
8. Puissant A, Robert G, Fenouille N, et al. Resveratrol promotes autophagic cell death in chronic myelogenous leukemia cells via JNK-mediated p62/SQSTM1 expression and AMPK activation. *Cancer Res.* 2010;70(3):1042-1052.
9. Vakana E, Altman JK, Glaser H, Donato NJ, Platanias LC. Antileukemic effects of AMPK activators on BCR-ABL-expressing cells. *Blood.* 2011;118(24):6399-6402.

● ● ● THROMBOSIS & HEMOSTASIS

Comment on Feys et al, page 3611, and on Callewaert et al, page 3603

Blocking VWF platelet binding to treat TTP

Isabelle I. Salles and James T. B. Crawley IMPERIAL COLLEGE LONDON

Two articles in this issue of *Blood* from Feys et al and Callewaert et al, respectively, have employed very similar and elegant strategies in attempts to ameliorate the symptoms of thrombotic thrombocytopenic purpura (TTP).[1,2]

TTP is associated with severe deficiency in ADAMTS13, the metalloprotease that regulates von Willebrand factor (VWF) multimeric size and its platelet-tethering function.[3,4] ADAMTS13 deficiency induces the persistence of pathogenic ultra-large (UL)–VWF in plasma that precipitates the formation of microvascular platelet-rich thrombi that variably cause microangiopathic haemolytic anaemia, thrombocytopenia, neurologic abnormalities, fever, and renal dysfunction. TTP is most frequently an acquired autoimmune disorder, arising through the formation of inhibitory antibodies against ADAMTS13. Untreated, the mortality rate of TTP is approximately 90%.

TTP is currently most effectively treated by plasma exchange, which serves to remove anti-ADAMTS13 antibodies and UL-VWF, and also replenish plasma ADAMTS13 activity. Steroids are used as immunosuppression to attain complete remission and, more recently, the use of rituximab has also proved highly efficacious in treating acquired TTP.[5] Despite the appreciable reduction in mortality (to 10%-20%) using these therapeutic approaches, treatments are individualized and may be associated with further risks and complications. Therefore, new safer and simpler strategies to treat TTP are desirable.

In mice, ADAMTS13 deficiency alone is insufficient to precipitate TTP-like symptoms, making it a difficult model in which to study novel therapeutic approaches.[6] To circumvent this, and to better mimic the human scenario, Feys et al recently developed a baboon model of acquired TTP by infusing an anti-ADAMTS13 monoclonal antibody that efficiently inactivates plasma ADAMTS13.[7] In baboons, this causes elevated plasma UL-VWF, platelet-rich thrombi in the microvasculature resulting in thrombocytopenia and haemolytic anaemia (see figure panel A). Although this nicely models early-stage human TTP, baboons do not develop life-threatening disease, or signs of neurologic or renal dysfunction. The clinical features of TTP are primarily linked to elevated plasma UL-VWF. For this reason, both Feys et al and Callewaert et al rationalized that targeting the glycoprotein Ib binding site in the VWF A1 domain might specifically prevent TTP.[1,2]

Feys et al employed a humanized mouse monoclonal antibody against the VWF A1