A Novel Role for a Familiar Protein in Apoptosis Induced by Proteasome Inhibition

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In this issue of *Cancer Cell*, Nickeleit et al. (2008) identify a new proteasome inhibitor, argyrin A, and show that it induces apoptosis and inhibits angiogenesis via p27-dependent mechanisms. Their observations challenge current thinking about how this class of promising cancer therapies works and why they selectively kill cancer cells.

The proteasome is a multisubunit catalytic complex consisting of three major enzymatic activities that is responsible for the degradation of a large proportion of total cellular proteins (Adams, 2004). Small-molecule inhibitors of the proteasome have been useful research tools for several years, but recently they have gained attention as cancer therapies with the US Food and Drug Administration's approval of bortezomib (also known as PS-341 or Velcade) for the treatment of multiple myeloma (MM) and mantle cell lymphoma. Proteasome inhibition is also highly relevant to the molecular mechanisms involved in the tissue damage associated with neurodegenerative disorders (Bennett et al., 2007). Therefore, mechanistic insights gained from studies of cancer cells are potentially relevant to our understanding of the mechanisms involved in neurodegeneration, and vice versa.

Some of the enthusiasm for developing bortezomib as a therapy for cancer came from work showing that the inflammation-associated transcription factor NF-κB is constitutively active in many different advanced cancers, coupled with the knowledge that its physiological inhibitor (IκBα) is regulated primarily at the level of the proteasome (Adams, 2004) (Figure 1). Subsequent studies have confirmed that bortezomib and other proteasome inhibitors block NF-κB, and recent work has linked the clinical activity of the drug to activating mutations in the NF-κB pathway in patients with MM (Keats et al., 2007). However, given the large number of pathways that could potentially be affected by proteasome inhibition, it seemed unlikely that NF-κB inhibition would account for all of bortezomib's cytotoxic effects (Hideshima et al., 2002), and more recently attention has turned toward mechanisms that might be more universally shared by cells when proteasome function is blocked, including the possible involvement of the "proteotoxic" stress that is caused by misfolded protein buildup. Several studies have shown that bortezomib-induced apoptosis in MM cells and other cancers is associated with the activation of an endoplasmic reticulum (ER) stress-response pathway known as the unfolded protein response (UPR) (Obeng et al., 2006), and apoptosis in many models is mediated by caspase-4, an ER-resident member of this family of death proteases that is activated by ER stress (Nawrocki et al., 2005). Furthermore, chemical and molecular inhibitors of the coupling between the proteasome and autophagy (the other major route of intracellular protein degradation) can synergize to promote apoptosis in cancer cells. One such approach involves combining proteasome inhibitors with inhibitors of histone deacetylases, since the coupling between the proteasome and autophagy requires the cytosolic deacetylase HDAC6 (Nawrocki et al., 2006). Clinical trials have opened to test the effects of proteasome inhibitor and HDAC inhibitor combinations in patients, and the preliminary results are encouraging.

In their study, Nickeleit and colleagues (2008) set up a high-throughput screen to identify small molecules capable of increasing the expression of $p27^{kip1}$, a polypeptide cyclin-dependent kinase inhibitor, in cancer cells. Their screen identified argyrin A, a cyclic peptide derived from the myxobacterium *Archangium gephyra*. Functional studies revealed that argyrin A is a potent inhibitor of the proteasome's three major enzymatic activities and that all of the measured biological effects of the compound were dependent on p27 but not on stabilization of IκBα (Figure 1). Importantly, the investigators found that bortezomib's cytotoxic effects were not p27 dependent, and gene expression profiling suggested that the effects of argyrin A were more similar to the effects of proteasome subunit knockdown than the effects of bortezomib were. They concluded that argyrin A is a "purer" proteasome inhibitor than bortezomib and that bortezomib's off-target effects might explain why it kills cells via a p27-independent mechanism. The expression profiling results confirmed that bortezomib increases the expression of genes associated with ER stress but that argyrin A and proteasome subunit knockdown do not.

This paper generates two conclusions that are potentially paradigm shifting if they can be confirmed. First, the general explanation for why proteasome inhibitors display selectivity for cancer cells is that uncontrolled cell-cycle progression makes these cells particularly vulnerable to their effects (Drexler, 1997). In a more general sense, the relationship between cell-cycle progression and apoptosis has been appreciated for over a decade as a result of studies performed by Gerard Evan, Doug Green, Scott Lowe, John Cleveland, and others showing that Myc and viral oncogenes that function like Myc sensitize cells to apoptosis (Green and Evan, 2002). Indeed, recent work from M.S. Soengas's group (Figure 1) and our own observations have implicated Myc in the proapoptotic effects of bortezomib (Nikiforov et al., 2007), which would seem to contradict the conclusion that an inhibitor of cell-cycle progression

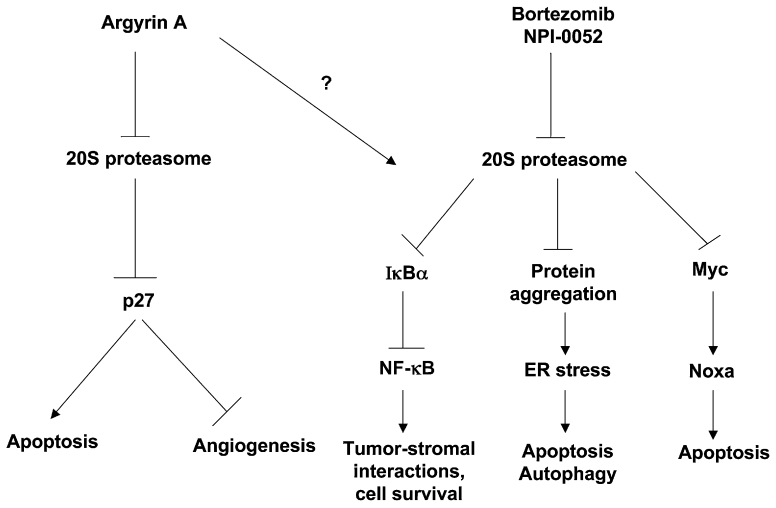

Figure 1. Candidate Mechanisms Underlying Cell Death Induced by Proteasome Inhibitors
In this issue, Nickeleit and colleagues (2008) demonstrate that argyrin A is a novel proteasome inhibitor and that it induces apoptosis and inhibits angiogenesis via p27-dependent mechanisms (left pathway). On the other hand, studies with bortezomib or the structurally distinct inhibitor NPI-0052 (right pathways) have implicated NF-κB inhibition, proteotoxic stress, and stabilization of Myc and downstream upregulation of the BH3-only BCL-2 family member Noxa (among many other mechanisms) in cell death. The extent to which proteasome inhibition versus off-target effects of each compound dictate their downstream effects in cancer cells requires further investigation. Clearly, these compounds have diverse effects on cells, and attempting to identify final common pathways involved in their antitumor activities could be difficult. However, the Nickeleit et al. study will cause a significant reevaluation of past results and could have important implications for our understanding of how the proteasome might best be exploited in specific cancers.

like p27 would be centrally involved in the cytotoxic effects of proteasome inhibitors in cancer cells. Here, the potential off-target effects of bortezomib or an extranuclear cdk-independent mechanism of p27 could explain the apparent discrepancy. Several other structurally unrelated proteasome inhibitors are now in clinical development, and it will be interesting to compare their on- and off-target effects to bortezomib, argyrin A, and proteasome subunit knockdown by expression profiling and other approaches in future studies. One would imagine that they would have very different off-target effects that are not attributable to proteasome inhibition.

The other striking conclusion is that the effects of bortezomib on ER stress pathway genes (Figure 1) may be related to its off-target effects, since these effects were not observed in cells exposed to argyrin A or siRNAs targeting proteasome subunits. While this is conceivable, the bulk of the available evidence here suggests that protein aggregation probably does play an important role. Perhaps the best evidence supporting the aggregation hypothesis has come from recent studies of neurodegeneration, where elegant work has established that the misfolded proteins associated with disease cause proteasome inhibition (Bennett et al., 2007) and that the extent of protein buildup dictates the extent of tissue damage. Furthermore, studies in a *Drosophila* eye model have directly demonstrated that proteasome subunit knockdown induces a stress that is caused by protein aggregation (Pandey et al., 2007). Thus, many investigators will remain attracted to a model in which the protein aggregation caused by proteasome inhibition plays a central role in cell death. It is certainly possible that p27 modulates these effects, potentially by regulating autophagy. One can imagine that protein aggregation and features of ER stress could occur without obvious changes in mRNA expression as measured in microarrays.

Finally, the translational significance of this work to proteasome inhibitor-based therapies could be quite significant. Even in disease sites like MM where it is active, bortezomib induces major clinical responses in a minority of patients (<40%), and no patient has been cured with the drug. Recent studies indicate that structurally distinct proteasome inhibitors like NPI-0052 kill cells via mechanisms that are distinct from those elicited by bortezomib (Chauhan et al., 2005), and based on these data, it seems clear that argyrin A also works by a distinct mechanism. Therefore, by combining these agents or using them in different subsets of tumors, we may be able to better attack the intertumoral heterogeneity that limits therapeutic efficacy in MM and other proteasome inhibitor-sensitive cancers.

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