Bortezomib paradigm shift in myeloma

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Bortezomib’s unprecedented antitumor activity in myeloma has long been attributed to NF-κB inhibition. A new study from the group that put the drug on the map directly challenges this assumption.

Bortezomib (Velcade, formerly PS-341) is a peptide boronate inhibitor of the proteasome that was developed for cancer therapy by Julian Adams, Peter Elliott, and their colleagues. At the time, this was considered a very bold move, since loss of proteasome function was thought to be incompatible with viability in normal cells. However, using a novel assay that directly measures 20S proteasome activity, they demonstrated that rodents and primates tolerated doses of bortezomib resulting in up to 80% proteasome inhibition without obvious toxicity. They also used this assay to guide dose escalation in cancer patients in phase 1 clinical trials in solid and hematologic malignancies. Although single-agent activity was modest in most tumors, Anderson’s group led a phase 2 clinical trial that showed that bortezomib was active in relapsed refractory multiple myeloma (MM), leading to FDA approval of the drug for the treatment of MM in 2003.

Although it was assumed from the start that bortezomib would have diverse effects on cancer cell biology, the most common mechanism attributed to its antitumor actions was inhibition of the inflammation-associated transcription factor, NF-κB. Work performed by several groups implicated NF-κB activation in the maintenance of cancer cell survival, and other studies demonstrated that conventional cancer therapies also commonly activated NF-κB, undermining their therapeutic potential. Preclinical studies and clinical trials confirmed that bortezomib downregulated tumor cell expression of known NF-κB transcriptional targets (IL-6, etc), consistent with the hypothesis. Furthermore, a more recent high-profile study demonstrated that the subset of primary MMs that possesses activating mutations within the so-called noncanonical NF-κB pathway was especially sensitive to bortezomib, adding further support to the concept.

A cautionary note was introduced by work performed several years ago, showing that NF-κB inhibition did not fully account for bortezomib’s cytotoxic effects in MM cells. Nonetheless, it is still generally accepted that bortezomib is a potent and general NF-κB inhibitor and that these effects contribute to cytotoxicity. In this issue of Blood, the new work by Hideshima et al turns this idea on its head. Using human MM cell lines and primary tumor specimens, they now show that bortezomib actually activates 2 upstream NF-κB–activating kinases (RIP2 and IKKβ), promotes down-regulation of NF-κB’s inhibitor (IkBα), and increases NF-κB DNA binding in vitro. Another structurally unrelated proteasome inhibitor (lactacystin) induces the same effects, strongly suggesting that NF-κB activation is an “on-target” effect of the drug. Furthermore, they present evidence that bortezomib also fails to block NF-κB in vivo, determined by measuring nuclear localization of NF-κB’s RelA/p65 subunit. Exposure of cells to bortezomib in the presence of a selective IKK antagonist (MLN120B) blocks NF-κB activation and promotes cell death. Together, these results provide compelling evidence that NF-κB inhibition is probably irrelevant to the effects of bortezomib in MM cells.

Why did it take so long for this long-held assumption to be overturned? One reason is that bortezomib’s documented NF-κB inhibitory effects were almost always measured in cells exposed to cytokines that are known to promote proteasome–dependent degradation of IkBα (ie, TNFα), while investigators largely ignored bortezomib’s lack of inhibitory effects on basal NF-κB activity. In addition, the possibility that nonproteasomal degradation of IkBα might contribute to NF-κB activation was not explored. So, if NF-κB inhibition is not involved in bortezomib’s cytotoxic effects, what other mechanisms might explain its unique potency in MM cells? One attractive explanation (and the simplest) is that cell death results from protein build-up and aggregation, as is the case in neurodegenerative diseases. In this model, the high levels of immunoglobulin production and ER–Golgi...
protein transport would sensitize MM cells to proteotoxic stress, providing an attractive explanation for bortezomib’s clinical activity and a potential means of identifying bortezomib-based combination approaches that will display even greater antitumor effects.

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REFERENCES

Comment on Graham et al, page 1083

Granules and thrombus formation

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In this issue of Blood, Graham and colleagues demonstrate the importance of platelet dense granule secretion for in vivo platelet accumulation following laser injury, which is mediated by the SNARE protein Endobrevin/VAMP-8.

Each human platelet contains 50 to 80 α-granules, 3 to 8 dense (β-) granules, and a few lysosomes. α-granules carry more than 300 releasable proteins, including adhesion molecules, chemokines, cytokines, fibrinolytic regulators, immunologic modulators, and an assortment of coagulation, complement, growth, and proangiogenic and antiangiogenic factors. Dense granules contain mostly small molecules, including calcium, magnesium, polyphosphate, ATP, ADP, GTP, GDP, and serotonin. Platelet granule release is important for hemostasis, because patients with inherited granule defects have bleeding problems. α-granules are absent in the gray platelet and ARC syndromes, while deficient δ-granules are observed in isolation, in combination with α-granule deficiency, or as part of a syndrome in the Hermansky-Pudlak, Chediak-Higashi, and Griscelli syndromes.

The molecular mechanisms involved in platelet granule secretion are complex and incompletely understood. Platelet activation involves several rapid events, including shape change, aggregation, and granule secretion, and it is difficult to discern the cellular mechanisms involved. Electron microscopy studies have revealed that platelet shape changes are accompanied by cytoskeletal rearrangements and centralization of granules. Platelet exocytosis involves fusion of granules with the plasma membrane or the open canalicular system (extensive channels of internal membranes that communicate with the outer surface) to release their contents into the extracellular space (see figure). Fusion of granule vesicles requires soluble N-ethylmaleimide–sensitive attachment protein receptors (SNAREs), lipid components, and SNARE regulatory proteins (eg, NSF, Sec1/Munc18 proteins). SNAREs from opposing membranes assemble into a complex consisting of parallel 4-helix bundles, which catalyzes the apposition and fusion of the vesicle with the target membrane. Platelets contain vesicle-membrane SNAREs (v-SNAREs), including VAMP-2, -3, -7, and -8, and target-membrane SNAREs (t-SNAREs), including syntaxin 2, 4, 7, and 11, and SNAP-23, -25 and -29. Studies have shown that syntaxin 2 and SNAP-23 are involved in all 3 platelet granule secretion events, whereas syntaxin 4 mediates α-granule and lysosome release. VAMP-8 is the primary v-SNARE required for secretion of all platelet granules.

Platelet granule secretion requires SNARE proteins. Platelets contain α-granules, dense (β-) granules, and a few lysosomes. Platelet activation results in cytoskeletal rearrangements, centralization of granules, and fusion of granules with the target membrane (plasma membrane or the open canalicular system). Secretion involves the assembly of granule v-SNAREs (eg, VAMP-8) and target membrane t-SNAREs (eg, syntaxin 2, SNAP-23) into a complex allowing membrane fusion and exocytosis.
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