THERAPEUTICS

Opening the door to a new class of proteasome inhibitors

The first proteasome-targeted drug, bortezomib, was approved in 2003 for the treatment of multiple myeloma and this validated the 20S proteolytic subunit of the proteasome as an anticancer target. Now, reporting in *Nature Medicine*, Linder and colleagues present a small-molecule inhibitor of proteasomal deubiquitylases that induces tumour cell apoptosis, potentially paving the way for the development of a new class of proteasome inhibitors.

The small molecule b-AP15 was identified in a screen for inhibitors that induce the lysosomal apoptosis pathway. *In vitro* experiments with tumour cell lines showed that b-AP15 induced gene expression signatures that closely matched those of cells treated with well-characterized proteasome inhibitors. Cells treated with b-AP15 rapidly accumulated polyubiquitylated proteins, with similar kinetics to cells treated with bortezomib; however, polyubiquitylated proteins showed higher molecular masses. b-AP15 treatment also led to an upregulation of apoptotic markers and the accumulation of cell-cycle regulatory proteins, leading to a G2/M phase cell cycle arrest. Decreased cell viability was observed at concentrations that induced polyubiquitin accumulation, indicating a link between proteasome inhibition and cytotoxicity.

On further investigation of the mode of proteasomal inhibition, the authors found that b-AP15 acts as a reversible inhibitor of the deubiquitylases ubiquitin thioesterase L5 (UCHL5) and ubiquitin-specific-processing protease 14 (USP14), which form part of the 19S regulatory subunit of the 26S proteasome. Interestingly, the inhibition of the proteasome via the 19S subunit can induce apoptosis regardless of mutations or deletions in the tumour suppressor p53 or amplifications in the *BCL2* oncogene, which are involved in resistance mechanisms to bortezomib. Testing b-AP15 in the NCI60 panel of cancer cell lines also revealed a different therapeutic range compared with bortezomib with regard to tumour type, with the highest sensitivity observed in colon carcinoma and central nervous system tumours.

In vivo experiments showed that daily subcutaneous injections of b-AP15 significantly slowed tumour growth in severe combined immunodeficient mice with human squamous carcinoma xenografts. In mice with colon carcinoma xenografts overexpressing BCL-2, b-AP15 treatment led to significantly delayed tumour onset, with two of six mice completely disease-free at the end of the study period compared with none in the control group. The authors also tested less frequent administration protocols (2 days on, 2 days off; and 1 day on, 3 days off) in a syngeneic mouse model of lung carcinoma and in mice with orthotopic breast carcinomas, and found significant growth inhibition in both models. In an aggressive model of acute myeloid leukaemia, tumour regression was found in 8 of 10 mice compared with none in the control group.

These findings show that the deubiquitylating activity of the 19S regulatory particle is a promising target for cancer treatment. As the cellular response to inhibition of proteasomal deubiquitylases is distinct from inhibitors of the catalytic core of the proteasome, 19S inhibitors might also expand the range of cancers that could be treated with proteasome-targeted drugs.

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