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Culmination of MG132-induced apoptosis in human lung cancer NCI-H1703 cells depends on a positive feedback of caspase activation and Mcl-1 cleavage

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Abstract

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Non-small cell lung carcinoma (NSCLC) is the leading cause of cancer death in both men and women in the United States. NSCLC is caused by cigarette smoking as well as occupational and environmental carcinogens. The low sensitivity of NSCLCs, especially in late stage, to different therapeutic modalities requires development of new treatments for this malignancy. Proteasome inhibitors (PIs) have become a rising hope for treating different types of human cancer which are refractory to currently available chemotherapies. Induction of apoptosis via caspase activation is believed to be the major mechanism of PIs' ability to kill cancer cells. To investigate mechanisms of action of PIs in treating NSCLCs, we treated NCI-H1703, a human NSCLC cell line, with MG132, the most commonly used proteasome inhibitor. It was observed that MG132 concentrations of greater than 0.25 μM caused a significant apoptosis as evidenced by DNA damage, cleavage of caspases 3, 7, 9, 10, Bid and PARP, and mitochondrial release of Smac/DIABLO and Cytochrome c. Among the antiapoptotic Bcl-2 family proteins tested, Bcl-2 and Bcl-XL exhibited no response to MG132 treatment while Mcl-1 showed an increase in protein level to low dose MG132 (0.25 μM) and a decrease in protein level to higher dose MG132 (>0.25 μM). MG132-induced apoptosis was inhibited by over-expression of Bcl-XL, but not by dominant negative FADD, the mediator of death receptor-initiated apoptosis, suggesting that MG132-induced apoptosis is initiated by activation of the mitochondrial pathway. Mcl-1 siRNA enhanced MG132-induced apoptosis, demonstrating that Mcl-1 is a major inhibitor the apoptosis induction. Inhibition of caspases 3 and 9 by

either specific caspase inhibitors or corresponding siRNAs inhibited MG132-induced apoptosis and PARP cleavage. It also inhibited cleavage of caspase 10 and Bid, mitochondrial release of Smac/DIABLO and reduction of Mcl-1 to higher dosages of MG132, suggesting the existence of a positive feedback in caspase activation by the initially activated caspases 3 and 9, which leads to further activation of caspases 3 and 9 through caspase 10 and the mitrochondria. The over-activated casapase 3 then subsequently resulted in Mcl-1 cleavage. The note of positive feedback is supported by an observation on the kinetics of changes of all relevant proteins in which cleavage of both caspase 10 and Mcl-1 were found to be late events. Furthermore, in agreement with this note, over-expression of a short form of Mcl-1, equivalent to Mcl-1's cleavage product, significantly enhanced apoptosis induced by low dose MG132. Collectively, this study demonstrates that culmination of MG132-induced apoptosis depends on a positive feedback for an enhanced caspase activation which converts Mcl-1 from an anti-apoptotic protein into a proapoptotic protein.

Footnotes

- 99th AACR Annual Meeting-- Apr 12-16, 2008; San Diego, CA

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