CELLULAR AND MOLECULAR BIOLOGY 36: Anticancer Therapies Targeting Autophagy, Proteasome, and Survivin

#3350 Apoptosis is induced in T-cell non-Hodgkin's lymphoma cell lines after treatment with vorinostat and bortezomib. Jennifer L. Cultrera, Lauren M. Marquis, Barbara Pro, David J. McConkey. *U. T. M. D. Anderson Cancer Center, Houston, TX.*

Introduction: T-cell non-Hodgkin's lymphomas are a heterogenous group of hematologic malignancies with distinct morphologic and clinical properties whose prognosis is worse than for their B-cell counterparts. Optimal therapy is currently unknown and long term survival rates are lower than 10%, illustrating a great need to expand treatment options beyond conventional chemotherapy. In these experiments we assessed the synergy between vorinostat and bortezomib in enhancing apoprosis in T-cell lymphoma (TCL) cell lines. There is evidence that both of these agents demonstrate single agent clinical activity in TCL. Vorinostat is a histone deacetylase inhibitor that enhances transcription of target genes through histone hyperacetylation and interacts with non-histone targets such as signal transduction mediators and transcription factors. Bortezomib is a reversible inhibitor of the chymotrypsin-like activity of the 26S proteasome in mammalian cells, which plays an essential role in several signaling cascades involved in apoptotic pathways. The combination of vorinostat and bortezomib has shown significant synergy in preclinical studies in multiple myeloma and leukemia. Methods: HH (cutaneous TCL) and Karpas 299 (K299, ALK + ALCL) are cell lines derived from TCLs and are good models for evaluating molecular mechanisms of activation involved in apoptosis. To assess the activity of these agents in TCL, sub-confluent K299 and HH cell cultures were treated with varying concentrations of vorinostat (0.1 μM - 10 μM) and bortezomib (0.5 nM - 10 nM). After incubation for 24 hours, cells were harvested and stained with propidium iodide and evaluated with flow cytometry to assess the percentage of cells undergoing apoptosis. Further studies were undertaken to assess the change of DR5 expression in response to drug treatment. HH cells were treated with varying concentrations of vorinostat and bortezomib then stained with DR5 IgG, and DR5 expression was assessed using flow cytometry. Results: In K299 line, cells treated with both vorinostat (at $\geq$ 1 μM) and bortezomib (at $\geq$ 5 nM) demonstrate increased apoptosis as compared to untreated cells. HH cells also undergo apoptosis at $\geq$ 1 μM of vorinostat and $\geq$ 10 nM of bortezomib. Upon combination, the concentration necessary for similar levels of apoptosis is reduced, demonstrating synergism between the two agents (vorinostat 0.5 μM and bortezomib 0.5 nM). Analysis using the Chou - Talalay method for assessing two agents that have independent modes of action yielded a combination index of 0.181 (ED50) indicating synergism in HH cells. Conclusions: This data suggests that vorinostat and bortezomib produce synergistic effects when combined in TCL. The mechanism may be due to upregulation of the extrinsic apoptotic pathway through increased expression of DR5. Further studies are merited to explore the effects of these agents in TCL *in vitro* and in clinical trials.

#3351 HSP70 inhibits apoptosis in pancreatic cancer cells by two Mechanisms: stabilizing lysosomes and attenuating cytosolic Ca^{2+}. Vikas Dudeja, Phoebe Phillips, Rajinder Dawra, Selwyn M. Vickers, Ashok Saluja. *University of Minnesota, Minneapolis, MN.*

We have previously shown that overexpression of Heat Shock Protein 70 (HSP70) in pancreatic cancer cells induces resistance to apoptosis in by attenuating release of cytochrome c (cyt c). However, the mechanism by which HSP70 inhibits release of cyt c is unknown. We have evaluated the role of Ca^{2+} and lysosomal enzymes, the two main regulators of cyt c release, in apoptosis induced by downregulation of HSP70. We hypothesize that HSP70 overexpression in pancreatic cancer cells prevents apoptosis by two separate and independent mechanisms i.e. stabilizing the lysosomes and attenuating cytosolic Ca^{2+}. Methods - MiaPaCa-2 or PANC-1 (pancreatic cancer cells) were pretreated with either BAPTA-AM (10μM, an intracellular Ca^{2+} chelator) or CA074me (10μM, a cell permeable Cathepsin B inhibitor) or with both followed by HSP70 inhibition by triptolide (0.2μM) or quercetin (100μM) for 24h. Cell viability, Annexin V and caspase 3 were measured as markers of apoptosis. Results - Downregulation of HSP70 significantly decreased MiaPaCa-2 viability and induced apoptosis reflected by increased Annexin V and Caspase 3 levels (Table). Pre-treatment with BAPTA-AM or CA074me prior to HSP70 downregulation significantly improved MiaPaCa-2 viability (Table) and reduced apoptosis (data not shown) and caspase 3 activation (Table). Pre-treatment with both BAPTA-AM or CA074me prior to HSP70 downregulation increased viability and decreased apoptosis significantly, over and above that induced by BAPTA-AM or CA074me alone (Table). Conclusion - Lysosomal enzymes and Ca^{2+} both independently play a role in the apoptosis induced by inhibition of HSP70 expression.

Table: Viability and Caspase 3 in MiaPaCa-2 (% of control. Mean±SE. n=3. *p<0.

	Treatment	Viability	Caspase 3
Triptolide	alone	42.3±3.5	1600.7±67.7
	+ BAPTA AM	57.5±2.3*	1191.2±14.5
	+ CA074me	59.0±4.6*	630.0±76.5*
	+ BAPTA AM + CA074me	81.1±1.8*	347.7±36.2*

#3352 Culmination of MG132-induced apoptosis in human lu NCI-H1703 cells depends on a positive feedback of caspase activ Mcl-1 cleavage. Bao-Zhu Yuan, Joshua Chapman, Steven H. Reynolds. *for Occupational Safety & Health, Morgantown, WV.*

Non-small cell lung carcinoma (NSCLC) is the leading cause of canc both men and women in the United States. NSCLC is caused by cigaret as well as occupational and environmental carcinogens. The low s NSCLCs, especially in late stage, to different therapeutic modalities req opment of new treatments for this malignancy. Proteasome inhibitors become a rising hope for treating different types of human cancer which tory to currently available chemotherapies. Induction of apoptosis via c vation is believed to be the major mechanism of PIs' ability to kill canc investigate mechanisms of action of PIs in treating NSCLCs, we tre H1703, a human NSCLC cell line, with MG132, the most commonly teasome inhibitor. It was observed that MG132 concentrations of greate μM caused a significant apoptosis as evidenced by DNA damage, c caspases 3, 7, 9, 10, Bid and PARP, and mitochondrial release of Smac and Cytochrome c. Among the antiapoptotic Bcl-2 family proteins te and Bcl-XL exhibited no response to MG132 treatment while Mcl-1 increase in protein level to low dose MG132 (0.25 μM) and a decrease level to higher dose MG132 (>0.25 μM). MG132-induced apoptosis ited by over-expression of Bcl-XL, but not by dominant negative F mediator of death receptor-initiated apoptosis, suggesting that MG13 apoptosis is initiated by activation of the mitochondrial pathway. Mc enhanced MG132-induced apoptosis, demonstrating that Mcl-1 is a m itor the apoptosis induction. Inhibition of caspases 3 and 9 by either spec inhibitors or corresponding siRNAs inhibited MG132-induced apo PARP cleavage. It also inhibited cleavage of caspase 10 and Bid, mit release of Smac/DIABLO and reduction of Mcl-1 to higher dosages o suggesting the existence of a positive feedback in caspase activation by activated caspases 3 and 9, which leads to further activation of caspas through caspase 10 and the mitrochondria. The over-activated casp subsequently resulted in Mcl-1 cleavage. The note of positive feedback is by an observation on the kinetics of changes of all relevant protei cleavage of both caspase 10 and Mcl-1 were found to be late events. Furth agreement with this note, over-expression of a short form of Mcl-1, eq Mcl-1's cleavage product, significantly enhanced apoptosis induced b MG132. Collectively, this study demonstrates that culmination of M duced apoptosis depends on a positive feedback for an enhanced caspase which converts Mcl-1 from an anti-apoptotic protein into a proapopto

#3353 The proteasome inhibitor PS-341 (Velcade) stabilizes t receptor DR5 mRNA through the 3'-untranslated region. Karthik dasamy, Andrew S. Kraft. *Medical Univ. of South Carolina, Charleston.*

Addition of proteasome inhibitors PS-341 (VELCADE, bortezomib) cancer cells enhances cell death mediated by TNF-related apoptosis-i gand (TRAIL). PS-341 sensitizes prostate cancer cells to TRAIL-induce by increasing TRAIL receptors (DR5), inhibiting protein degradation. ing DR5 mRNA. Investigations into how PS-341 regulates the stabili mRNA revealed that PS-341 increased DR5 mRNA by extending its ha 4 to 10 h. The 2.5-kb 3′-untranslated region (UTR) of the DR5 gene heterologous gene in LNCaP human prostate cancer cells, suggesting tance of this mRNA sequence. In contrast, human prostate cancer cell and DU145 do not show this stabilization, suggesting cell specifi treatment of LNCaP cells increases specific cytoplasmic mRNA bin di including AUF-1 isoforms, hnRNP C1/C2, and HuR proteins. In UV ing experiments, after PS-341 treatment, the HuR protein markedly in ing to specific sequences in the DR5 3′-UTR. In LNCap cells treated w siRNA-mediated knockdown of HuR markedly decreases the half-l mRNA, indicating that HuR is essential for mRNA stabilization. HuR ubiquitinated, suggesting that PS-341 increases this protein by preven radation. These experiments implicate modulation of mRNA stability mechanism by which proteasome inhibitors function, sensitizing ca anti-neoplasmic agents.

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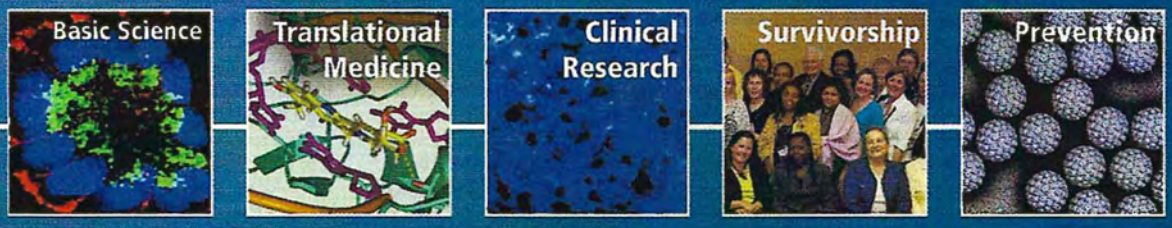

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