and their significant role in cancer progression, *CCR5* and *TNF* gene expression studies are being investigated.

580 POSTER

Reolysin induces endoplasmic reticular stress in multiple myeloma and enhances the activity of bortezomib

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Background: While the approvals of bortezomib (BZ) and lenalidomide have significantly improved the treatment of multiple myeloma (MM), it remains an incurable disease. MM cells have high protein synthesis rates due to their heavy engagement in immunoglobulin production, which renders them hypersensitive to endoplasmic reticulum (ER) stress-mediated apoptosis. Reolysin is a proprietary formulation of the human reovirus that has shown anticancer efficacy in clinical studies. We hypothesized that Reolysin treatment would lead to the accumulation of viral products in MM cells, stimulate ER stress, and significantly enhance BZ-induced apoptosis.
Materials and Methods: The antimyeloma activity of Reolysin was assessed by MTT assay, propidium iodide staining followed by flow cytometry, and measuring active caspase-3 levels in a panel of MM cell lines. Dual reovirus and ubiquitin accumulation were visualized by confocal microscopy. Intracellular Ca2+ levels were quantified by flow cytometry and increases in ER stress-related genes were determined by quantitative real-time PCR and immunoblotting. Xenograft (RPMI-8226) and syngeneic (5TGM1) models of MM were used to evaluate the effects of Reolysin and bortezomib in vivo.
Results: Reolysin induced viral replication and apoptosis selectively in MM cell lines and not in normal peripheral blood mononuclear cells (PBMCs). Reolysin treatment stimulated ER stress as measured by increased expression of GADD153, GADD34, and XBP-1s, ER swelling visualized by electron microscopy, and an elevation of intracellular Ca2+ levels. Co-treatment with Reolysin and BZ promoted the simultaneous accumulation of viral and ubiquitin-conjugated proteins, which resulted in enhanced levels of ER stress and cell death. Importantly, the Reolysin and bortezomib combination significantly reduced tumor burden in both xenograft and syngeneic MM mouse models.
Conclusion: Reovirus replication in MM cells induces an accumulation of viral products that stimulates ER stress and apoptosis. Co-treatment with Reolysin and bortezomib stimulated a simultaneous accrual of viral and ubiquitinated proteins leading to enhanced ER stress-mediated apoptosis. Reolysin is a promising anticancer agent that displays activity against MM alone and in combination with bortezomib and warrants further investigation for the treatment of MM and other malignancies.

581 POSTER DISCUSSION

Use of functional human cancer cell line mitochondria to explore the mechanisms of ABT-737-induced mitochondrial membrane permeabilization

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Current limitations of chemotherapy include toxicity on healthy tissues and multidrug resistance of malignant cells. A number of recent anti-cancer strategies aim at targeting the mitochondrial apoptotic machinery to induce tumor cell death. In this study, we set up protocols to purify functional mitochondria from various human cell lines to analyze the effect of peptidic and xenobiotic compounds described to harbour either Bcl-2 inhibition properties or toxic effects related to mitochondria. Mitochondrial inner and outer membrane permeabilization were systematically investigated in cancer cell mitochondria *versus* non-cancerous mitochondria. The truncated (t-) Bid protein, synthetic BH3 peptides from Bim and Bak, and the small molecule ABT-737 induced a tumor-specific and OMP-restricted mitochondrio-toxicity, while compounds like HA-14.1, YC-137, Chelerythrine, Gossypol, TW-37 or EM20-25 did not. We found that ABT-737 can induce the Bax-dependent release of apoptotic proteins (cytochrome c, Smac/Diablo and Omi/HtrA2 but not AIF) from various but not all cancer cell mitochondria. Furthermore, ABT-737 addition to isolated cancer cell mitochondria induced oligomerization of Bax and/or Bak monomers already inserted in the mitochondrial membrane. Finally immunoprecipatations indicated that ABT-737 induces Bax, Bak and Bim desequestration from Bcl-2 and Bcl-xL but not from Mcl-1L. This study investigates for the first time the mechanism of action of ABT-737 as a single agent on isolated cancer cell mitochondria. Hence, this method based on MOMP (mitochondrial outer membrane permeabilization) is an interesting screening tool, tailored for identifying Bcl-2 antagonists with selective toxicity profile against cancer cell mitochondria but devoid of toxicity against healthy mitochondria.

582 POSTER

Disruption of autophagic and autolysosomal signaling pathways leads to synergistic augmentation of erlotinib-induced apoptosis in wild type EGFR human non-small cell lung cancer cell lines

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Background: autophagy is a tightly regulated lysosomal self-digestion process that may promote cell survival and/or be involved in type II cell death. Erlotinib, an orally bioavailable EGFR TK inhibitor, has modest but real efficacy in wild type EGFR NSCLC tumors. The potential role of autophagy in erlotinib-induced cytotoxicity has not been previously investigated.
Materials and Methods: four wild type EGFR NSCLC cell lines were used: erlotinib-sensitive H322 and H358, and erlotinib-resistant H460 and A549.
Results: erlotinib at a clinically achievable concentration (2 mM) induced autophagic features including an increase in the formation of AVO and MDM staining, conversion of LC3-I (the cytosolic form) to LC3-II (the lapidated form associated with autophagosome), and the formation of autophagic vacuoles in both erlotinib-sensitive and -resistant cell lines. The combination of erlotinib with chloroquine, an autophagy inhibitor, was synergistic in all tested human NSCLC cell lines. Co-treatment with 3-methyladenine (3-MA), an inhibitor of early stage autophagy or with bafilomycin A1, an inhibitor of late stage autophagy, and down-regulation of Atg-5 and Beclin-1 gene expression by siRNA resulted in enhanced apoptotic cell death and cytotoxicity as compared with erlotinib alone in H460 and A549 cells. Moreover, co-treatment with lysosomal inhibitors, ammonium chloride, E-64, Pepstain-A and Z-LA-fmk also resulted in the enhancement of erlotinib-induced cytotoxicity. The combined treatment with chloroquine did not alter erlotinib-induced EGFR pathway inhibition or G1 cell cycle arrest, but significantly induced apoptosis associated with the activation of caspase-9 and caspase-3, and increase in cleavage of PARP protein in all tested cells. Interestingly, the combination of erlotinib and chloroquine induced up-regulation of Bim protein expression, and down-regulation of Bim gene expression by siRNA attenuated cell death induced by the drug combination. In addition, inhibition of autophagy by chloroquine treatment enhanced erlotinib-induced ROS generation, and activation of p38 but not JNK signaling. Inhibition of ROS generation by NAC, or inhibition of p38 by SB202190 resulted in the attenuation of the potency of the combination, suggesting that ROS generation and p38 activation are potential mediators of the effect of this combination.
Conclusion: inhibition of autophagy by chloroquine represents a novel strategy to enhance erlotinib efficacy in wild type EGFR NSCLC tumors.
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583 POSTER

PI3-kinase inhibition enhances ABT-737 induced apoptosis in colorectal cancer cell lines

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Background: Although context dependent, the PI3-kinase signalling pathway is widely reported to promote cell survival, proliferation and migration. This pathway is up-regulated in several solid tumours via mechanisms including PTEN deletion, RAS or PIK3CA mutation and aberrant activation of receptor tyrosine kinases. As such, inhibitors of PI3-kinase and its down-stream effectors are of great interest as tractable anti-cancer agents. In colorectal cancer (CRC) cells inhibition of the PI3-kinase pathway did not induce apoptotic cell death. Moreover, CRC cells were relatively resistant to the pro-apoptotic BH-3 mimetic ABT-737. This study examined whether PI-3K inhibition and ABT-737 combined reduced the threshold of apoptosis in CRC cells.
Methods: The PI3-kinase pathway was inhibited with PI-103 (PI3-kinase inhibitor), AKTi1/2 (AKT inhibitor) or rapamycin (mTORC1 inhibitor). Apoptosis was assessed by activating conformational changes in BAK, caspase 3 cleavage, and phosphatidylserine exposure. Interactions between Bcl-2 BH3 family members were assessed by co-immunoprecipitation. Expression of the ABT-737 resistance factor Mcl-1 was knocked down by transfection of siRNA.
Results: PI3-kinase inhibition enhanced apoptosis induced by ABT-737 upstream of cytochrome *c* release in CRC cells. PI3-kinase inhibition reduced the levels of Mcl-1. However, PI3-kinase inhibition further enhanced ABT-737 induced apoptosis in CRC cells were Mcl-1 was