depleted by siRNA or in Mcl-1 −/− MEFs suggesting mechanisms in addition to Mcl-1 downregulation contributed to ABT-737 sensitisation. Co-immunoprecipitation revealed that PI3-kinase inhibition increased Bcl-x_L-bound Bim which also occurred in Mcl-1 depleted CRC or Mcl-1 −/− MEFs. ABT-737-induced apoptosis was independent of AKT and mTOR in CRC cells as it was unaffected by PI-3K pathway inhibition using AKTi1/2 and rapamycin.
Conclusion: PI3-kinase inhibition enhanced ABT-737 induced apoptosis via a Mcl-1 independent mechanism, consistent with a mechanism that altered the affinity for Bim to Bcl-x_L. ABT-737 sensitisation by PI3-kinase inhibition occurred via an Akt/mTORC1 independent arm of the PI3-kinase signalling pathway.

584 POSTER

c-FLIP, a critical target for histone deacetylase inhibitors in mesothelioma

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Background: Mesothelioma typically presents at a late stage and therefore carries a poor prognosis in the majority of cases. Chemotherapy is the mainstay of treatment, but only one quarter of patients respond to first line treatment (cisplatin/pemetrexed). Resistance to apoptosis is a key mechanism underlying the failure of anti-cancer therapies, and targeting the death receptor apoptotic pathway is a novel strategy to overcome this problem in mesothelioma. The caspase 8 inhibitor c-FLIP is a key inhibitor of death receptor signalling, which has previously been shown to be important in regulating apoptosis and drug resistance in several cancers. Here, we investigated the role of c-FLIP in regulating the response of mesothelioma cells to the histone deacetylase (HDAC) inhibitor Vorinostat, which has been found to be active in mesothelioma in early phase clinical trials.
Mesothelioma typically presents at a late stage and therefore carries a poor prognosis in the majority of cases. Chemotherapy is the mainstay of treatment, but only one quarter of patients respond to first line treatment (cisplatin/pemetrexed). Resistance to apoptosis is a key mechanism underlying the failure of anti-cancer therapies, and targeting the death receptor apoptotic pathway is a novel strategy to overcome this problem in mesothelioma. The caspase 8 inhibitor c-FLIP is a key inhibitor of death receptor signalling, which has previously been shown to be important in regulating apoptosis and drug resistance in several cancers. Here, we investigated the role of c-FLIP in regulating the response of mesothelioma cells to the histone deacetylase (HDAC) inhibitor Vorinostat, which has been found to be active in mesothelioma in early phase clinical trials.
Materials and Methods: Four mesothelioma cell lines were studied: REN, E58, H28 and MSTO. Cells were maintained in 5% CO2 at 37^0 in F-12 or RPMI medium supplemented with 10% FCS, 1% penicillin/streptomycin. Stably transfected clones were maintained in selection medium containing 600μg/ml G418. c-FLIP expression was analysed by Western blot. Apoptosis was measured by PARP cleavage and flow cytometry (protium iodide, PI staining). Silencing of caspase 8 and c-FLIP was achieved by caspase 8 and c-FLIP specific siRNA. Expression of c-FLIP mRNA was measured by QRT-PCR.
Results: The IC_{50} doses of Vorinostat were found to be in the 5μM range, as determined by MTT assay. We found that c-FLIP was down-regulated at the protein and mRNA level, 6−12 hours after exposure to Vorinostat in a dose-dependent manner, with potent down-regulation observed at the IC_{50} dose. Vorinostat-induced down-regulation of c-FLIP correlated with caspase 8 activation and induction of apoptosis. Importantly, apoptosis induced by Vorinostat was significantly reduced in cells in which caspase 8 and the key death receptor adapter protein FADD were silenced. Furthermore, siRNA-mediated silencing of c-FLIP was found to be sufficient to activate caspase 8 and induce apoptosis in the mesothelioma cell lines. These results are consistent with c-FLIP down-regulation being a major mechanism of Vorinostat-induced apoptosis in mesothelioma. Moreover, Vorinostat was not found to affect expression of other proteins involved in apoptotic pathway, such as Mcl-1, Bcl-2, Bcl-XL, BAK, and XIAP.
Conclusions: c-FLIP is down-regulated at both a transcriptional and post-transcriptional level in response to Vorinostat. This appears to be a major mechanism leading to apoptosis induction by this agent in mesothelioma cell lines and suggests that c-FLIP, caspase 8 and other death receptor signalling molecules may be potential biomarkers of response to Vorinostat in mesothelioma.

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NOXA as mediator for drug-induced apoptosis − molecular studies in patient-derived ALL cells

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Background: Chemotherapy is given as polychemotherapy, where drugs are combined to yield super-additive anti-tumor effects. Betulinic acid is a natural compound which is intensively studied as potent novel anti-cancer drug. We were first to show that Betulinic acid induced apoptosis in leukemia cells (Ehrhardt et al, Leukemia 2004). Here, we studied which conventional cytotoxic drugs cooperate with Betulinic acid and which signaling pathways are responsible for positive interaction.
Materials and Methods: Leukemic cell lines and primary tumor cells from 35 children with acute leukemia were studied in vitro. Classical cell death assays were performed. For release of mitochondrial factors, subcellular compartments were isolated and subjected to Western Blot analysis. Primary acute leukemia cells were amplified in NOD/SCID mice. As a completely new method, xenograft cells were transiently transfected by electroporation.
Results: Betulinic acid synergized for apoptosis induction with doxorubicin, asparaginase and vincristine, but not other conventional cytotoxic drugs. Clinically most important, these drugs induced cooperative apoptosis also in primary tumor cells freshly obtained from children with acute lymphoblastic leukemia (ALL). To characterize the mechanism responsible for effective apoptosis induction by drug combinations with Betulinic acid, we found that apoptogenic factors like Cytochrome c, Smac and OMI/HtrA2 were released from mitochondria and enhanced caspase activation and DNA-fragmentation. Upon combinatorial treatment, p53 was activated which lead to the upregulation of the pro-apoptotic Bcl-2 family member NOXA. Knockdown of either p53 or NOXA disabled synergistic apoptosis induction proving that both proteins mediate the cooperative pro-apoptotic effect. Most importantly and beyond tumor cell lines, we tested this signaling mechanism in primary leukemia cells derived from children with acute leukemia after amplification of the cells in NOD/SCID mice. These data represent the first data ever obtained in patient-derived ALL cells. Knockdown of either p53 or NOXA in patient-derived xenograft leukemia cells disabled both upregulation of NOXA as well as synergistic apoptosis induction by Betulinic acid and conventional cytotoxic drugs.
Conclusion: Our data suggest that NOXA represents an important, p53-regulated molecular target for the combination therapy of Betulinic acid and conventional cytotoxic drugs which enables apoptosis induction, e.g., in primary, patient-derived leukemia cells. If incorporated into polychemotherapy protocols for treatment of ALL, Betulinic acid should be applied in close proximity to doxorubicin, asparaginase or vincristine in order to take advantage of the favorable regulation of p53 and NOXA.

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Lucanthone: A novel inhibitor of autophagy that induces cathepsin D-mediated apoptosis

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Background: Cellular stress induced by nutrient deprivation, hypoxia, and exposure to many chemotherapeutic agents activates an evolutionarily conserved cell survival pathway termed autophagy. This pathway enables cancer cells to undergo self-digestion to generate ATP and other essential biosynthetic molecules to temporarily avoid cell death. Therefore, disruption of autophagy may sensitize cancer cells to cell death and augment chemotherapy-induced apoptosis. Chloroquine and its analog hydroxychloroquine are the only clinically relevant autophagy inhibitors. Since both of these agents induce ocular toxicity, novel inhibitors of autophagy with a better therapeutic index are needed. Here we demonstrate that the small molecule lucanthone inhibits autophagy and induces apoptosis in breast cancer models.
Materials and Methods: Inhibition of autophagy was visualized by electron microscopy and lysosomal membrane permeabilization was measured by quantification of acridine orange fluorescence. The anticancer efficacy of lucanthone was determined by MTT assay and propidium iodide staining followed by flow cytometry. Gene expression arrays, quantitative real-time PCR, immunocytochemistry, and immunoblotting were used to measure cathepsin D expression. siRNA targeting cathepsin D was used to evaluate its contribution to lucanthone-mediated apoptosis.
Results: Lucanthone inhibits autophagy, induces lysosomal membrane permeabilization, and possesses significantly more potent anticancer activity compared to chloroquine. Exposure to lucanthone resulted in processing and recruitment of microtubule-associated protein 1 light