chain 3 (LC3) to autophagosomes, but impaired autophagic degradation as revealed by electron microscopy and the accumulation of p62/SQSTM1. Lucanthone stimulated a large induction in cathepsin D expression, which correlated with cell death. Accordingly, knockdown of cathepsin D reduced lucanthone-mediated apoptosis. Subsequent studies using p53+/+ and p53−/− HCT116 cells established that lucanthone induced cathepsin D expression and reduced cancer cell viability independently of p53 status.
Conclusion: Our results demonstrate that lucanthone is a novel autophagic inhibitor that induces apoptosis via cathepsin D accumulation in breast cancer models.

587 POSTER

Down-regulation of FLT3 kinase phosphorylation and survivin expression contributes to the activity of imidazoacridinone C-1311 against leukemia cells with FLT3 ITD mutation

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Background: Antitumor imidazoacridinone C-1311, is a DNA-reactive inhibitor of topoisomerase II, undergoing phase II clinical trials. Recent studies against recombinant receptor tyrosine kinases (RTKs) showed that C-1311 is a potent and selective inhibitor of Fms-like tyrosine kinase 3 (FLT3). Since activating mutations of FLT3 occur in one third of acute myeloid leukemias (AML) and are associated with poor prognosis, we studied the effect of C-1311 on proliferation, cell cycle and mode of cell death in human AML models.
Materials and Methods: C-1311 was tested on two leukemic cell lines with contrasting FLT3 status. MV4;11 cells harbor internal tandem duplications (ITD) resulting in constitutively activated FLT3 while RS4;11 cells express wild type FLT3. The direct effect of C-1311 on FLT3 was examined based on its phosphorylation status using ELISA. Apoptosis was determined based on DAPI staining, annexin-V assay and caspase-3 activation. RNA levels of 84 different apoptosis-related genes were quantified using real-time PCR.
Results: Cytotoxic activity of C-1311 against MV4;11 cells was 4-fold greater compared with RS4;11, indicating more potent inhibition against cells with constitutively activated FLT3 ITD. In MV4;11 cells, C-1311 strongly reduced FLT3 autophosphorylation in a dose- and time-dependent manner with IC_{50} values of 14 μM and 0.7 μM after 3 h and 24 h of treatment, respectively. Importantly, down-regulation of FLT3 phosphorylation was achieved at biologically active doses of C-1311, indicating the inhibition of FLT3 activity crucial for cytotoxic effect of the drug. Sequence of cellular responses in MV4;11 cells treated with 1 μM C-1311 (IC_{90} concentration) included transient accumulation of cells in G1 phase followed by caspase-3 activation and cell death via apoptosis. The recruitment of the mitochondrial apoptosis pathway was suggested by the moderate up-regulation of proapototic genes e.g., Bax, BBC3, and APAF-1. Surprisingly, C-1311 markedly down-regulated antiapototic survivin gene expression (>30 difference over control), suggesting survivin as a new target for C-1311 action.
Conclusions: Inhibition of FLT3 by C-1311 resulted in reduced proliferation and induction of apoptosis in FLT3 ITD cells. Our finding that C-1311 decreases survivin expression is of particular interest since survivin regulates development of ITD FLT3-positive AML. How dual targeting of survivin and FLT3 contributes to the activity of C-1311 needs further studies.

588 POSTER

Trans-membrane-p53 peptide therapy for malignant glioma

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Background: Malignant gliomas are the most aggressive primary brain tumor in adults. The prognosis of patients with malignant gliomas remains poor despite decades of basic and clinical research. Malignant gliomas are still fatal disease.
It is known that glioblastoma cells have multiple genetic alterations associated with the p53/MDM2/ERK pathway, which is related to cell-cycle regulation and apoptosis. The goal of therapies such as p53 protein transfer is to recover the function of this tumor suppressor gene in cancer cells. Recent studies have reported the efficiency of protein transduction therapy for several cancers. In our present study, we use p53 peptide including Mdm2 binding site and examined its effects on the proliferation of three different glioblastoma cell lines.
Materials and Methods: We use p53 peptide including Mdm2 binding site with the 11 polyarginine PTD (protein transfer domain) and the SV40 large T-antigen NLS (nuclear localization signal) (p53-NLS-11R).We examined effects on the proliferation of three different glioblastoma (YKG, T98, U87) cell lines and peptide delivery into the nucleus was also confirmed using a fluorescently labeled peptide.
Results: p53-NLS-11R peptide was delivered into cells within 30 minutes and showed growth inhibitory effect in 1−10 μM on three cell lines. The growth inhibitory effect was achieved by the apoptosis with p53-NSL-11R peptide.
Conclusion: Direct intracellular delivery of p53-NSL-11R peptide is a possible useful method as a glioma therapy.

589 POSTER

HDAC inhibitors induce FLIP down-regulation and caspase 8 dependent apoptosis in colorectal cancer cells

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Introduction: c-FLIP is an anti-apoptotic protein that blocks death receptor mediated apoptosis by inhibiting Caspase 8 activation. FLIP is often over-expressed in colorectal (CRC) cells, and it has previously been shown that high FLIP expression limits the effectiveness of chemotherapy treatment. HDAC inhibitors (HDACi) such as SAHA have been shown to induce apoptosis via the intrinsic apoptotic pathway, and are currently in phase I trials. We have shown that the HDAC inhibitors SAHA and CMPHB down regulate the expression of c-FLIP at the protein level in a panel of cell lines. Our aims are to assess the effect of HDACi on the extrinsic apoptotic pathway, elucidate the mechanism of c-FLIP down-regulation and investigate the potential of combination therapies with TRAIL and chemotherapy.
Methods: HCT116 parental and HCT116 $FLIP_L$ over-expressing colorectal cancer (CRC) cell lines were utilised. Levels of FLIP expression were analysed by Western Blot. Apoptosis was measured by PARP cleavage and Flow cytometry (PI staining). Inhibition of Caspase 8 activation was achieved by siRNA and the Caspase 8 inhibitor IETD. Death Receptor expression was inhibited using DR4/5 targeted siRNA transfection. Expression of FLIP mRNA was analysed by RT-PCR. Proteosomal inhibition was achieved using MG132.
Results: It was found that SAHA and CMPHB induce apoptosis in HCT116 cells in a manner that is dependent on Caspase 8 and the ability of the compound to reduce c-FLIP protein expression. Furthermore, the apoptosis induced by both HDAC inhibitors was found to be dependent on the TRAIL receptors DR4 and DR5. Further analysis revealed that SAHA and CMPHB down regulate FLIP expression post-transcriptionally by triggering its degradation via the ubiquitin- proteasome system. Combination of SAHA and CMPHB with TRAIL indicates that treatment with HDAC inhibitors synergistically sensitise CRC cells to TRAIL and chemotherapy induced apoptosis.
Conclusions: We have previously shown that reduction of FLIP expression using siRNA synergistically enhances the response of CRC cells to chemotherapy or TRAIL. The results presented here indicate that HDAC inhibitors induce apoptosis in CRC cells by down regulating c-FLIP and thereby activating DR4/5-dependent Caspase 8-mediated apoptosis. Since the clinical down regulation of FLIP expression by siRNA is not yet feasible, the use of small molecules such as HDAC inhibitors which have a profound effect on c-FLIP expression may have important therapeutic implications.

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Transcription factor, Id4, induced cellular reprogramming of cancer cells using an advanced prostate cancer cell model

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Id4 belongs to a family of genes that are dominant negative regulators of basic helix-loop-helix (bHLH) transcription factors. Id1−3 expression correlates with various advanced metastatic cancers and act as tumor promoter genes. Many studies have suggested that loss of Id4 expression in many types of metastatic cancers is the result of promoter hypermethylation. The Id genes have been linked to a number of cellular responses including differentiation and proliferation. Id4 may act as a tumor suppressor by linking several cellular events. In the DU145 prostate cancer cell line, Id4 down regulation is responsible for increased cell survival, metastasis and decreased apoptosis. In this study we used advanced prostate cancer cell line DU145, negative for Id4 to observe the effects that ectopic Id4 expression plays in cellular reprogramming. Therefore, the role Id4 in cell cycle control, apoptosis, senescence, proliferation and hormone regulation were all aspects of the focus of this experiment. FACS analysis highlighted the ability of Id4 to regulate key stages of the cell cycle. Apoptosis assays, and observation of key apoptotic genes support the tumor suppressor role of Id4. Induction of senescence was a major characteristic of Id4 expressing DU145 cells, which simultaneously decreased proliferation. RT-PCR and western blot analysis were used to study gene expression of downstream apoptosis and cell cycle control genes E-cadherin, p21,

p27, p53, MDM2, E2F1 and, Androgen receptor. Du145+Id4 cells have significantly decreased proliferation due to an S-phase arrest suggested by an E2F-1 intermediary response. The increased expression of E-cadherin, p27, p21 and p53 strengthens the hypothesis of Id4 as a tumor suppressor by regulating key cell cycle control and apoptosis associated genes. A functional androgen receptor marked a dramatic change in the androgen independent prostate cancer cell type. Id4 ectopic expression resulted in a significant decrease in Id1 and Id3, which are known contributors to metastasis and cell survival. Id4's role may be to inhibit bHLH transcription factors involved in proliferation, metastasis, apoptosis and senescence. Id4 induces apoptosis whether dependent/independent of the mutated p53 gene in DU145 cells. The presence of senescent cells in Id4 transfected cell lines suggest that Id4 may also play a role in autophagic cell death. As a transcription factor Id4 has the capability of inducing a total cellular reprogramming, by influencing a number of key cellular pathways. We conclude that the tumor suppressor function of Id4 is responsible for causing a reversal in the cancer phenotype of the cell by inducing apoptosis, senescence, S-phase mediated cell cycle arrest resulting in a molecular and morphological change.

591 POSTER

Berberine sensitizes TRAIL-induced apoptosis through proteasome-mediated down-regulation of c-FLIP and Mcl-1 proteins

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Berberine is an isoquinoline alkaloid used in traditional Chinese medicine and has been isolated from a variety of plants, such as *Coptis chinensis* and *Phellodendron amurense*. It has a wide spectrum of clinical applications such as in anti-tumor, anti-microbial, and anti-inflammatory activities. We showed that co-treatment with subtoxic doses of berberine and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induced apoptosis in human renal cancer cells, Caki cells, but not in normal tubular kidney cells. Treatment of Caki cells with berberine down-regulated c-FLIP and Mcl-1 proteins in dose- and time-dependent manners. Interestingly, berberine-induced decreases in c-FLIP and MCl-1 protein levels were involved in proteasome dependent pathways, which was confirmed by the result that pretreatment with proteasome inhibitor, MG132, inhibited berberine-induced down-regulations of both c-FLIP and Mcl-1 proteins. Pretreatment with N-acetyl-l-cysteine (NAC) significantly inhibited the cell death induced by the combined treatment with berberine and TRAIL as well as recovered the expression levels of c-FLIP and Mcl-1 down-regulated by the combinatory treatment with berberine plus TRAIL, suggesting that berberine-stimulated TRAIL-induced apoptosis appears to be dependent on the generation of reactive oxygen species for down-regulation of c-FLIP and Mcl-1. Taken together, the present study demonstrates that berberine enhances TRAIL-induced apoptosis in human renal cancer cells by ROS-mediated c-FLIP and Mcl-1 down-regulations.

592 POSTER

Characterisation of novel, small molecule antagonists of XIAP, cIAP1 and cIAP2 generated by fragment based drug discovery (FBDD)

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The inhibitor of apoptosis (IAP) family of proteins are important regulators of cancer cell survival, making them attractive targets for cancer therapy. They are characterized by one to three baculovirus IAP repeat (BIR) domains, which are necessary for the antiapoptotic activity of most IAPs. Several small molecule BIR antagonists mimic the N-terminal sequence of SMAC (second mitochondrial activator of caspases), an endogenous inhibitor of the IAPs. These peptidomimetic compounds have the ability to sensitise and/or promote apoptosis in cancer cells and inhibit tumor growth *in vivo*. Using our fragment-based screening approach, Pyramid™, we identified a range of diverse, non-peptidomimetic chemotypes which bind to the P1′−P2′ pocket in the BIR3 domain of XIAP. Alanine-like fragments have also been identified with excellent Ligand Efficiency (LE) values, which are superior to LE of Ala-Val (natural substrate) and to LEs of published competitor compounds. Optimisation of these hits using a structure based approach led to novel series (both alanine and non-alanine) which bound with sub μM potency to both XIAP and cIAP1. The most potent compounds were characterised further in proliferation assays using two sensitive human breast cancer cell lines EVSA-T and MDA-MB-231 (with an insensitive cell line, HCT116, as a control). Anti-proliferative compounds were investigated further for their ability to induce cIAP1 degradation and to increase the levels of cleaved caspase-3 in EVSA-T cells. cIAP1 degradation occurred rapidly at low compound concentrations in all cell lines tested; whilst caspase-3 induction closely paralleled the anti-proliferative data. In conclusion fragment-based screening has enabled the identification of non-peptidomimetic ligands that inhibit this protein:protein interaction. These chemotypes represent promising start points for novel, selective IAP antagonists.

593 POSTER

Notch-1 fragment peptide induces autophagy and caspase-independent cell death in leukemic cell lines

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Background: Non-apoptotic cell death such as autophagic cell death or necrosis is an important physiological process. Potent inducers of non-apoptotic cell death promise to be a valuable tool for development of novel chemotherapeutic agents. In this study, we found that a 25mer peptide conjugate induces a caspase-independent cell death in multiple human leukemic cell lines.
Material and Methods: All the peptides were synthesized by using an Fmoc chemistry-based automatic peptide synthesizer. Human monocytic cells and leukemic cell lines were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum. Measurements of cell viability and mitochondrial membrane potential were determined by WST-8 chromogenic assay and fluorescence-activated cell sorter using the JC-1 dye, respectively.
Results: To suppress Notch signaling which is overactivated in T-cell leukemia and glioma, we designed Tat-Notch-1 fragment peptide (Tat-NF), which is a cell penetrating HIV-1 tat-conjugate with a 13mer peptide fragment (R-R-Q-H-G-Q-L-W-F-P-E-G-F) derived from Notch-1 intracellular domain. Unfortunately, the conjugate has no inhibitory effect on the Notch signaling in malignant gliomas, whereas the gamma-secretase inhibitor blocked the signal. However, the Tat-NF rapidly killed almost the leukemic cells (Jurkat-T, CCRF-CEM, Molt-4 etc.) tested in a tumor cell-specific manner. Single alanine substitutions of the LWF motif caused a significant decrease of the peptide-inducing cell death. Pharmacological inhibition of caspase activity did not prevent the cell death, although mitochondrial membrane potential was significantly decreased. In the cells undergoing such cell death, we observed the conversion of the soluble LC3-I to the autophagic vesicle-associated LC3-II and the formation of lysosomes/autophagosomes.
Conclusions: The 13mer peptide conjugated with HIV-1 tat has an ability to induce autophagy and caspase-independent cell death without affecting the Notch signaling. These data suggest that the conjugate is useful to elucidate the molecular mechanism of non-apoptotic cell death and to develop novel chemotherapeutic agents for treating leukemia with apoptotic defects.

Biomarkers

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In vivo detection of mammary tumor and its lung metastases in the 4T1 metastasis mouse model by PET imaging using [F-18]-D-FMT (BAY 869596)

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Background: Detection and localization of metastatic lesions and their differentiation from therapy induced inflammation is still a difficult obstacle for diagnosis and subsequent treatment decisions. Positron emission tomography (PET) allows the sensitive detection of radioactive labeled molecules, which specifically accumulate in tumor tissue. This offers a promising tool to assess molecular details about the disease comprehensively and contributes to an optimized cancer patient management and therapy control. At present [F-18]-fluorodeoxyglucose (FDG) is the most frequently used PET tracer in oncology. However, FDG has limited specificity by accumulating also in inflammatory cells due to their increased glucose metabolism. To overcome this problem, amino acids have been investigated. D-[F-18]-fluoromethyl tyrosine (D-FMT, (R)-2-amino-3-(4-[F-18]fluoromethoxy-phenyl)-propionic acid) has shown good uptake into HeLa tumors in nude mice with no accumulation in sterile induced inflammation sites. The aim of this study was to investigate D-FMT in a metastasis model for its capability to detect the primary tumor as well as metastatic lesions and differentiate metastatic from inflammatory sites.
Material and Methods: 2.5×10^5 4T1 mouse mammary carcinoma cells were implanted subcutaneously in NMRI mice, which generated several