cisplatin and rhTRAIL. No differences in protein expression levels of the major constituents of the TRAIL pathway were seen. Membrane expression levels of the TRAIL receptors showed similar results: DR4 and DcR1 were not expressed at both cell lines. DR5 was expressed, whereas low levels of DcR2 were detected. To develop an in vivo BLI-model, we inoculated nude mice ip with different concentrations of A2780-luc (resp. 1×10^7 and 2×10^6 cells). In both groups the bioluminescent signal correlated well with the ip tumor load as assessed by visual inspection of the peritoneal cavity at necropsy. Progressive tumor growth could be monitored by repeated imaging of a defined group at several time points.
Conclusion: This study shows that in vivo BLI is a reliable and feasible method to monitor noninvasively ip tumor growth. We are currently performing the experiments with iv and ip rhTRAIL therapy, cisplatin and the combinatory regimen. The results of these drug modulation studies will be presented.

491 POSTER

Targeting XIAP in paediatric cancers

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Cancer remains one of the commonest causes of death in children in the UK. In selected types of childhood tumours de novo drug resistance is a major problem. One potential cause of pleiotropic drug resistance is a failure to engage apoptosis after cytotoxic drug-induced damage. Endogenous inhibitor of apoptosis proteins (IAPs) prevent apoptosis by inhibiting both initiator (caspase-9) and effector (caspases-3 and 7) caspases. Down-regulating X-linked IAP (XIAP), the most potent endogenous inhibitor of caspases, sensitises adult tumour cells to drug-induced apoptosis. A novel XIAP antisense oligonucleotide is currently in adult phase I trial.
Although little is known about the function of XIAP in paediatric tumours, high levels of XIAP expression correlate with poor survival in childhood AML. We have screened a panel of paediatric tumour cell lines for expression of XIAP and its endogenous inhibitor XAF-1, and found near universal expression of XIAP. The small molecule XIAP inhibitor TPI-1396−11 (xiapuradamib) was effective against neuroblastoma, osteosarcoma, and Ewing's sarcoma cell lines in short term growth assays (SRB) and long term clonogenic assays, with IC50 values ranging from 2.1 to 7.25 μM. The combination index equation was used to define synergistic interactions between xiapuradamib and clinically relevant cytotoxic agents. Clear synergy was seen between xiapuradamib and etoposide in 791T osteosarcoma cells. NGP neuroblastoma cells with stable shRNA repressed XIAP were sensitised to etoposide in clonogenic assay. We are extending these studies into rhabdomyosarcoma, medulloblastoma and lymphoma cell lines and aim to take forward promising combinations into xenograft and ultimately clinical studies.

Differentiation

492 POSTER

Induction of myeloid differentiation by a novel sterol mesylate compound (NSC 67657)

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Background: Inducers of differentiation can offer a relatively non-toxic means of chemotherapy and are of proven value in settings such as acute promyelocytic leukemia. The CEBPa transcription factor plays a key role in the regulation of normal myeloid cell differentiation and thus constitutes a target for discovery of novel differentiation inducing agents.
Materials and Methods: We conducted a high-throughput screening campaign to identify activators of CEBPa signaling using a clone of U937 cells transfected with a luciferase reporter driven by four copies of the CEBPa response element.
Results: Screening of more than 135,000 samples from the National Cancer Institute's repository of chemical compounds identified a novel sterol mesylate (NSC 67657) as a potent activator of CEBPa signaling. Secondary testing in U937 and HL60 cell lines demonstrated that this compound could induce myeloid differentiation manifest as increased expression of CD11b and CD14 cell surface markers, increased NBT activity, and morphologic evidence of differentiation. Transcriptional profiling demonstrated a distinctly different pattern from that produced by retinoic acid and suggested a predominantly monocytic mode of differentiation. Initial studies with cryopreserved leukemic blasts from patients with AML have demonstrated induction of CD11b and CD14 in two FAB type M5 samples by flow cytometry.
Conclusions: Xenograft studies as well as additional ex vivo studies using AML patient samples will be pursued to establish a case for clinical development of NSC 67657 or an optimized derivative.
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493 POSTER

Down regulation of topoisomerase IIβ in myeloid leukemia cell lines leads to activation of apoptosis following all-trans retinoic acid-induced differentiation/growth arrest

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Background: Among the topoisomerase (topo) II isozymes (α and β), topo IIβ has been suggested to regulate differentiation. In this study we examined the functional role of topo IIβ in all-trans retinoic acid (ATRA)-induced differentiation/growth arrest and apoptosis of myeloid leukemia cells.
Materials and Methods: Topo IIβ was inhibited with ICRF-193 or stably down-regulated with an si-RNA in the myeloid leukemia cell lines HL-60, KG-1 and AP-1060, to determine the role of this enzyme in ATRA-induced differentiation/growth arrest and apoptosis. Differentiation was assessed by microscopy based on reduction of nitroblue tetrazolium. Apoptosis was determined by fluorescent microscopy of cells stained with Hoechst 33342 + propidium iodide. mRNA and protein expression was determined by real-time RT-PCR and Western blot analysis, respectively. Gene expression profiles in topo IIβ-expressing and topo IIβ-deficient cells were compared by cDNA microarray analysis. Reactive oxygen species (ROS) was measured by flow cytometry using the dye dihydroethidium.
Results: Inhibition of topo IIβ activity with ICRF-193 in HL-60, KG-1 or AP-1060 cells or si-RNA mediated down-regulation of topo IIβ protein in HL-60 or KG-1 cells, significantly ($p < 0.05$) enhanced ATRA-induced differentiation/growth arrest and apoptosis. In contrast, down-regulation of topo IIα did not alter ATRA-induced differentiation or apoptosis. ATRA-induced apoptosis in topo IIβ-deficient cells led to activation of caspase 3 and was rescued by ectopic expression of topo IIβ. Gene expression profiling of topo IIβ-expressing and topo IIβ-deficient cells led to the identification of peroxiredoxin 2 (PRDX2) as a candidate gene that was down-regulated in topo IIβ-deficient cells. Reduced expression of PRDX2, validated at the mRNA and protein level, correlated with increased accumulation of ROS following ATRA-induced differentiation and apoptosis. Overexpression of PRDX2 in topo IIβ-deficient cells, prevented accumulation of ROS and partially reversed ATRA-induced apoptosis.
Conclusions: These results support a role for topo IIβ in survival of myeloid leukemia cells following ATRA-induced differentiation/growth arrest. Reduced expression of topo IIβ induces apoptosis in part by impairing the anti-oxidant capacity of the cell due to down-regulation of PRDX2. Thus, suppression of topo IIβ and/or PRDX2 levels in myeloid leukemia cells provides a novel approach for improving ATRA-based differentiation therapy.

494 POSTER

MMTV-RANK transgenic mice show increased mammary epithelial proliferation and impaired alveolar differentiation during pregnancy and a higher incidence of chemically induced mammary tumors

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Background: RANK and RANKL, the key regulators of osteoclasts differentiation and activation, also have an important role in the control of proliferation, differentiation and survival of mammary epithelial cells.
Materials and Methods: We have generated transgenic mice that overexpress RANK under the mouse mammary tumor virus (MMTV) promoter, and characterized their mammary gland development during pregnancy and their susceptibility to mammary tumors induced by medroxiprogesterone acetate (MPA) and DMBA. We have also characterized the