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

differentiation of primary mammary epithelial cells (MECs) from these mice in 3D cultures.
Results: We show that the mammary epithelial cells of MMTV-RANK transgenic mice show higher levels of proliferation than wild-type mice during pregnancy and impaired differentiation of lobulo-alveolar structures resulting in a marked decrease in the expression of the milk proteins β-casein and WAP and a lactation defect. Analysis of the protein expression by immunohistochemistry demonstrates that not only RANKL, but also RANK protein expression is strictly regulated in a spatial and temporal manner during mammary gland development. MMTV-RANK mice also show a significantly higher incidence of mammary tumors induced by medroxyprogesterone acetate and DMBA. Using acinar cultures of primary mammary cells we show that RANKL treatment results in enhanced proliferation, a dramatic increase in size and lack of luminal apoptosis.
Discussion: These results show that signaling through the RANK receptor promotes proliferation, inhibits terminal differentiation of the mammary epithelial cells and impairs apoptosis increasing the susceptibility of the gland to chemically induced tumorigenesis.

495 POSTER

Identification and characterization of a phenyl-thiazolyl-benzoic acid derivative as a novel RAR/RXR agonist

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Background: Acute promyelocytic leukemia (APL) is characterized by a chromosomal translocation of t(15;17)(q22;q21) which results in the fusion of retinoic acid receptor alpha (RARα) to promyelocytic leukemia gene. For differentiation-inducing therapy of APL, all-trans retinoic acid (ATRA) has been used. However, APL cells become resistant to ATRA due to its susceptibility to P450 enzyme, induction of P450 enzyme, increased sequestration by cellular retinoic acid binding protein and increased expression of P-glycoprotein. Small molecule compounds without these undesired profiles are long-coveted for the treatment of APL.
Methods: In this study, we identified a phenyl-thiazolyl-benzoic acid derivative as a potent agonist for RXRα and RARα by virtual screening. The compound was evaluated in binding and reporter gene assays, and NB4 in vivo model. All procedures in this study were in compliance with the regulations of Animal Welfare Committee in Novartis Institutes for BioMedical Research Tsukuba.
Results: The compound bound directly to RXRα and RARα, but not to PPARα, δ (β) or γ. It activated reporter genes with enhancer elements for RXRα/RXRα and RARα/RXRα, and partially activated reporter genes with enhancer elements for PPARδ (β) and PPARγ. Furthermore, the compound induced differentiation, and inhibited the growth of human APL cells in vitro and in vivo.
Conclusion: The identified compound was a dual agonist of RXRα and RARα and worked as both a differentiation inducer and a proliferation inhibitor to leukemic cells. Thus, the compound is a novel class of RAR/RXR agonist with potential therapeutic application.

496 POSTER

Gene expression profiles of TEL/AML1-positive pediatric leukemia: new insights in leukemia's molecular processes

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Background: The t(12;21)(p13;q22) translocation occurs in 25% of pediatric B acute lymphoblastic leukemia (B-ALL). This rearrangement induces the fusion of *ETV6* (*TEL*) and *RUNX1* (*AML1*) genes and defines a relatively uniform category. The *TEL*/*AML1*-positive patients are thus an appropriate subgroup for studying the relationship between biological mechanisms and clinical outcome.
Material and Methods: We analysed 33 pediatric B-ALL patients treated as part of the FRALLE 2000 trial using Agilent whole genome oligo-chips (44K-G4112A). Among them nine presented the *TEL*/*AML1* rearrangement. Combination of unsupervised and supervised clustering analyses of the training-set data (26 samples) were used to identify discriminating genes, characterizing the *TEL*/*AML1*-positive ALL. These genes were further functionally annoted. Validation of the arrays-results was assessed in two steps: RT-PCR quantification of biological relevant genes and unsupervised classification of a test-set (seven new samples).
Results: Gene enrichment analysis of the 74 genes discriminating *TEL*/*AML1* positive ALL highlighted five enriched Gene Ontology categories: apoptosis, response to wounding, cell proliferation, cell differentiation and cell motility, characterized by 14 genes, able to discriminate the *TEL*/*AML1* sub-group: *RUNX1, TCFL5, TNFRSF7, CBFA2T3*, *CD9*, *SCARB1, TP53INP1, ACVR1C, PIK3C3, EGFL7*, *SEMA6A, CTGF, LSP1, TFPI* (Figure 1). The robustness of these 14 genes was proved by their RT-PCR expression in the training-set and the accurate segregation of the test-set patients. Over-expression of *RUNX1* was investigated further and is proposed to be a predominant and stratifying surrogate marker.

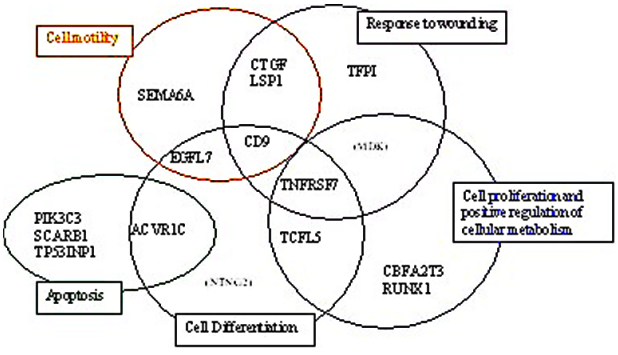

Fig. 1. Schematic representation of enriched GO term analysis ($p < 0.05$) obtained by comparison of the *TEL*/*AML1* gene-set to the Webgestalt pre-stored human genome gene-set.

Conclusion: Our results give new insights into the *TEL*/*AML1* molecular process and link molecular data to *TEL*/*AML1* clinical outcome, suggesting additional classification with new therapeutic prospect.

DNA repair

497 POSTER

Modulation of the repair of cisplatin-induced DNA interstrand crosslinks by trastuzumab

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HER2 (c-erbB2, HER-2/neu) is a member of the EGFR tyrosine kinase receptor family, which is activated through the formation of heterodimers with other members of the EGFR family. HER2 is overexpressed in 25−30% of breast cancers and is associated with poor clinical outcome. Currently, trastuzumab (Herceptin®) is the only approved antibody targeting specifically the extracellular domain of HER2. Trastuzumab acts by preventing the formation of HER2 heterodimers and accelerates the rate of endocytosis, inhibiting cell proliferation. Combination treatments of trastuzumab with various chemotherapeutic agents have shown synergy, both in vivo and clinically. In this study, we investigated the role of HER2 in drug-induced DNA damage repair. Using MCF-7, MDA-MB-453 and SK-BR-3 breast cancer cell lines, proliferation of cells following treatment was assessed using the Sulphorhodamine B assay. Single treatment with trastuzumab caused 20% inhibition of proliferation for MDA-MB-453 and 40% for SK-BR-3 at 10ug/ml, but not for MCF-7 which expresses low level of HER2. Combination treatments, with chemotherapeutic drugs (cisplatin, melphalan, etoposide, doxorubicin and paclitaxel), produced inhibition of proliferation by up to 70 fold, compared to the individual drug alone. Formation and repair of DNA interstrand crosslinks (ICLs) produced by cisplatin were measured using the single cell gel electrophoresis (COMET) assay. Treatment with trastuzumab did not alter the peak level (9 hours) of crosslinks produced by cisplatin. In the three cell lines, 35% (MCF-7) to 50% (MDA-MB-453) of crosslinks produced by cisplatin were repaired (unhooked) after 24 hours, which was reduced to 9% (SK-BR-3) to 18% (MDA-MB-453) combined with trastuzumab (20ug/ml). Repair was dose dependently inhibited in MDA-MB-453 cells at clinically relevant trastuzumab doses, between 20 and 100ug/ml, resulting in 63% to 97% inhibition of repair at 24 hours. In contrast, repair of ICLs produced by melphalan was not inhibited. Interestingly, repair of etoposide-induced strand breaks was not altered. FACS analysis, with cisplatin and trastuzumab, revealed that delay in DNA repair was not due to a cell cycle arrest. These findings establish a link between HER2 tyrosine kinase receptor and DNA damage repair in response to cisplatin.