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

S. Hatakeyama[1], C. Koshiishi[1], T. Kanazawa[2], T. Honda[1]. *[1]Novartis Institutes for BioMedical Research, Discovery Biology, Tsukuba, Ibaraki, Japan; [2]Novartis Institutes for BioMedical Research, Global Discovery Chemistry, Tsukuba, Ibaraki, Japan*

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

V. Gandemer[1,2], A. Rio[1], V. Sibut[1], B. Ly Sunnaram[3], C. Henry[4], A. Monnier[5], A. LeTreut[7], C. Berthou[6], J. Mosser[1,5,7], M. Galibert[1,7]. *[1]CNRS UMR6061, oncogenomics, Rennes, France; [2]CHU, Department of OncoPediatrics, Rennes, France; [3]CHU, Laboratory of Hematology, Rennes, France; [4]CHU, Laboratory of Cytogenetics, Rennes, France; [5]IFR 140, Ouest-Genopole, transcriptomic platform, Rennes, France; [6]CHU, Laboratory of Hematology, Brest, France; [7]CHU, Laboratory of Biochemistry and Genomics, Rennes, France*

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.

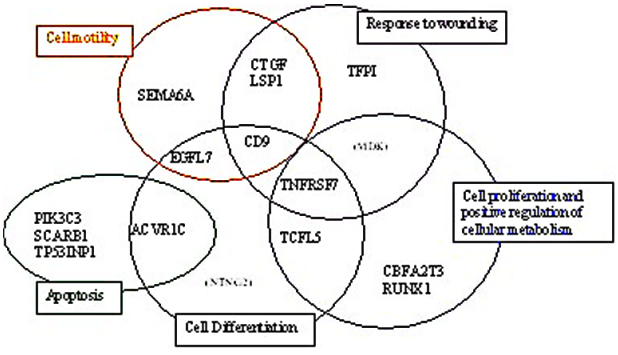

Fig. 1. Schematic representation of enriched GO term analysis ($p < 0.05$) obtained by comparison of the *TEL*/*AML1* gene-set to the Webgestalt pre-stored human genome gene-set.

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

J.J.M. Boone, D. Hochhauser, J.A. Hartley. *University College London, Oncology, London, United Kingdom*

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.