

*abd-B* gene after overexpression of DmCyp33 in the *Drosophila* SL2 cell line. Overexpression of a truncated form of DmCyp33 resulted in overexpression of the *abd-B* gene. This modulation was also sensitive to cyclosporin-A. The chimeric MLL proteins resulting from leukemia associated translocations lose the PHD fingers. Such loss may interfere with modulation of *HOX* gene regulation mediated by the interaction of MLL and Cyp33.

**Abstract# 1296****Poster Board #-Session: 346-II**

**ANALYSES OF THE JUN-DIMERIZATION PROTEIN p21<sup>SNFT</sup> IMPLICATE IT IN HTLV PATHOGENESIS.** Linda S. Feng\*,<sup>1,2</sup> Lee H. Remington-Boone\*,<sup>1,2</sup> Vong Huynh\*,<sup>2</sup> Alice Yu,<sup>2,3</sup> Angela Manns\*,<sup>4</sup> Steven Baird\*,<sup>1,3,5</sup> Hur-Song Chang\*,<sup>1,2</sup> William Wachsman.<sup>1,2,3</sup> <sup>1</sup>Research Service, VA San Diego Healthcare System, San Diego, CA; <sup>2</sup>Hematology-Oncology, UCSD School of Medicine, La Jolla, CA; <sup>3</sup>Cancer Center, UCSD, La Jolla, CA; <sup>4</sup>Division of Cancer Epidemiology and Genetics, NCI, Bethesda, MD; <sup>5</sup>Pathology, UCSD School of Medicine, La Jolla, CA.

Human T-cell leukemia virus, type 1 (HTLV-1) is etiologic for adult T-cell leukemia/lymphoma (ATLL) that occurs in 2-5% of infected individuals 20-30 years after primary infection. Little, if any, viral replication is detected soon after infection or in freshly isolated ATLL cells, suggesting that the provirus resides in a latent state. In addition, it has been shown that there is slow clonal expansion of HTLV-infected T cells without evidence of transformation *in vivo*. Previously, we isolated the p21<sup>SNFT</sup> (small nuclear factor of T cells) protein as part of a heteromultimeric complex formed with JunB bound to the 21-basepair Tax-responsive repeat sequences in the HTLV long terminal repeat (LTR). Analysis of SNFT cDNA, cloned from HTLV-1-transformed T cells, revealed that it is a member of a new subfamily of basic region, leucine zipper (bZIP) proteins, including Marek Disease Virus EcoQ, B-ATF, and JDP-1, all of which dimerize with Jun family proteins. TaqMan real-time RT-PCR was used to measure expression of SNFT mRNA relative to GAPDH mRNA in B- and T-cell tumors. Low levels of SNFT were found in quiescent peripheral blood mononuclear cells (PBMC). In comparison to PBMC, expression of SNFT was 5 to 200-fold higher in ATLL, significantly lower in T-cell acute lymphoblastic leukemia, and low to basal in chronic lymphocytic leukemia and B-cell non-Hodgkin's lymphoma. Cotransfection assays showed that p21<sup>SNFT</sup> in the presence of JunB inhibits Tax-mediated transactivation of the HTLV-1 LTR in Jurkat T cells in a dose-dependent manner. Although p21<sup>SNFT</sup> and JunB together had no significant effect on the level of *tax* message, we found that they markedly reduced the level of Tax protein, providing a novel mechanism for inhibition of Tax function. Lastly, analysis of Jurkat T cells stably transfected with SNFT showed, in comparison to vector transfected control cells, a 25% reduction in caspase-3 activity following UV-irradiation. Our data demonstrate that expression of p21<sup>SNFT</sup> is induced in HTLV-1 infected T cells and suggest that this novel bZIP protein facilitates establishment of HTLV-1 latency while inhibiting host T-cell apoptosis. Based on these results, we postulate that p21<sup>SNFT</sup> plays a role in HTLV pathogenesis and development of ATLL.

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**VEGFR-1 (Flt-1) AND VEGFR-2 (KDR) STIMULATE THE PROLIFERATION OF AML CELLS VIA THE PI3-KINASE AND AKT/PROTEIN KINASE-B (PKB) SIGNAL PATHWAY.** A.F. List,<sup>1</sup> B. Glinsmann-Gibson\*,<sup>1</sup> C. Stadheim\*,<sup>1</sup> E. Meuillet\*,<sup>1</sup> W. Bellamy\*,<sup>1</sup> G. Powis\*,<sup>1</sup> <sup>1</sup>Arizona Cancer Center, University of Arizona, Tucson, AZ, USA.

Vascular endothelial growth factor (VEGF) interacts with two type III receptor tyrosine kinases (RTK) on endothelial cells to initiate separate and distinct biological responses: VEGFR-1(Flt-1), permeability; and VEGFR-2(KDR/Flk-1), proliferation. We previously reported that AML cells express VEGF and one or both VEGF receptors [Bellamy et al. Blood (Suppl 1) 1999]. Recent reports indicate that VEGF is essential to AML engraftment in SCID mouse models, and that myeloblast VEGF content inversely correlates with treatment outcome. To characterize the biological effects of VEGF in AML, we investigated clonogenic response to rhu-VEGF in receptor competent KG1 (Flt-1<sup>+</sup>/KDR<sup>+</sup>) and HL60 (Flt-1<sup>+</sup>/KDR<sup>-</sup>) AML cell lines, and analyzed the roles of phosphoinositide-3-kinase (PI3K), Akt/Protein kinase-B (PKB), and ras mitogen-activated protein kinase kinase (MAPKK/MEK) in receptor signal transduction. Rhu-VEGF [0.1 to 50 ng/ml] stimulated colony formation up to 2.5-fold and increased colony size in each cell line in methylcellulose cultures. To discern receptor specificity of clonogenic response, we assessed colony formation after stimulation with receptor-specific agonist antibodies. 24-hour exposure to anti-VEGFR-1 or anti-VEGFR-2 triggered equivalent and concentration-dependent stimulation of colony recovery in KG1 cells; whereas clonogenic response in KDR-negative HL60 cells was restricted to Flt-1 engagement. Preincubation with the irreversible PI3K inhibitor wortmannin [1-50nM] inhibited VEGF-induced colony formation in a concentration dependent fashion. In serum deprived KG1 and HL60 cells, rhu-VEGF stimulated rapid (1H) and sustained (24H) phosphorylation of Akt/PKB that was inhibited by wortmannin pre-incubation. Rhu-VEGF-induced clonogenic response and Akt-phosphorylation was abolished by the selective VEGF-RTK inhibitor SU-5416 (Sugen, Inc; San Francisco, CA) at concentrations >10µM; whereas the MAPKK/MEK inhibitor PD98059 [1µM and 10µM] had no effect. These data indicate that VEGF ligation of either VEGFR-1 or VEGFR-2 stimulates a receptor tyrosine kinase dependent clonogenic response in AML cells that is mediated by PI3-kinase dependent activation of Akt/PKB. VEGF-RTK inhibitors such as SU-5416 represent novel therapeutics that merit clinical investigation in AML.

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**THE RARα-PLZF CHIMERA INHIBITS C/EBPα FUNCTION AND THE EXPRESSION OF THE G-CSF RECEPTOR.** Nathalie Girard\*,<sup>1</sup> Nathalie Bouchard\*,<sup>1</sup> Haman Andre\*,<sup>1</sup> Labrecque Jean\*,<sup>1</sup> Sai-Juan Chen\*,<sup>2</sup> Xiang-Jiao Yang\*,<sup>3</sup> Trang Hoang.<sup>1</sup> <sup>1</sup>Hematopoiesis and Leukemia, <sup>2</sup>Clinical Research Institute, Montreal, QC, Canada; <sup>3</sup>Shanghai Institute of Hematology, Shanghai, China; <sup>4</sup>Molecular Oncology Group, Royal Victoria Hospital, Montreal, QC, Canada.

Acute promyelocytic leukemia (APL) harbouring the t(15;17) translocation is responsive to differentiation therapy with retinoic acid (RA) while the t(11;17) APL is a more aggressive disease with poor prognosis. The latter produces two fusion proteins, PLZF-RARα which forms RA-insensitive complexes with co-repressors, and RARα-PLZF that has retained the DNA binding properties of PLZF as well as an interaction domain with HDAC and mSin3a. The present study is designed to address the function of the RARα-PLZF through ectopic expression in the hemopoietic cell line 32D that undergoes granulocyte differentiation in response to granulocyte colony stimulating factor (G-CSF) and to RA. Our results indicate that the RARα-PLZF represses expression of the gene encoding the G-CSF receptor (G-CSFR), which fulfills an important function in cell survival and differentiation along the granulocytic lineage. Repression of the G-CSFR promoter activity in transient transfection assays or of the endogenous G-CSFR gene in chromatin can be relieved by TSA, suggesting the recruitment of histone deacetylase. Interestingly, repression of G-CSFR by PLZF and RARα-PLZF is dependent on the binding of the myeloid transcription factor C/EBPα to its cognate sequence at position -49 relative to the transcription start site. Since PLZF and RARα-PLZF recruit co-repressors, our observations suggest that RARα-PLZF inhibit G-CSFR gene expression through association with C/EBPα and recruitment of a histone deacetylase complex. Significantly, G-CSFR mRNA is low to undetectable in leukemic cells from patients with t(11;17) translocation, whereas those characterized by the t(15;17) translocation exhibit elevated levels of G-CSFR mRNA.

In summary, our studies suggest that in t(11;17) APL, leukemic cells are arrested at the promyelocyte stage due to the concerted effect of PLZF-RARα and RARα-PLZF that repress two critical pathways for granulocyte differentiation, the RARα/γ and the C/EBPα-G-CSFR pathways, consistent with the poor prognosis of t(11;17) APL.

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**TRANSCRIPTIONAL TARGETS OF CORE BINDING FACTOR FUSION PROTEINS.** Koichi Sasaki,<sup>1</sup> Trista North\*,<sup>2</sup> Nancy Speck\*,<sup>2</sup> Todd R. Golub.<sup>1,3</sup> <sup>1</sup>Dana-Farber Cancer Institute, Boston, MA, USA; <sup>2</sup>Department of Biochemistry, Dartmouth Medical School, Hanover, NH, USA; <sup>3</sup>Whitehead/MIT Center for Genome Research, Cambridge, MA, USA.

Core Binding Factor (CBF) is a heterodimeric transcription factor complex that is frequently targeted by chromosomal translocation in acute leukemia. The alpha subunit (AML1) commonly forms an AML1/ETO fusion in t(8;21) AML, and a TEL/AML1 fusion in t(12;21) ALL. Similarly, the beta subunit (CBFB) forms a CBFB/MYH11 fusion in inv(16) AML. Remarkably few downstream effectors of these fusion proteins are known. To address this, we established ecdysone-inducible NIH 3T3 cells which express the 3 fusion proteins, and we performed oligonucleotide microarray expression profiling of approximately 11,000 murine genes and ESTs 24 hours following fusion protein induction. First, we noted that AML1/ETO but not TEL/AML1 or CBFB/MYH11 resulted in G1 growth arrest. Consistent with this, AML1/ETO induced the growth arrest gene GADD45, whereas the other two did not. Interestingly, a relatively small group of genes were similarly regulated by AML1/ETO and CBFB/MYH11, including the angiogenic factor Connective Tissue Growth Factor (CTGF), and Heparin-Binding EGF-like Growth Factor (HB-EGF), the receptor for diphtheria toxin. The regulation of angiogenic genes by AML1/ETO and CBFB/MYH11 is also consistent with the recent demonstration of AML1-dependent hematopoietic stem cells being critical for normal angiogenesis in the mouse. The kinetics of induction by CBFB/MYH11 lagged that of AML1/ETO, perhaps due to CBFB/MYH11 sequestering alpha subunits rather than direct transcriptional activity of the fusion protein. Both genes were also found to be expressed in primary bone marrow specimens of acute leukemia patients. Of note, these genes were not induced by TEL/AML1, suggesting that the mechanism of transformation by AML1/ETO and CBFB/MYH11 may not be identical to the ALL-associated TEL/AML1 fusion despite their being reported to function similarly in promoter-reporter assays of CBF function.