Lymphoma biology

373 B-CELL LYMPHOMA CELLS OVEREXPRESSING BMI-1 ARE CORRELATED WITH DRUG RESISTANCE THROUGH ENHANCED EXPRESSION OF SURVIVIN AND ARE MORE EFFECTIVELY ELIMINATED BY T CELLS WITH CHIMERIC RECEPTOR AGAINST CD38 THAN CD19

K. Mihara[1], J. Bhattacharyya[1], M. Ohtsubo[2], Y. Takihara[2], M. Hoshi[1], A. Kimura[1],
[1]Dept of Hematology-Oncology, RIRBM, Hiroshima University, Hiroshima, Japan, [2]Dept of Stem Cell Biology, RIRBM, Hiroshima University, Hiroshima, Japan

BMI-1 is essential for self-renewal and proliferation of leukemic and hematopoietic stem and progenitor cells. The expression of BMI-1 is well correlated with disease progression and poor prognosis in cancer patients. We showed that ectopic expression of BMI-1 in B-cell lymphoma cell lines, HT and RL, conferred resistance to etoposide and oxaliplatin, known to enhance sensitivity by targeting the survivin gene, but not to irinotecan, which is not relevant to the downregulation of survivin expression. Survivin has been reportedly implicated in resistance to chemotherapeutic agents. The expression of survivin was not only augmented in cells transduced with BMI-1, but persisted in the presence of etoposide in cells overexpressing BMI-1. By contrast, the mock-transduced cells succumbed in the medium with anti-cancer drugs with an accompanying decrease in BMI-1 and survivin expression. BMI-1 overexpression stabilized survivin post-translationally without an accompanying rise in the mRNA, suggesting survivin to be a potential target for BMI-1.Knockdown of either BMI-1 or survivin restored sensitivity to etoposide in the BMI-1-overexpressing lymphoma cells. An analysis of 6 patients with B-cell lymphoma showed that in the drug-resistant patients, levels of BMI-1 and survivin were maintained even after drug administration. Downregulation of both BMI-1 and survivin expression was, however, observed in the drug-sensitive patients. Thus, BMI-1 may facilitate drug resistance in B-cell lymphoma cells through the regulation of survivin. Next, we examined whether B-cell lymphoma cells overexpressing BMI-1 are abrogated by the immunotherapy with T cells containing either an anti-CD19 or -CD38 chimeric receptor (CR) *in vitro*. Interestingly, these B-cell lymphoma cells were effectively eliminated by specific T cells against the B-cell lymphoma cells bearing CD38. Alternatively, T cells with the anti-CD19-CR killed B-cell lymphoma cells overexpressing BMI-1 less effectively than T cells with the anti-CD38-CR. T cells with each of CRs may work in a somewhat different way. These results suggest that the immunotherapy, especially with T cells bearing the anti-CD38-CR, is useful for treatment of patients with B-cell lymphoma cells overexpressing BMI-1, which are refractory to chemotherapeutic reagents. BMI-1 could, thus, be an important prognostic marker as well as a future therapeutic target in the treatment of drug-resistant lymphomas.

374 ANALYSIS OF PHENOTYPE OF CELLS IN PRIMARY CULTURES DERIVED FROM LYMPH NODE BIOPSIES OF PATIENTS WITH LYMPHOMAS

T. Loja[1], A. Janikova[1], J. Mayer[1], M. Klabusay[1].
[1]Internal Medicine - Hematooncology, Masaryk University, Brno, Czech Republic

Introduction: Biopsy of lymph node is critical for establishment of diagnosis of malignant lymphomas. Except of classical histology, modern diagnostic methods including PCR, FISH, RNA microarrays or flow cytometry became inevitable for diagnostic process. Immunophenotyping of BM (bone marrow) or PB (peripheral blood) by flow cytometry is a standard method lymphoma staging, but immunophenotyping of lymph node biopsies is not commonly used. Because of a complex nature of lymph node tissue, analysis may be difficult to perform because of presence of various types of contaminating cells.

Material and Methods: In our study, we decided to establish primary cultures derived from lymph node biopsies of patients with newly diagnosed lymphoma, and to monitor phenotypic markers in both native lymph node biopsies, as well as in primary lymphoma-derived short-term cultures. 45 biopsy samples were successfully analyzed and cultured. In 28 samples the diagnosis of lymphoma was confirmed by histology. 32% of samples were diagnosed as Hodgkin's lymphoma, 29% as follicular lymphoma (FL), 25% as diffuse large B-cell lymphoma (DLBCL) and 14% as mantle cell lymphoma (MCL) or other type of lymphoma. By multicolor flow cytometry analysis, the expression of following antigens was analyzed: CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD23, CD34, CD38, CD45, HLA-DR, TCR-αβ.

Results: Short-term primary cultures were successfully established in 65% of all samples. *In vitro* cultivation of lymphoma-derived cells and theirs phenotypic analysis was performed within the range 2 to 5 weeks. We repeatedly analyzed viability of cells and changes in ratios of cell populations (B-lineage, T-lineage, $CD34^+$ cells, lymphoma cells). No long-term culture was established. Comparison with histology, molecular biology and FISH analysis was made.

Conclusions: It is feasible to derive short-term cell cultures from native lymph node biopsies of patients with lymphomas. Immunophenotyping by flow cytometry may help in the diagnostic process and may reveal the pathophysiology of lymphoma cells under conditions of *in vitro* cultivation.
This work was supported by grants IGA NS9670 and MSM 0021622430.

375 WITHDRAWN

376 U-DCS, THE FIRST HUMAN CELL LINE DERIVED FROM AN INTERDIGITATING DENDRITIC CELL SARCOMA

P. Möller[1], S. Brüderlein[1], F. Leithäuser[1], I. Melzner[1], K. Zimmermann[1], P. Wiegand[2], M. Buck[1], S. Corbacioglu[3], M. Malaise[3], A. Viardot[4]
[1]Deot of Pathology, University of Ulm, Ulm, Germany, [2]Institute for Forensic Medicine, University of Ulm, Ulm, Germany, [3]Paediatric Clinic, University of Regensburg, Regensburg, Germany, [4]Dept of Hematology and Oncology, University of Ulm, Ulm, Germany

Introduction: Human dendritic cells are a phenotypically diverse group of antigen-presenting cells found in tiny fractions in tissues throughout the body. The low numbers of dendritic cells present in blood mononuclear cells has hampered a detailed molecular analysis of dendritic cell function. No stable human dendritic cell lines could have been established so far. Interdigitating dendritic cell sarcoma (IDCS) is an extremely rare neoplasm that mostly occurs in the lymph nodes. Only 184 cases have been reported worldwide.

Materials and Methods: Here we describe a novel permanent human IDCS cell line, U-DCS, derived from an interdigitating dendritic cell sarcoma of a male patient. Serial passaging of the cells gave rise to a fast proliferating cell line, which was characterized by immunocytochemical, molecular, cytogenetic and Elisa techniques.

Results: The cell line maintained stable phenotypic characteristics during the last one year *in vitro*. U-DCS is growing adherent with typical dendritic morphology, expresses MHCI- and MHCII- proteins, CD68, CD80, CD11c, S100 protein, and, in a subset, CD163 and CD205 but not CD40. mRNA expression analysis revealed transcripts for the toll-like receptors TLR3, -4, -9, and RIG-I (DDX58) but not for TLR2. The cells react with T and B lymphocytes. Karyotype analysis, m-FISH and FACS analysis revealed chromosomal instability, a hypotetraploid karyotype with about 130 chromosomes and multiple aberrations. CGH analysis showed more than three-fold loss of chromosome 4q and more than three-fold gain of chromosome Y. In contrast, short tandem repeat (STR) analysis revealed that U-DCS is stable on the molecular level. U-DCS forms slow growing tumors in the mouse NOD. Cg-$Prkdc^{scid}Il2rg^{tm1Wjl}$/Sz which are deficient in mature lymphocytes and NK cells, thereby conserving its phenotype after recultivating *in vitro*.

Conclusion: U-DCS is the first permanent human dendritic cell line and could be useful and helpful in future dendritic research.

377 CONCENTRATION OF SELECTED CYTOKINES IN BONE MARROW AND PERIPHERAL BLOOD OF PATIENTS WITH LYMPHOMA

M. Klabusay[1], J. Chovancova[1], V. Hrabcakova[1], O. Stehlikova[1], P. Coupek[2], J. Mayer[1]
[1]Internal Medicine - Hematooncology, Masaryk University, Brno, Czech Republic, [2]Div. of Informatics, Czech Geology Survey, Brno, Czech Republic

Introduction: Cytokines participate as important mediators in immune responses. They influence development and survival of many cells and they could play a critical role in progression of cancer. Some of them are thought to be useful markers of hematological malignancies, particularly lymphomas.

Material and Methods: In our study, we investigated the plasma concentration of following cytokines, their receptors and co-stimulatory molecules: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-18, TNF-α, VEGF, IFN-γ, G-CSF, sIL-2R, sIL-6R, sCD23 and sCD40L using an enzyme-linked immunosorbent assay (ELISA).
52 samples of peripheral blood and bone marrow were evaluated with following diagnoses: chronic lymphocytic leukemia (CLL/SLL), diffuse large B cell lymphoma (DLBCL), marginal zone lymphoma (MZL) and mantle cell lymphoma (MCL). Control group consisted of 30 healthy volunteers.

Results: Plasma levels of IL-4 and IL-6R were significantly higher in DLBCL ($p=0.0015$, $p=3\times10^{-5}$) and MZL patients ($p=0.0022$, $p=0.00014$), as compared to healthy controls. In DLBCL patients, levels of sCD40 were elevated ($p=0.018$). On the other side, MZL patients showed significantly higher levels of IL-2 ($p=0.019$). Patients with CLL/SLL diagnosis embodied increment in IL-2 ($p=0.025$), sIL-2R ($p=0.00014$) and IL-18

© The Author 2011. Published by Oxford University Press on behalf of the European Society for Medical Oncology.
All rights reserved. For permissions, please email: journals.permissions@oup.com

Downloaded from http://annonc.oxfordjournals.org/ by guest on May 21, 2012

(p=0.0013). Elevations in concentration of IL-18 and sIL-2R (p=0.0011, p=0.0043) were also found in MCL patients. Further, no significant differences were found comparing concentrations of selected cytokines in peripheral blood to those in bone marrow.

Conclusions: These changes in cytokine concentrations of particular hematological malignancy may help in the diagnostic process and in our understanding of changes in immune responses in lymphoma patients.

This work was supported by grant IGA MZCR NS 9671-4 and MSM 0021622430.

378 WITHDRAWN

379 THE ROLE OF AID FOR THE PROGRESSION OF FOLLICULAR LYMPHOMA

H. Shikata[1], Y. Yakushijin[1], N. Matsushita[2], A. Sakai[3], A. Sugita[4], N. Nakamura[5], M. Yasukawa[1]
[1]Department of Bioregulatory Medicine, Ehime University Graduate School of Medicine, Toon, Japan, [2]Functional Genomics Core–Laboratory, Ehime University Proteo Medicine Research Center, Toon, Japan, [3]Department of Hematology and Oncology, Research Institute Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan, [4]Department of Pathology, Ehime University Hospital, Ehime University, Toon, Japan, [5]Department of Pathology, Tokai University School of Medicine, Isehara, Japan

Background: Follicular lymphoma (FL), a heterogeneous entity with a cell origin in germinal center B (GCB) lymphocytes, may develop into a progressive or transformed disease in some patients. Secondary genetic events have been associated with this transformation such as c-myc amplification or mutation. Activation induced cytidine deaminase (AID) is required for somatic hyper mutation and class switch recombination of immunoglobulin gene and c-myc translocation of GCB-cell lymphoma. However, the role of AID in transformed FL has not been established. Here we tried to identify the significance of AID associated with c-myc in the progression of FL.

Materials and Methods: A total of thirty-six clinical samples obtained from the patients with FL were divided into three groups; patients with FL in grade 1 or 2 (n=15), patients with FL in grade 3 who could survive more than two years (n=14), and patients with rapidly progressed FL in grade 3 (RPFL) who died within two years after diagnosis (n=7). AID and c-myc expression among those patients with FL were examined with RT-PCR and quantitative real-time PCR. In order to examine the role of AID expression for RPFL, the full length of AID transcription was transfected into cell lines established from the patients with RPFL, and their cell proliferation and survival were compared to the controls in vitro.

Results: Tissues from the patients with FL in grade 3 and the patients with RPFL expressed relatively higher levels of c-myc than those from the patients with FL in grade 1 or 2 (p= .02 and p< .01 representatively). Also, high levels of AID expression was observed among the tissues from the patients with FL in grade 3, but not significantly. Interestingly, six of seven samples with RPFL expressed low levels of AID or did not express AID even though they expressed high levels of c-myc. Moreover, transfection of AID full length into AID-negative cell lines, which were established from three patients with RPFL, reduced cell proliferation and survival in vitro.

Conclusions: Our results indicate that AID may play a role of negative regulator on cell survival of FL when sufficient c-myc is expressed in the tumor. Switch off or low expression of AID after c-myc amplification may correlate to rapidly progressive and clinical outcomes of FL.

380 INTEGRATIVE GENOMIC PROFILING OF HIGH-RISK DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS LESS THAN 65 YEARS OLD TREATED WITH DOSE-DENSE CHEMOIMMUNOTHERAPY AND CNS PROPHYLAXIS

S. Koivula[1], M. Taskinen[1], R. Louhimo[1], P. Chen[1], J. Delabie[2], H. Holte[3], M. Karjalainen-Lindsberg[4], M. Björkholm[5], Ø. Fluge[6], L. M. Pedersen[7], M. Jerkeman[8], M. Eriksson[8], S. Hautaniemi[1], S. Leppä[9].
[1]Genome Scale Biology Program, University of Helsinki, Helsinki, Finland, [2]Department of Pathology, Rikshospitalet, Oslo, Norway, [3]Department of Oncology, Radiumhospitalet, Oslo, Norway, [4]Department of Pathology, University of Helsinki, Helsinki, Finland, [5]Department of Medicine, Karolinska University Hospital, Stockholm, Sweden, [6]Department of Oncology, Haukeland University Hospital, Bergen, Norway, [7]Department of Hematology, Odense University Hospital, Odense, Denmark, [8]Department of Oncology, Lund University Hospital, Lund, Sweden, [9]Department of Oncology, Helsinki University Central Hospital, Helsinki, Finland

Background: Several gene-expression signatures with prognostic significance in DLBCL have been identified. Here, we have integrated the high-resolution array comparative genomic hybridization (aCGH) data with transcription profiles to define novel prognostic markers for homogeneously treated DLBCL patients.

Patients: 59 de novo high-risk DLBCL patients less than 65 years were treated with six courses of R-CHOEP14 followed by two courses of systemic CNS prophylaxis (Mtx and Ara C). After a median follow-up of 36 months, 3-year relapse free survival (RFS) was 72% and overall survival 78%.

Results: The most frequent amplifications occurred in 1q44 (19%) and 20q11.22 (17%). Recurrently deleted regions were located at 2p11.2 (22%) and 9p21.3 (17-22%). When aCGH data were combined with the information from exon array analysis, we found 61 genes in which the expression changes were associated with copy number aberrations. Of these, 56 were overexpressed due to copy number gains at 2p14-16, 2p25, 11p15, 14q32, 16q22, and 18q21-23. In contrast, 5 genes were underexpressed due to copy number losses at 6p21, 9p21 and 15q15. When the prognostic value of genomic alterations was evaluated, patients (n=6) with gain in chromosome 2p15 locating to CCT4 (p=0.007), COMMD1 (p=0.007), USP34 (p=0.040) and XPO1 (p=0.007) had significantly inferior RFS compared to patients with no gain.

Conclusions: By this approach genes not previously recognized by expression profiling alone are identified and data highlight the value of an integrated comprehensive analysis of genetic alterations in this disease.

381 BCL2 MUTATIONS IN DIFFUSE LARGE B-CELL LYMPHOMA

J. Schuetz[2], N. Johnson[1], R. Morin[2], M. Marra[2], J. Connors[3], A. Brooks-Wilson[2], R. Gascoyne[4]
[1]Hematology and Oncology, Jewish General Hospital, Montreal, Canada, [2]Genome Sciences Centre, BC Cancer Agency, Vancouver, Canada, [3]Medical Oncology and Centre for Lymphoid Cancer, BC Cancer Agency, Vancouver, Canada, [4]Pathology and Centre for Lymphoid Cancer, BC Cancer Agency, Vancouver, Canada

Background: BCL2 is deregulated in diffuse large B-cell lymphoma (DLBCL) by the t(14;18) translocation, promoter hypermutation, gene amplification, and as a target gene of NF-kB signaling. BCL2 overexpression also affects DLBCL prognosis.

Methods: 31 DLBCL cases were subject to RNA-seq and *BCL2* variants were determined using SNVmix. The *BCL2* gene (exons 1-3) was resequenced by Sanger method in 542 samples: 348 primary DLBCL, 30 small lymphocytic lymphoma (SLL), 26 follicular lymphoma (FL), 25 mantle cell lymphoma (MCL), and 25 peripheral T cell lymphoma (PTCL) patient samples; 8 purified centroblasts; 51 germline DNAs from DLBCL or FL patients in this study; and 24 DLBCL cell lines. t(14;18) presence was determined by fluorescence *in situ* hybridization in 140 DLBCL samples and by karyotype in 26 FL samples. BCL2 protein expression was determined by clone 124 (Dako) in 310 cases. Association between the presence of mutations and other variables was determined by Fisher's exact test and association to overall survival was determined by log rank test.

Results: We show that BCL2 is highly mutated in DLBCL, particularly in GCB molecular subtype, and in FL which, like many GCB tumors, is characterized by t(14;18) and arises from germinal center B cells. In ABC DLBCL, which seldom show t(14;18), we see very low levels of BCL2 mutation. We do not see high rates of BCL2 mutation in MCL, SLL or PTCL, which do not typically show t(14;18) and are derived from other stages of lymphocyte development. This strongly suggests that t(14;18) translocation, rather than deregulation of the BCL2 gene through other mechanisms such as the transcriptional up-regulation seen in ABC type DLBCL or deregulation of miR 15-16 in CLL, plays a pivotal role in the acquisition of BCL2 mutations in GCB type DLBCL and in FL. However, 26% of DLBCL cases without detectable t(14;18) contained BCL2 mutations, indicating BCL2 mutations can also occur by independent mechanisms. Many mutations cause amino acid changes; these are rarely found in the regions encoding BH domains.

Conclusions: BCL2 mutations in DLBCL and FL are much more prevalent than previously anticipated and appear to be a consequence of on-going somatic hypermutation in the germinal center. The functional and clinical significance of these mutations should be studied further, given that BH3 mimetics may eventually be considered as therapeutic options in these patients.

382 WITHDRAWN

383 WHOLE BLOOD GENE EXPRESSION PROFILING IN ADULT DLBCLS, LESS THAN 60, AT DIAGNOSIS: COMPARISON WITH A MATCHED COHORT OF BLOOD DONORS

T. Fest[1], X. Cahu[2], C. Pangault[1], J. De Vos[3], D. Maucort-Boulch[4], P. Roy[4], S. Le Gouill[5], D. Damotte[6], G. Semana[7], T. Lamy[2], N. Milpied[8], D. Rossille[9]
[1]Laboratoire d'Hématologie, CHU Rennes & INSERM U917, Rennes, France, [2]Hématologie Clinique, CHU Rennes, Rennes, France, [3]IRB, CHU Montpellier, Montpellier, France, [4]Biostatistiques, CHU Lyon, Lyon, France, [5]Hématologie Clinique, CHU Nantes, Nantes, France, [6]Anatomo-Pathologie, Hôtel-Dieu de Paris, Paris, France, [7]EFS Bretagne, Etablissement Français du Sang, Rennes, France, [8]Hématologie et Thérapie Cellulaire, Hôpital Haut-Lévêque, Pessac, France, [9]Laboratoire d'Hématologie & Département d'Informatique Médicale, CHU Rennes, Rennes, France

Introduction: Patients with diffuse large B cell lymphoma (DLBCL) often exhibit disturbances of their biological parameters including blood count and biochemistry.

Downloaded from http://annonc.oxfordjournals.org/ by guest on May 21, 2012

The GOELAMS 075 is a phase 3 trial focusing on patients between 18 and 60 years old with advanced *de novo* DLBCL. Our project aims at understanding the gene expression disturbances which take place at diagnosis in the blood of patients included in this trial.

Material and methods: Peripheral blood samples were collected in PAXGene™ tubes from 95 patients with DLBCL at diagnosis and 93 sex- and gender-matched blood donors as controls. After RNA isolation and globin reduction, cDNA was hybridized onto Affymetrix GeneChip® Human Exon 1.0 ST arrays. After quality check, core-annotated probesets without any SNP were preprocessed using the Robust Multi-Average (RMA) method and filtered using detection above background. Unsupervised analysis was performed using the Principal Component Analysis (PCA). Differential expression between controls and DLBCLs was determined with Mann-Whitney U tests corrected with a Benjamini and Hochberg false-discovery rate <5%. Genes were further selected according to the absolute Fold Change. Biological data were interpreted using the Broad Institute MSigDBv3.0 database.

Results: 80 patients and 87 samples met quality criteria. 6270 transcript-ID were found differentially expressed among the 8680 filtered transcripts. PCA revealed a clear cut between control and DLBCL samples in a two-dimensional scatterplot. With an absolute FC > 2, 96 genes were upregulated including TNFSF13B, interferon-induced proteins. 50 genes were downregulated including IL2RB and lymphoid-related transcription factors. With an absolute FC > 1.5, the number of genes increased reaching 501 upregulated and 529 downregulated genes.

Conclusions: This GEP study reveals profound disturbances in the gene expression in whole blood of patients with DLBCL compared healthy controls. They are mainly related to inflammation and inhibition of lymphoid processes. Ongoing analyses focus on characterizing specific pathways and cell origin that drive this DLBCL whole blood pattern. Specificity and sensibility of this pattern will be analyzed as well as the prognostic value in DLBCL of specific genes.

384 INTEGRATED MOLECULAR PROFILE OF BURKITT LYMPHOMA

P. Piccaluga[1], G. De Falco[2], M. Rossi[1], M. Laginestra[1], M. Sapienza[1], C. Bellan[2], S. Lazzi[2], L. Tumwine[3], M. Mawanda[4], M. Ogwang[4], V. Calbi[4], A. Gazzola[1], L. Leoncini[2], S. A. Pileri[1]
[1]*Hematology and Oncological Sciences, Bologna University, Bologna, Italy,* [2]*Human Pathology and Oncology, Siena University, Siena, Italy,* [3]*Pathology, Makerere University, Kampala, Uganda,* [4]*Human Pathology and Oncology, St. Mary Hospital, Lacor, Gulu, Uganda*

Background: Endemic Burkitt lymphoma (BL) is the commonest pediatric cancer in Africa. Recently, we showed that Endemic BL differs from the sporadic variant of BL, which occurs in Western Countries, for a series genes involved in relevant functional programs, including immune response, BCR signaling, and cell cycle regulation. However, the exact contribution of EBV (which is currently considered a main etiopathogenetic factor of the disease) to the molecular profile of the disease has not been clearly established.

In this study we aimed to further explore the molecular profile of BL subtypes, by focusing on the possible roles of EBV and other possible pathogenetic events (i.e. chronic immune stimulation) as determinants of the differences in GEP among the different BL subtypes.

Material and Methods: We studied 48 BL cases, including endemic (N=24), sporadic (N=12) and HIV-related forms (N=12). For the latter two subgroups, cases with or without evidence of EBV infection were enrolled. We performed whole genome GEP by using the Illumina DASL technology, and extensive miRNA profiling by using the Affymetrix Human miRNA arrays. Results were validated by immunohistochemistry (IHC) and in situ hybridization (ISH) on a large series of cases, as well as by functional assays on BL cell lines.

Results: Unsupervised and supervised analyses have been performed, evaluating differences in both gene and miRNA expression among the different BL types. In addition, we specifically focused in differences between EBV positive vs. EBV negative cases. Finally, we validated our findings by IHC, ISH and functional assays. All the results will be preliminary presented at the ICML in Lugano.

Conclusions: BL subtypes differ for their molecular profiles. High throughput techniques allowed a identify specific pathways deregulated in each groups.

385 DLEU1 SIRNA GENE KNOCKDOWN IN PEDIATRIC BURKITT LYMPHOMA (PBL) IS ASSOCIATED WITH A SIGNIFICANT DECREASE IN DRUG INDUCED APOPTOSIS: IMPLICATION OF DLEU1 AS A TUMOR SUPPRESSOR GENE

N. S. Day[1], J. Ayello[1], M. S. Lim[2], S. Perkins[3], R. R. Miles[3], C. Van de Ven[1], W. G. Sanger[4], L. Harrison[1], S. Goldman[5], M. S. Cairo[6]
[1]*Pediatrics, Columbia University, New York, United States,* [2]*Pathology, University of Michigan, Ann Arbor, United States,* [3]*Pathology, University of Utah, Salt Lake City, United States,* [4]*Munroe Meyer Institute for Genetics and Rehabilitation, University of Nebraska Medical Center, Omaha, United States,* [5]*Pediatric Hematology Oncology, Medical City Children's Hospital, Dallas, United States,* [6]*Pediatrics, Pathology and Medicine, Columbia University, New York, United States*

Background: PBL represents 40% of pediatric NHL and is characterized by an 8q24 (*c-myc*) gene rearrangement. Most PBL patients (70%) also have a secondary chromosomal aberration (Poirel/Cairo et al Leukemia, 2009). Children with PBL who have a 13q14.3 deletion have a significantly poorer survival (Nelson/Cairo/Perkins/Sanger et al BJH 2010). DLEU1, a Burkitt classifier (Dave/Staudt et al. NEJM, 2006), is located within 13q14.3. cMYC binds to the promoter of DLEU1 to initiate the expression of DLEU1. DLEU1 in turn negatively regulates cMYC. Thus, DLEU1 may act as a tumor suppressor, and if deleted in children with 13q- PBL may allow the deregulation of several signaling pathways leading to drug/immune therapy resistance and inhibit apoptosis. We investigated the effects of DLEU1 on chemotherapy (cyclophosphamide; CY) and antibody (rituximab) -induced BL apoptosis.

Methods: Ramos PBL cell line was transiently transfected (24 hrs) with DLEU1 siRNA (5'-AUACUUGGCAUGAAUGAACUUAUGU-3' and 3'-UAUGAACCGUACUUACUUGAAUACA-5') as previously described (Day/Cairo SIOP 2008). The siRNA transfected cells were then treated with various concentrations of CY or rituximab for additional 4 hrs. Cells were evaluated for apoptosis using Annexin V-FITC and Propidium Iodide followed by FACS using BD LSRII. Statistics was conducted by one-way ANOVA followed by Dunnett multiple comparisons test.

Results: There was a significant reduction in apoptosis in the CY treated PBL transfected DLEU1 siRNA vs mock control cells (89.5 nM CY: 10.26+0.23% reduction, p<0.05 to negative control; 895 nM CY, 10.86+0.67% reduction, p<0.01; 8950 nM, 9.85+0.32% reduction, p<0.05. There was a similar significant reduction in rituximab induced apoptosis in the PBL transfected DLEU1 siRNA vs mock control cells (4 microgram/mL Rituximab: 25.45+2.55% reduction, p<0.01 to negative control; 40 microgram/mL Rituximab, 18.31+5.13% reduction, p<0.04; 400 microgram/mL Rituximab, 32.33+1.77% reduction, p<0.02).

Conclusion: DLEU1 siRNA gene knockdown studies resulted in significantly less apoptosis in CY and rituximab treated PBL cells. Deletion of 13q14.3, which contains DLEU1, in PBL may confer a phenotype of drug resistance and suggests a tumor suppressor role of DLEU1.

386 WITHDRAWN

387 ANALYZING THE ROLE OF RELB IN B CELL LYMPHOMA

R. Marienfeld[1], C. Platzer[1], C. Schulz[1]
[1]*Institute of Pathology, Ulm University, Ulm, Germany*

Introduction: NF-kB family member RelB is crucial for the development and function of normal B cells. Functionally, RelB acts as a repressor as well as activator of NF-kB-dependent gene-expression. RelB exerts its negative effect by a) sequestering RelA in inactive complexes and b) epigentic silencing of NF-kB target genes. In contrast, the positive effect of RelB is mainly connected to the alternative NF-kB signalling pathway. This alternative pathway is characterized by a signal-induced processing of p100 and activation of RelB-p52 heterodimers. Importantly, a deregulated alternative NF-kB pathway was observed in several types of B cell lymphoma. For instance, an increased alternative NF-kB pathway has been reported in anaplastic large cell lymphoma cells as well as in H-RS cells. However, the exact role of RelB in B cell lymphoma biology remains largely unclear.

Materials and methods: Cell lines: Karpas 1106, MedB1, L450, L428, L1236, SupHD1, S107. Immunoblot analysis, EMSA, qPCR, siRNA knock down, luciferase-reporter gene-assays.

Results: To characterize the function of RelB in B cell lymphoma, we first determined the activity of nuclear RelB in a panel of different B cell lymphoma cell lines (both Hodgkin and non-Hodgkin cell lines). Overall expression of RelB was similar in all cell lines analyzed. However, the nuclear localization was distinct with highest levels of nuclear RelB observed in L1236, SupHD1 and Karpas 1106. To identify RelB-target genes in B cell lymoma cells, we generated a S107 murine plasmacytoma cell line stably overexpressing RelB as a model system. Analysis of a panel of apoptosis-regulating genes revealed a group of genes down regulated by RelB as well as several genes induced by RelB. Whereas anti-apoptotic genes like Bax and Birc3 were only slightly induced, the expression Traf1 was highly augmented. In contrast, expression of TRAF-family members Traf2, Traf4 and Traf5 was attenuated in S107 RelB cells.

Conclusions: The members of the TRAF family play a crucial role in the signal transduction pathways initiated upon engagement of surface receptors of the TNF receptor superfamily like TNFRI, CD40 or CD30. Enhanced TRAF1 expression is known to modulate the signalling through these receptors by affecting the formation of TRAF-trimers. Thus, RelB –and therefore the alternative NF-kB signalling pathway- might attenuate signalling by these receptors by enhancing TRAF1 expression and suppressing TRAF2 expression. Experiments are under way to determine the activation of JNK and MAPK pathways in S107 and S107 RelB cells as well as after siRNA-mediated suppression of RelB in Hodgkin and non-Hodgkin B lymphoma cell lines.

Downloaded from http://annonc.oxfordjournals.org/ by guest on May 21, 2012

388 MONOCYTIC-MYELOID DERIVED SUPPRESSOR CELLS ARE INCREASED IN THE PERIPHERAL BLOOD IN DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) BUT NOT IN INDOLENT B –CELL LYMPHOMAS OR CLL

R. Fell[1], D. Attias[1], A. Polliack[2], T. Tadmor[1]
[1]*Hematology, Bnai-Zion Medical Center, Haifa, Israel,* [2]*Hematology Department, Hadassah University Hospital, Jerusalem, Israel*

Background: Different tumors have evolved a variety of mechanisms to generate a suppressive network to evade the host immune response and counteract the anti-tumor effect of cytotoxic T cells thereby promoting tumor dependent angiogenesis, tumor invasion and metastasis. Myeloid derived suppressor cells (MDSCs) are an heterogeneous population of immature myeloid cells, macrophages, granulocytes, and other cells at different stages of maturation, found both in mice and humans which express both Gr-1^+ and CD 11b+. MDSC suppress immune responses both in vitro and in vivo and can be subdivided into 2 sub-populations: granulocytic and monocytic. Recently it has been demonstrated that M-MDSCs possess stronger suppression activity.

Materials and Methods: Flowcytometry (FC) analysis was performed in order to quantitate immune suppressive MDSCs in the peripheral blood (PB) from 12 patients with DLBCL, 10 with low grade lymphoma, 10 with CLL and 12 healthy volunteers. Surface staining for $CD14^+$ HLA $DR^{-/Low}$ was used to define this population of MDSCs. In the patients with DLBCL, analyses were performed using two time points: before, and at the end of chemotherapy. Statistical analyses were done using the two-tailed Student t-test, and P< 0.01 was kept as a threshold for the level of significance.

Results: Patients with DLBCL had significantly more MDSCs in their peripheral blood (mean 9.07%±3.91) than patients with low grade lymphoma (mean 6.0 %±1), healthy volunteers (mean 5.5%±1.4) (P<0.01), and patients with chronic lymphocytic leukemia (CLL) who unexpectedly had a lower proportion of MDSCs (mean 0.94 %±0.8)(p<0.001). Levels of MDSCs returned to within normal limits in patients with DLBCL after the completion of chemotherapy (5.7%±1).

Conclusions: These preliminary but consistent results in a relatively small cohort of patients illustrate that at the time of initial diagnosis patients with DLBCL have significantly higher numbers of MDSCs in their peripheral blood than those with indolent lymphoma and CLL and normal individuals. Successful treatment of the lymphoma was accompanied by a decrease in the numbers of MDSCs to within the normal range. A larger study is ongoing in our institute to validate these results, to determine the functional Significance of these findings in DLBCL, to establish whether the numbers of MDSCs present in the peripheral blood can be used as a marker of disease activity in DLBCL and to determine their more exact role in tumor surveillance.

389 REGULATORY T-CELLS IN GASTRIC MALT LYMPHOMA

M. Garcia[1], B. Bellosillo[1], B. Sánchez-González[2], A. Seoane[3], A. Torné[1], L. Martinez[1], A. Ferrer[2], E. Gimeno[2], F. Sole[1], C. Besses[2], S. Serrano[1], A. Salar[2]
[1]*Pathology, Hospital del Mar, Barcelona, Spain,* [2]*Hematology, Hospital del Mar, Barcelona, Spain,* [3]*Gastroenterology, Hospital del Mar, Barcelona, Spain*

Background: Gastric MALT lymphoma (gMALT) is an indolent lymphoma acquired in the course of *H pylori* infection. In this immune setting T Reg cells (FOXP3+) may play a role in the disease as a component of tumor microenvironment. The aim of this study was to analyze by immunohistochemistry the presence of these cells in gMALT at the time of diagnosis and during follow-up, and to evaluate their clinical significance in outcome as well as the effect of the treatment in this cell type population.

Methods: Thirty-three patients with gMALT were included. Sections were immunostained for CD20 and FOXP3. The number of CD20+ tumor cells and FOXP3+ infiltrating cells was quantified using a micrometric ocular.

Results: The median age was 63 y (range 32-83) with 52% being male. Stage: I in 66%, II in 25% and IV in 9%; B-symptoms in 6%. At diagnosis, the mean (± standard deviation) number of CD20+ tumor cells and FOXP3+ infiltrating cells was 680±232 and 30±29 cells/cm^2, respectively.

Number of treatments analyzed was 37 (treatment not available in 2 pts and 5 pts received more than 1 treatment). Treatment regimens included: eradication therapy (n=11); interferon+ribavirin (n=1); single or combined agent chemotherapy without rituximab (n=5); rituximab alone or CHOP-like with rituximab (n=4); fludarabine or bendamustine with or without rituximab (n=16).

The first response evaluation showed an overall clinical response rate of 84% (CR 76%).

Overall, the mean (±SD) CD20+ tumor cells and FOXP3+ infiltrating cells was significantly reduced after treatment (730±208 vs 285±343, p<0.0001; 34±41 vs 21±36, p=0.016, respectively). The mean number of CD20+ tumor cells and FOXP3+ infiltrating cells decreased in responders (722 vs 210, p<0.001 and 37 vs 24, p=0.063, respectively) but not in non responders (p=0.279 and p=0.249). Treatment with fludarabine or bendamustine with or without rituximab induced a quick and profound depletion in the number of CD20+ cells in comparison with the other treatments (8-fold vs 2-fold reduction), but FOXP3+ cells remained relatively unchanged (1.2-fold vs 2-fold reduction). During a median of 18 months of follow-up, CD20+ cells remain undetectable in patients treated with fludarabine or bendamustine with or without rituximab, whereas numbers of FOXP3+ cells are stable regardless of treatment regimen.

Conclusion: Regimens with fludarabine or bendamustine with or without rituximab induce a quick and deep reduction of CD20+ tumor cells and infiltrating FOXP3+ cells. This effect is maintained during follow-up.

T Reg cells might play some role in the development and sustainment of gMALT, but because of the good behaviour of the disease, we have not been able to find T Reg cells as a suitable prognostic marker.

390 HIGH-RESOLUTION COPY-NUMBER ANALYSIS REINFORCES THE EXISTENCE OF A SPECIFIC MOLECULAR SIGNATURE IN PRIMARY CNS LYMPHOMAS (PCNSL)

E. Braggio[1], R. Robetorye[1], W. R. Macon[2], M. B. Lopes[3], D. Schiff[3], B. P. O'Neill[4], R. Fonseca[1]
[1]*Cancer Center, Mayo Clinic, Scottsdale, United States,* [2]*Pathology, Mayo Clinic, Rochester, United States,* [3]*Medical Center, UVA, Charlottesville, United States,* [4]*Neuro oncology, Mayo Clinic, Rochester, United States*

Introduction: Unlike nodal DLBCL, histogenetic origin and molecular pathogenesis of PCNSL are poorly understood. It is still a matter of debate if there is a genomic signature specific of PCNSL.

Materials and Methods: 17 EBV and HIV negative and immunocompetent PCNSL were studied by array-based comparative genomic hybridization (aCGH) with an average resolution of 3Kb. B-cell differentiation status was characterized for CD10, MUM-1, and BCL-6 immunostains. aCGH profile was compared with 59 nodal DLBCL.

Results: All 7 GCB and 10 non-GCB PCNSL analyzed have a complex karyotype with a median of 21 copy-number abnormalities (CNA) per case (range 10–49). Overall, 18 regions (10 losses and 8 gains) were affected in >20% of patients. Focal deletion of 9p21 (*CDKN2A*) was the most common CNA, found in 14 of 17 cases (82%). Other common CNAs were deletion of 6q23.3 (*TNFAIP3;* 59%), 6p21 (*HLA* genes; 53%), 6q21 (*PRDM1;* 47%) and gain of 12q21-q24 (53%), 7q21-q31 (35%) and 19q13 (35%). Five novel recurrent focal CNAs were found in PCNSL; loss of 3p21.3, 3q26.3 (*TBL1XR1*), 10p14–p15, 12q24.3 (*BCL7A*) and 16q12–q21 (29% of cases each). All the novel CNAs, excluding –16q12-q21, were only found in less than 5% or absent in nodal DLBCL, thus suggesting to be unique to PCNSL. Moreover, –3p21.3 and –10p14-p15 were mutually exclusive with +12q21-q24 (*p=0.0004*). At the gene level, recurrent homozygous deletions were found in *CDKN2A*, *TMEM30A*, *CD58* and *TOX*, the latter two involved in T-cell development and activation. *CD58* was also recurrently affected by monoallelic losses from 15 nt to 1-2 exons as was confirmed by DNA resequencing. Another 34 genes, including *B2M, ETV6*, HLA genes, *PRDM1, TNFRSF10A* and *TNFRSF10B* were also homozygously deleted. Pathway analysis shows an enrichment of genes associated with immune response, apoptosis and lymphocyte differentiation and proliferation.

Conclusions: We showed evidence of a highly complex genome and identified a subset of genes with potential relevance in PCNSL pathogenesis. The genomic profile described here reinforces the existence of a specific molecular signature in PCNSL, thus helping to genetically differentiate this entity from the nodal DLBCL and related lymphomas.

391 WITHDRAWN

392 SPECULATE: SYNCHROTRON TECHNOLOGY IN CHRONIC LYMPHOCYTIC LEUKEMIA

M. Bosch[1], L. Bolster[1], A. Saxena[2]
[1]*Medicine, University of Saskatchewan, Saskatoon, Canada,* [2]*Pathology, University of Saskatchewan, Saskatoon, Canada*

Background: Chronic lymphocytic leukemia (CLL) is the most prevalent form of leukemia. It is a diverse disease with prognosis ranging from a survival of two to eleven years despite modern therapy. Single genetic abnormalities do not always correlate well with disease outcomes. By the addition of synchrotron radiation to vibration spectroscopy, tissue may be analyzed at cellular and even sub cellular resolutions. By collecting these synchrotron infrared microscopy spectra or a "synchrotron fingerprint" and relating it to nuclear activity, it may be possible to predict more accurate clinical outcomes.

Methods: Blood samples were collected from 27 CLL patients at hematology clinics in Saskatoon, Saskatchewan and from 10 normal volunteers. Thirteen CLL samples were mounted on low e- microscope slides (Kevley Technologies, USA). Subsequently 14 CLL and ten normal samples were then processed via Histopaque-1077 (Sigma-Aldrich, USA) extraction and then mounted on low e slides. Comparison between the synchrotron fingerprint of normal lymphocytes and lymphocytes of CLL patients were carried out with the mid Infrared (midIR) beamline at the Canadian Light Source, Saskatchewan. From each sample 14 cells were scanned and their spectrum's averaged. Spectral analysis was performed using The Unscrambler® X Software. Charts from all CLL patients were reviewed to obtain their flow cytometry, cytogenetic characteristics and clinical course.

Downloaded from http://annonc.oxfordjournals.org/ by guest on May 21, 2012

Results: The Mid-IR bands in the DNA regions (900-1300 cm-1) and the protein bands (1300-1700 cm-1) did not reveal any significant differences in cytogenetics, flow or lymphocyte count in the samples without histiopaque processing. Cluster analysis of the histiopaque slides in the protein regions (1300-1700 cm-1) had a tendency to cluster into 2 groups. One predominately CLL (10/13) and the other predominately normals (6/10). By looking at the DNA band region (900-1300 cm-1) via cluster analysis two groups emerged. The average of lymphocyte counts was 10x10^9/L in group 1 and 41x10^9/L in group 2 (P-value 0.0236).

Discussion: While current technology for predicting disease severity and clinical outcomes provides information for some CLL patients, many CLL patients have no known prognostic markers, making their clinical course unpredictable. The clustering analysis of the difference in lymphocyte count is promising and would be aided with a future look at cytogenetic profiles. By identifying patients with high levels of nuclear activity through infrared spectroscopy, it may be possible to predict severe disease and prognosis in patients with CLL.

393 DIFFERENT PROTEIN EXPRESSION PATTERNS MAY INDICATE DIFFERENT DISEASE MECHANISMS IN OLDER AND YOUNGER CHL PATIENTS

M. Ludvigsen[1], P. Kamper[2], S. J. Hamilton-Dutroit[3], K. Bendix[3], F. A. D'Amore[2], B. Honoré[1]
[1]Department of Medical Biochemistry, University of Aarhus, Aarhus, Denmark,
[2]Department of Haematology, Aarhus University Hospital, Aarhus, Denmark,
[3]Institute of Pathology, Aarhus University Hospital, Aarhus, Denmark

Introduction: In western countries, the age distribution of Hodgkin lymphoma (HL) follows a characteristic bimodal curve showing the first peak at 15-20 years and a second peak in late life, 70-80 years. The mortality curve does not show the same bimodal pattern but increases with age. Is this observation just a function of age or does it also relates to different underlying biological processes for classical HL (cHL). HL shows heterogeneity in tumor cell number and morphology. A variable, but usually only minor proportion of cHL tumor tissue is composed of neoplastic Hodgkin/Reed-Stenberg cells, the surrounding cellular microenvironment are presumed to be important components in the development and course of the disease. Analysis using proteomic techniques may shed new light on the role of tumor and microenvironmental cells in key steps in HL-oncogenesis. Our aim is to provide hypothesis-generating data by comparing protein expression patterns in tumors from younger (≤60yrs) and older (>60yrs) cHL patients in order to identify possible age-related differences in the biology of cHL.

Methods: Frozen tissue samples from 10 patients with stage I or II cHL were identified in the pathology archives and clinical data were obtained from the Danish Lymphoma Group database. Tissues were studied from 5 younger and 5 older patients. Tissues were subjected to high-resolution two-dimensional gel electrophoresis. Individual protein spots were visualized with silver staining and expression profiles in the younger and older groups were compared by computer analysis. Proteins with two fold or more differential expression between the two clinical groups were identified by liquid chromatography-tandem mass spectrometry and further studied by immunological methods.

Results: This study confirms the feasibility of using archival frozen tissues from cHL patients for proteomic analysis. The protein expression profiles of the two clinical groups analyzed showed significant and distinct differences. Preliminary results suggest differential expression among proteins belonging to functional families such as the MAPK signalling pathway, cell cycle regulation and cancer proto-oncogenes.

Conclusions: Proteomic analysis found significant differences in the protein composition of cHL tumor tissues from younger compared with older patients. Differential expression of a number of these proteins may provide further insights into the age-specific pathophysiology of cHL.

Downloaded from http://annonc.oxfordjournals.org/ by guest on May 21, 2012