''focus on...'' session: profiling and prognosis

033 DIFFERENT SUBTYPES OF AGGRESSIVE B CELL LYMPHOMAS SHARE AN EPIGENETIC SIGNATURE ENRICHED FOR POLYCOMB TARGETS IN STEM CELLS

J.I. Martin-Subero[1], M. Kreuz[2], M. Bibikova [3], S. Bentink [4], W. Klapper[5], E. Wickham-Garcia[3], M. Rosolowski [2], J. Richter[1], L. Lopez-Serra[6], O. Ammerpohl[1], E. Ballestar[6], H. Berger [2], J.B. Fan [3], M. Loeffler[2], L. Trümper[7], H. Stein [8], R. Spang[4], M. Esteller[6], D. Barker[3], D. Hasenclever[2], R. Siebert[1]

[1]Institute of Human Genetics, Christian-Albrechts University, Kiel, Germany, [2]Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany, [3]Illumina Inc, San Diego, United States, [4]Institute of Functional Genomics, University of Regensburg, Regensburg, Germany, [5]Institute of Pathology, Christian-Albrechts University, Kiel, Germany, [6]Cancer Epigenetics Laboratory, Spanish National Cancer Centre, Madrid, Spain, [7]Department of Hematology and Oncology, Georg-August University of Göttingen, Göttingen, Germany, [8]Institute of Pathology, Charité–Universitätsmedizin Berlin, Berlin, Germany

Introduction: Although gene inactivation by DNA methylation is well-known in lymphomas, its association with different lymphoma subtypes defined by morphological, genetic and transcriptional features is currently unknown.

Material and methods: Microarray-based DNA methylation analyses of 807 cancer-ralated genes were performed in 83 aggressive B cell lymphomas characterized by transcriptional and genetic profiling, 7 B cell lymphoma cell lines and 10 non-malignant hematopoietic tissues. Immunohistochemistry for DNMT and MBD proteins was performed in selected cases.

Results: DNA methylation profiles were not strictly associated with any morphologic, genetic or transcriptional features. By supervised analyses, we identified genes de novo methylated in all lymphoma subtypes (n=56) or in a subtype-specific manner (n=22). Genes de novo methylated across different lymphoma subtypes were highly enriched for targets of the polycomb repressor complex in stem cells (OR=8.2) and for biological processes deregulated in different cancers. Furthermore, these genes were expressed at low levels in normal hematopoietic tissues. Initial expression analyses suggest differences in DNMT and MBD levels between lymphoma entities.

Conclusions: These findings, especially the high enrichment for polycomb targets in stem cells, suggest that different aggressive B cell lymphomas might derive from precursor cells with stem cell-like features.

034 GENE EXPRESSION SIGNATURES PREDICT SURVIVAL IN DIFFUSE LARGE B CELL LYMPHOMA FOLLOWING RITUXIMAB AND CHOP-LIKE CHEMOTHERAPY

G. Lenz[1], G. Wright[2], S. Dave[2], A. Kohlmann[3], W. Xiao[2], J. Powell[2], H. Zhao[2], W. Xu[2], R.D. Gascoyne[2], J.M. Connors[2], L. May[2], J. Iqbal[2], J. Vose[2], D. Weisenburger[2], T. Greiner[2], J.O. Armitage[2], M. Bast[2], K. Fu[2], E. Campo[2], E. Montserrat[2], A. Lopez-Guillermo[2], P. Jares[2], A. Martinez[2], L.M. Rimsza[2], R.I. Fisher[2], R.M. Braziel[2], R. Tubbs[2], T. Miller[2], J. Cook[2], B. Pohlman[2], J. Sweetenham[2], G. Troen[2], E.B. Smeland[2], J. Delabie[2], S. Kvaloy[2], H. Holte[2], D. Dierickx[2], G. Verhoef[2], E.S. Jaffe[2], W.H. Wilson[2], E. Hartmann[2], A. Rosenwald[2], G. Ott[2], H.K. Muller-Hermelink[2], D. Wrench[2], T.A. Lister[2], M. Williams[3], L. Wieczorek[3], W.C. Chan[2], L.M. Staudt[1]

[1]CCR, NCI, Bethesda, United States, On Behalf of LLMPP, [2]LLMPP, [3]Molecular Systems, Roche, Pleasanton, United States

Introduction: Gene expression profiling (GEP) has been used to distinguish germinal center B cell-like (GCB) and activated B cell-like (ABC) DLBCL. Prognostic gene expression signatures include the lymph node, germinal center, and proliferation signature. R-CHOP has significantly improved outcome of DLBCL patients. Conflicting reports of immunohistochemistry based studies, if gene expression signatures remain relevant in R-CHOP treated patients, have recently been published. To investigate, if gene expression signatures remain predictive following R-CHOP, we performed GEP on 176 DLBCL biopsies using Affymetrix U133 arrays.

Methods: Samples were classified as GCB or ABC DLBCL and assessed for expression of molecular signatures. A Cox-proportional hazards model was used to determine the association of gene expression features with PFS/OS.

Results: 78 samples were classified as GCB DLBCL, 76 as ABC DLBCL, and 22 were unclassified. R-CHOP improved OS for both GCB and ABC DLBCL compared to historical controls. GCB DLBCL had a more favorable PFS and OS than ABC DLBCL (5-year PFS 72% vs. 43%; p=0.0001; 5-year OS 80% vs. 56%; p=0.04). The lymph node and germinal center signatures were associated with favorable PFS/OS and the proliferation signature with inferior PFS/OS.

Conclusions: In summary, the prognostic value of the lymph node, germinal center and proliferation signatures was maintained in the context of R-CHOP. An understanding of the biological attributes of DLBCL tumors reflected in these signatures is critical to improve OS of these patients.

035 GENE EXPRESSION PROFILING OF PERIPHERAL T-CELL LYMPHOMA INCLUDING GAMMA/DELTA T-CELL LYMPHOMA

K. Miyazaki[1], M. Yamaguchi[1], H. Imai[2], T. Kobayashi[1], S. Tamaru[1], K. Nishii[1], N. Katayama[1]

[1]Department of Hematology and Oncology, Mie University, Tsu, Japan, [2]Department of Pathologic Oncology, Mie University, Tsu, Japan

Background: Gamma/delta T cells are different from alpha/beta T cells in terms of development, tissue distribution, and function. Peripheral T-cell lymphoma (PTCL) with gamma/delta T-cell immunophenotype is extremely rare and presents unique clinicopathologic features. Several studies have elucidated the gene expression profile of PTCLs; however, GDTL has not been included in previous studies.

Materials and Methods: To clarify the difference between alpha/beta T-cell lymphoma (ABTL) and gamma/delta T-cell lymphoma (GDTL) in the gene expression profile, total RNA from 32 patients with PTCL including 10 PTCL-U with alpha/beta T-cell immunophenotype, 14 AILT, 1 hepatosplenic ABTL, 4 hepatosplenic GDTL, 1 cutaneous GDTL, 1 intestinal GDTL, and 1 thyroidal GDTL, was examined using Agilent 44K human oligo-microarrays. The expression of T-cell receptor heterodimer (alpha/beta or gamma/delta) in tumor cells was confirmed by means of immunohistochemistry using frozen sections. The clinicopathologic features of 6 out of 7 cases of GDTL have been reported previously (Yamaguchi M, et al., Int J Hematol 1999).

Results: Unsupervised analysis in the 32 PTCL cases classified hepatosplenic GDTL in a single cluster, whereasother GDTLs were scattered within ABTL. A classifier based on gene expression at supervised analysis correctly identified GDTL. Signature genes to distinguish between ABTL and GDTL were as follows: *LDHAL6B*, *KLRC4*, *FZD5*, *GPR37*, *CXCL12*, *CD16b*, *CD16a*, *BMP7*, *IFT2*, etc., were overexpressed in GDTL, and *POU5F1*, *OSMR*, *TNFRSF25*, *COL4A4*, *ADAM19*, *CCL19*, *RASGRP3*, etc., were overexpressed in ABTL. Of note, hepatosplenic ABTL could be discriminated among hepatosplenic GDTL. Enriched Gene Ontology categories in GDTL were defense response, response to biotic stimulus, response to stimulus, and immune response.

Conclusions: Our results suggest that hepatosplenic GDTL is distinct in its gene expression profile from ABTL and nonhepatosplenic GDTL. Nonhepatosplenic GDTL seems to be heterogeneous in their gene expression profile. Our study provides useful information to identify novel therapeutic targets and facilitate the diagnosis of GDTL.

036 ARRAY-CGH MAPPING OF AN OVERREPRESENTED 7Q REGION IN γ/δ HEPATOSPLENIC T-CELL LYMPHOMA

I. Wlodarska[1], H. Urbankova[1], P. Van Loo[2], S. Shetty[3], B. Roland[4], L. Krenacs[5], P. De Paepe[6], J. Billiet[6], M. Jarosova[7], P. Vandenberghe[1], C. De Wolf-Peeters[8]

[1]Dept of Human Genetics, Catholic University of Leuven, Leuven, Belgium, [2]Dept of Human Genetics; Flanders Interuniversity Inst. for Biotechnology; Dept. of Electrical Engineering, Catholic University of Leuven, Leuven, Belgium, [3]Dept of Medical Genetics, University of Calgary, Calgary, Canada, [4]Dept of Medical Genetics; Dept of Pathology and Laboratory Medicine, University of Calgary, Calgary, Canada, [5]Institute of Biotechnology, Bay Zoltan Foundation for applied Research, Szeged, Hungary, [6]Dept of Pathology, AZ St Jan AV, Brugge, Belgium, [7]Dept of Hemato-Oncology, Palacky University, Olomouc, Czech Republic, [8]Dept of Pathology, Catholic University of Leuven, Leuven, Belgium

Hepatosplenic T-cell lymphoma (HSTCL) is a rare, aggressive disease associated with a poor prognosis. Cytogenetically, this lymphoma is hallmarked by isochromosome 7q [i(7)(q10)] resulting in monosomy 7p and trisomy 7q. Molecular significance of this aberration remains largely unknown. Recently, we identified 3 cases of HSTCL with a ring chromosome 7 [r(7)] harbouring 3-5 additional copies of 7q31, as shown by FISH. To map the overrepresented 7q region and define the smallest commonly gained region (SCGR), we performed high resolution chromosome 7 array-CGH studies of 3 cases with r(7), 2 cases with i(7)(q10) and 1 case with i(7)(q10) and add(7)(q32). The analysis of first 2 cases showed the entire loss of 7p and duplication of the whole 7q, as expected. The third case revealed loss of 7p and a duplication of 7q11q31. All 3 cases with r(7) displayed a partial deletion of the telomeric region of 7p, duplication of 7q11q33 and an additional gain of the 7q21q33 region. The size of the latter region and amplification level of the targeted sequences differed from case to case. The mapped SCGR covered approximately 24 Mb at 7q21.1q31.1. FISH with the selected BACs showed presence of 3-8 additional signals in cases with r(7). In summary, high

resolution aCGH performed on 6 HSTCL cases (including 3 with r(7)) mapped the SCGR at 7q21.1q31.1. We hypothesize that this region harbours genes that play a critical role in pathogenesis of HSTCL. Transcriptomic analysis of the present cases aimed at identification of the overexpressed genes from the SCGR is ongoing.

037 GENE EXPRESSION PROFILING REVEALS DISTINCT MOLECULAR SIGNATURES FOR NASAL NK/T-CELL LYMPHOMAS AND HEPATOSPLENIC T-CELL LYMPHOMAS

Y. Huang[1], A. de Reynies[2], L. de Leval[3], D. Rickman[2], C. Gisselbrecht[4], J. Bosq[5], A. Lavergne[5], E. Macintyre[5], N. Brousse[5], J. Marquet[1], C. Schmitt[1], F. Berger[5], P. Gaulard[1]

[1]Pathology and Inserm U841, CHU Henri Mondor, Creteil, France, [2]Biostatistics, Ligue Nationale contre le Cancer, Paris, France, [3]Pathology, CHU Sart-Tilman, Liege, Belgium, [4]Hematology, GELA, Paris, France, [5]Pathology, GELA, Paris, France

Background: Nasal NK-/T-cell lymphomas (NK/T), hepatosplenic T-cell lymphomas (HSTL) and enteropathy-type T-cell lymphomas (ETL) are separate extranodal entities sharing a derivation from cytotoxic T or NK cell subsets. Their genetic alterations and molecular signatures are currently unknown.

Material and methods: With the aim to identify the molecular signature of each entity, RNAs of 22 tumor samples (NK/T: 7; HSTL: 9 including 3 sorted tumor cell suspensions; ETL: 4) and 2 NK/T tumor-derived cell lines were hybridized on Affymetrix HG-U133A Plus2.0 microarray, together with RNAs from normal NK and gammadelta T cells and from 16 cases of peripheral T-cell lymphoma unspecified (PTCL-U).

Results: Unsupervised analysis revealed 3 distinct clusters representative of each entity, in accordance with the pathological diagnoses. The molecular signature of each entity was defined by comparison to PTCL-U. NK/T was characterized by overexpression of genes encoding NK cell receptors (CD56, CD244, KIRs), cytotoxic molecules (Granzymes B, H, perforin), chemokines (CCL5, CCL4) and apoptosis-associated molecules such as Fas ligand. Apoptosis and Jak/Stat pathways were among the most differentially expressed pathways and phosphorylated Stat3 (Tyr705) was constitutively expressed in NK/T by immunohistochemistry. On the other hand, HSTL had overexpression of genes encoding cell to cell interaction molecules (integrins, VCAM1), NK cell receptors (KIRs), chemokines and PDGFR alpha. As expected, genes encoding cytotoxic functions were underexpressed in HSTL. VEGF, MAPK, Wnt signalling pathways were among the most overrepresented pathways in HSTL.

Conclusion: The current study (1) confirms that the molecular signature of each entity correlates with current WHO classification; (2) verifies the cell origins of NK/T (activated cytotoxic NK cells) and HSTL (non-activated cytotoxic T cells), and (3) suggests distinct signalling pathways implicated in the pathogenesis of these cytotoxic lymphomas.

038 GENOME-WIDE PROFILING OF FOLLICULAR LYMPHOMA (FL) BY ARRAY COMPARATIVE GENOMIC HYBRIDIZATION (ACGH) REVEALS PROGNOSTICALLY SIGNIFICANT DNA COPY NUMBER IMBALANCES

K. Cheung[1], S.P. Shah[1], C. Steidl[1], N. Johnson[1], T. Relander[1], A. Telenius[1], B. Lai[1], H. Qian[1], K. Murphy[1], W. Lam[1], M. Marra[1], J.M. Connors[1], R. Ng[1], R.D. Gascoyne[1], D.E. Horsman[1]

[1]Center for Lymphoid Cancers, British Columbia Cancer Agency, Vancouver, Canada

Background: The initial genetic event in ~85% of FL is the t(14;18)(q32;q21) translocation resulting in over-expression of the anti-apoptotic protein BCL-2.The secondary events associated with disease progression however are not well defined. To this end, we have generated a genome-wide profile of regional imbalances and identified significant prognostic correlates in relation to both overall survival (OS) and transformation risk (TR).

Materials and Methods: High resolution whole genome BAC aCGH was applied to 107 diagnostic FL specimens to characterize the recurrent regions of copy number change. An analytical approach that defined regions of copy number change as intersections between visual analysis and a Hidden Markov model-based computational approach was utilized to reduce false positive annotations.

Results: 68 distinct regional alterations recurrent in ≥10% of cases were found. These regions ranged in size from 200 kb to 44 Mb affecting chromosomes 1, 2, 5, 6, 7, 8, 10, 12, 17, 18, 19, and 22. Validation of this global profile was undertaken in an independent cohort of 37 FL cases. Cluster analysis showed that 44% of the 107 cases could be classified into subgroups determined by the presence of +1q, +6p/6q-, +7 or +18, providing support to a previous description of 4 secondary genetic pathways in FL development based on karyotype data. Survival analysis showed that 13 of the 68 regions correlated significantly with inferior OS ($p<0.05$). Of these 13 regions, 8 were identified to be significant independent predictors of OS using a multivariate Cox model that included the International Prognostic Index score. Two of these 8 regions (1p36.22-p36.33 and 6q21-24.3) were also predictors of TR and were validated by FISH.

Conclusions: Our study provides 1) a comprehensive copy number profile of 107 diagnostic FL cases by aCGH, 2) further insight into distinct genetic pathways related to FL development by cluster analysis, and 3) evidence of significant prognostic predictors that may ultimately be utilized for identifying high-risk patients as candidates for targeted risk-adapted therapies.