lymphoma biology

273 EXONIC MUTATIONS ARE COMMONLY FOUND IN PATIENTS WITH WALDENSTROM'S MACROGLOBULINEMIA AND FAMILY MEMBERS OF WM PATIENTS WITH HYPER-IGM SYNDROME

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Introduction: Waldenström's macroglobulinemia (WM) is an indolent B-cell disorder characterized by accumulation of IgM in serum of patients. Familial disease is common with 20% of WM patients demonstrating a first degree relative with WM or a closely related B-cell disorder. Moreover, IgA and IgG hypogammaglobulinemia are commonly encountered, and persist despite therapy suggesting that common variable immunodeficiency disorder (CVID) may be a predisposition to WM. Consistent with this, we have observed significantly higher IgM levels, decreased IgA levels, as well a 10-fold increased incidence of predominately IgM monoclonal gammopathy in family members of patients with familial but not sporadic WM. Given these findings, we sought to determine if patients with WM harbored CD40 mutations akin to those reported in patients with Hyper-IgM Syndrome.

Methods: we performed cloning and sequencingin of CD40 gene from bone marrow (BM) $CD19^{+}$cells from WM patients, family members of WM patients with polyclonal hyper-IgM, as well as healthy donors.

Results: in WM patients we detected at least one novel recurring mutation which predicted for amino acid changes. These mutations—3 missense (*on exon 3,5)*; and one frame-shift deletion leading to protein truncation (*on exon 5)*—occurring in WM cells taken from 5/14 (36%) WM patients. Overall mutated allele frequencies for the 3 missense mutations were 14%(Phe>Ser) at position 3997 and 21%(Phe>Ser) at position 4036 of exon 3, and 7%(Try>Arg) at position 4946 of exon 5, while no mutations were detected in any of the healthy donors (HD). Importantly, we detected the C>t missense mutation at position 4036 of exon 3 in individuals with polyclonal hyper-IgM who belonged to a family with familial WM.

Conclusions: Mutations in Exons 3 and 5 of CD40 are commonly found in patients with WM, as well as family members of WM patients with polyclonal hyper-IgM, suggesting that Hyper-IgM Syndrome may be a predisposition for WM.

274 FAMILIAL CHARACTERISTICS OF WALDENSTRÖM'S MACROGLOBULINEMIA (WM) AND LYMPHOPLASMACYTIC LYMPHOMA (LPL): A POPULATION-BASED STUDY IN SWEDEN

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Background: The causes of WM/LPL are largely unknown. A role for genetic factors in the etiology of WM and LPL has been suggested based on prior single center observations indicating evidence of familial aggregation. The aims of this large population-based familial case-control study were to quantify risks of WM, LPL, monoclonal gammopathy of undetermined significance (MGUS) and related lymphoproliferative malignancies (LP) among first-degree relatives of WM/LPL patients and to define characteristics of familial aggregation.

Methods: We identified 2,671 WM/LPL patients diagnosed in Swedish hospitals 1958-2005 (Cancer and local hospital-based registries), with linkable relatives; 8,279 frequency-matched controls (Population registry); and first-degree relatives of cases (n=6,172) and controls (n=24,612) (Multigenerational registry). Relatives of WM/LPL patients and controls were linked with the Cancer and local hospital-based registries to define occurrence of MGUS, WM/LPL and related LP tumors. Using a marginal survival model, we calculated relative risks (RR) and 95% confidence intervals (CI) as measures of familial aggregation.

Results: First-degree relatives of WM/LPL patients had a significantly increased risk for developing WM/LPL [RR=20.0; (95% CI, 4.1-98.4)], chronic lymphocytic leukemia [3.4; (1.7-6.6)], non-Hodgkin lymphoma [3.0; (2.0-4.4)], and MGUS [5.0; (1.3-18.9)]. We observed no increased risk of developing multiple myeloma or Hodgkin lymphoma. The risk estimates were virtually the same when we conducted analyses by gender of proband, by type of first-degree relative (parent, sibling, offspring), and by age at WM/LPL diagnosis (below/above 70 yrs) for probands.

Conclusions: In this first large population-based study we found over 3-fold increased risks of developing WM/LPL and associated LP disorders (CLL, NHL, and MGUS) among relatives of WM/LPL patients. These novel results support the hypothesis that there are common, strong, shared susceptibility genes that predispose to WM/LPL and related LP malignancies.

275 DETECTION OF EPSTEIN-BARR VIRUS INTEGRATION LOCI IN BURKITT'S LYMPHOMA CELL LINES

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Introduction: Epstein-Barr virus (EBV) is an oncogenic virus found in about 95% of the endemic Burkitt's lymphomas (BL). In latently infected cells, the EBV DNA can be maintained in episomal form, but integrated EBV could also be present. We herein report the identification of the EBV integration loci in the BL cell lines Seraphina and Naliaka using long distance-PCR (LD-PCR) and fluorescence in situ hybridization (FISH).

Material and Methods: For the synthesis of an EBV-DNA probe for FISH experiments, DNA of the EBV-positive BL cell line Raji was isolated and LD-PCR with specific primers for EBV was performed. The 3,000 bp fragment was further labelled with Biotin-16-dUTG by nick translation, and FISH was performed according to standard methods. FISH on metaphase spreads of the BL cell line Namalwa was performed to validate the quality of the EBV-FISH probe. Integration of EBV was defined by the presence of symmetrical doublet hybridization signals at the same chromosomal loci in both sister chromatids.

Results: The cell line Namalwa showed symmetrical doublet hybridization signals of our EBV-FISH probe on chromosome 1p35, as it has been shown in previous reports. In the cell line Seraphina, 11 out of 16 analyzed metaphases revealed a unique integration locus of EBV on chromosome 2p23. FISH experiments on 8 metaphase spreads of the cell line Naliaka revealed two integration loci of EBV. In the latter cell line, double hybridizations signals of the EBV-DNA probe were detected on chromosomes 7q11 and 17q25.

Conclusions: Our results identify for the first time chromosomes 2p23, 7q11, and 17q25 as the integration loci of EBV in the BL cell lines Naliaka and Seraphina. Whether the integration of EBV in these loci affects the expression of genes important for the pathogenesis of Burkitt's lymphomas remains to be determined.

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276 HUMAN HERPESVIRUS 8 K1-DERIVED PEPTIDES DISRUPT THE INHIBITORY FAS-K1 COMPLEX AND RESTORE FAS RECEPTOR-MEDIATED APOPTOSIS

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Background: Human herpesvirus 8 (HHV-8) infection is associated with the development of primary effusion lymphoma, Kaposi's sarcoma, and multicentric Castleman's disease. The *K1* gene of HHV-8 is expressed in tumor cells as a transmembrane protein with an immunoglobulin-like domain in its ectodomain and an immunoreceptor tyrosine-based activation motif (ITAM). We demonstrated that K1 protein activates nuclear factor-kappa B (NF-kB), and K1 expression in transgenic mice stimulated accumulation of lymphatic cells and development of lymphoma. How K1 blocks apoptosis and induces hyperplasia and lymphomas is not known. We hypothesized that K1 contributes to lymphoma development partly by suppressing apoptosis, and that this suppression combined with its NF-kB activation produces lymphoma.

Results: We found that K1 binds to Fas and in turn, inhibits Fas-mediated apoptosis. We mapped the region that K1 uses to bind to Fas as an immunoglobulin (Ig) chain-like domain by expressing deletion mutants of K1. Overexpression of an Ig domain-containing protein CD79b competed with K1-Fas binding in a dose-dependent manner. Two 20-amino acid peptides (N251, N253) representing the Ig domain of K1 competed with K1-Fas binding in immunoprecipitation/immunoblotting analysis. The N251 and N253 peptides (100 mM) enhanced anti-Fas antibody (CH-11, 50 ng/mL)-induced apoptosis of BJAB lymphoma cells that expressed K1 but not that of vector-transfected BJAB cells. Ig-deleted K1 (K1dIg)-transfected mice were not protected (0/6), and K1-transfected mice were protected (7/10, $P < 0.01$) against the lethal effects of agonistic anti-Fas (Jo2) antibody. K1dIg expressed in mice did not form complexes with Fas, suggesting that the Ig domain is essential for K1-Fas binding and suppression of apoptosis.

Conclusion: Collectively, these results indicate that K1 potently blocks apoptosis, and that this effect is mediated through the Ig-like domain of K1. Because viral proteins mimic cellular proteins, these results predict the presence of functional cellular homologs of K1 that have key roles in death receptor regulation.

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277 HIERARCHY OF HUMAN B CELL REGENERATION AFTER RITUXIMAB THERAPY

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Introduction: Constant immunoglobulin levels throughout life-time may be maintained by either long lived plasma cells or continuous formation of immunoglobulin secreting cells (ISC). Rituximab therapy targets CD20+ B cells, thus selectively spares B progenitor cells and plasma cells, providing an in vivo model to investigate human B cell homeostasis.

Methods and results: After weekly administration of rituximab in patients with follicular lymphoma within the trial SAKK 35/03, peripheral blood B cells are virtually absent (<0,01%), and no ISC are detected. In contrast, during rituximab maintenance therapy (administered in two months intervals) small numbers of B cells can be detected. These carry the phenotype of new formed plasma blasts (CD27++/CD38++/icIg+++ and Ki67+) and are identified as ISC in ex vivo ELISPOT assay. Surprisingly, ISC detected in treated patients were the same as in healthy donors in absolute numbers, while B cells were still reduced by 100-fold. Only 4 to 6 months after therapy human naïve and memory B cells were detected in peripheral blood again.

Conclutions: Regeneration of human peripheral blood B lymphocytes thus may follow a hierarchy: Recirculating ISC recover rapidly to normal steady state levels, while B cells with naïve or memory phenotype require longer time periods. This hierarchy may represent two elements within the B cells system: a primary system sustaining constant antibody levels and a secondary system providing a diverse repertoire for adaptive immune responses.

278 MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF 11Q23.1 AMPLIFICATION IN MULTIPLE MYELOMA (MM) AND DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

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Background: Genomic profiling by microarray-based comparative genomic hybridization is a powerful initial approach to identify DNA copy number changes and to detect candidate genes. We have previously analysed a series of MM and DLBCL clinical cases and cell lines with the Affymetrix GeneChip Human Mapping 10K arrays (Rinaldi et al, 2006; Lombardi et al, 2007). Here, we present genetic and functional analysis of a region at 11q23.1 identified as recurrent site of DNA amplification. The involved region overlaps with the breakpoint of a t(11;13)(q23;q12) chromosomal translocation that we had previously cloned in a chronic lymphocytic leukemia patient (Auer et al., 2005).

Material and Methods: 11q23.1 amplification was defined at genomic level with Affymetrix GeneChip Human Mapping 250K arrays, by quantitative genomic PCR and by FISH using three BAC clones overlapping the amplicon on two DLBCL (Karpas 422 and U-2932) and one MM cell line (JJN-3). Transcriptome mapping was performed with the Affymetrix GeneChip Human Tiling 2.0R arrays, using an RNA pool derived from normal lymph node, thymus and spleen, as reference sample. Validation of candidate genes expression was done by real-time PCR on lymphoma and MM cell lines and clinical samples.

Results: The minimally overlapping amplicon was 480 kb large, comprising the region from *POU2AF1* to *PPP2R1B*, including over 10 possible known transcripts. FISH analysis revealed different patterns of 11q23.1 structural rearrangement. Tiling expression arrays and real-time PCR identified three target genes: *POU2AF1*, *PPP2R1B* and *SNF1LK2*. Experiments aimed to silence the genes in DLBCL and MM cell lines are being performed using custom and validated Ambion siRNA molecules to evaluate the role of the genes in cell growth and proliferation.

Conclusions: Genomic profiling allowed the identification of a recurrent site of DNA amplification at 11q23.1. Detailed results will be presented on the molecular and functional characterization of the affected region.

279 A GENOMIC "BAR CODE" DEDICATED TO ANALYSE CHROMOSOME 9P21 LOCUS IN DIFFUSE LARGE B-CELL LYMPHOMAS (DLBCL) PROVIDES INFORMATION REGARDING MOLECULAR ALTERATIONS OF THE LOCUS AND MAY PREDICT RESISTANCE TO R-CHOP.

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CDKN2A (coding for $p16^{INK4A}$ and $p14^{ARF}$) and CDKN2B (coding for $p15^{INK4b}$) are tumor suppressor genes located on chromosome 9p21. This locus is part of a short region frequently deleted in tumors that encompasses other genes in its centromeric (DMRTA1) or telomeric (MTAP and MiR-31) boundaries. However, deletion frequencies of these respective genes, breakpoint locations and their relevance are unknown in DLBCL. We designed a single quantitative genomic PCR assay that provides a "bar code" of this locus and assess its signification in DLBCL.

Materials and Methods: A genomic multiplex PCR (Multiplex PCR of Short Fluorescent Fragments, QMPSF) was designed to analyse the 9p21 locus. This assay contains 10 primer pairs that cover a 2.8 Mb region and 5 relevant genes (miR-31/MTAP/CDKN2A/CDKN2B/DMRTA1). 93 DLBCL patients were analysed. QMPSF was validated by comparison with CGH-array (n=60). Allelic status was correlated to the GCB/non-GCB phenotype and $p16^{ink4A}$ expression. The prognosis value was assessed in 35 patients uniformly treated by CHOP plus rituximab (R-CHOP).

Results: A concordance between QMPSF and CGH was observed in all cases. Total or partial deletions of 9p21 locus were observed in 34 cases (36%). CDKN2A or CDKN2B homozygous deletions were detected in 16 cases and CDKN2A/CDKN2B were mostly codeleted. CDKN2A and CDKN1B codeletions were observed in 5 cases. Mir-31 loss was constantly detected in combination with CDKN2A loss. Telomeric breakpoints are mostly located between miR-31 and CDKN2A exon 3 (47%). Centromeric breakpoints are mainly located between CDKN2B exon1 and DMRTA1 (47%). A hot spot was identified between CDKN2A exon1a and alternative exon1b. CDKN2A/CDKN2B deletions were predominantly observed in the non-GCB subtype (81%, p=0.008) and correlated to a lower $p16^{ink4a}$ expression. CDKN2A/CDKN2B deletions strongly correlated to a shorter EFS and OS in patients treated by R-CHOP (p <0.005, median follow-up 28 months).

Conclusion: Our 9p21 QMPSF assay provided new information regarding molecular anatomy of the deletions involving this locus in DLBCL. CDKN2A/CDKN2B losses, detected in one third of patients and mainly in the non-GCB subtype, may contribute to the R-CHOP resistance.

280 MOLECULAR FEATURES DEFINING FOLLICULAR, DIFFUSE LARGE B-CELL, PRIMARY MEDIASTINAL B-CELL, AND MANTLE CELL LYMPHOMAS

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Introduction: The NHLs encompasses a wide variety of distinct pathologies. Several distinct subtypes have been given significant attention with recently developed high-throughput microarray technologies for describing genetic and gene expression changes. Recently, however, little attention has been given to looking at common changes amongst lymphomas and determining what changes are specific to what types. By characterizing these we will be able to gain an understanding of the biology of lymphoma in general as well as what drives the pathogenesis of each specific type of lymphoma.

Materials and Methods: 292 samples encompassing FL, DLBCL, PMBCL, and MCL were profiled for copy number alterations using array comparative genomic hybridization to a BAC array containing 26,819 clones for tiling-resolution coverage of the entire human genome. Alignment of profiles to identify regions of recurrent alteration was accomplished using a custom developed software package (SeeGH). Statistically significant distributions of these regions between subtypes were determined using Chi square and Fisher's exact tests.

Results: Thirty regions were investigated based on their prevalence in at least one of the lymphoma types being studied. Eight of these were found to be equally represented ($6p^{+}$, $6q16^{-}$, $6q23^{-}$, $7p^{+}$, $8q24.21^{+}$, $12q13^{+}$, $17p13.1^{-}$, and $18q31.23^{+}$), whereas, five regions were found to be widely disparate between lymphoma types. These included; $1p13^{-}$ found predominantly in MCL and PMBCL, $1q23^{+}$ found only in DLBCL and PMBCL, $2p15^{+}$ found in all types except MCL, $9p13^{+}$ found almost exclusively in PMBCL, and $13q14^{-}$ found predominantly in MCL.

Conclusions: The regions common to all types may play a role in lymphomagenesis or general cancer progression, whereas, the disparate alterations may drive biological pathways specific to different lymphoma subtypes. This is clear from the 2p15 gain found only within FL, DLBCL, and PMBCL which likely plays a major role in NF-kB activation. This pathway has not been shown to be active in MCL.

281 THE INHIBITORY RECEPTORS BTLA AND HVEM ARE EXPRESSED IN NEOPLASTIC CELLS FROM B-SMALL LYMPHOCYTIC LYMPHOMA AND B-MARGINAL ZONE LYMPHOMA BUT NOT IN NEOPLASTIC CELLS FROM MANTLE CELL LYMPHOMA

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Background: to prevent excessive activation, the immune response is regulated by membrane lymphoid inhibitory receptors, including the B and T Lymphocyte Attenuator (BTLA). The interaction of BTLA with its ligand, the Herpes Virus Entry Mediator (HVEM), leads to inhibition of lymphocyte activation. Recent data suggest that the BTLA/HVEM pathway may play a role in various autoimmune, infectious and neoplastic diseases. So far, there is no description of their expression in normal and neoplastic lymphoid tissues.

Material and Methods: We developed monoclonal antibodies against BTLA and HVEM and studied their expression in frozen tissues from benign lymph nodes (n=13) and lymphomas (n=86) using immunohistochemistry.

Results: *In hyperplastic lymph nodes,* both BTLA and HVEM are expressed in T lymphocytes within interfollicular areas and in mantle zone and marginal zone B-lymphocytes. Within germinal centers (GC), B lymphocytes are negative for both BTLA and HVEM, whereas T lymphocytes are BTLA positive and follicular dendritic cells are HVEM positive. *In lymphomas*, BTLA and HVEM are constantly expressed in Chronic Lymphoid Leukemia/Small Lymphocytic Lymphoma (CLL/SLL, 15/15), and more weakly on Marginal Zone Lymphomas (MZL, 8/8). Neither BTLA nor HVEM were detected in Mantle Cell Lymphoma (MCL, 0/5) and Follicular Lymphoma (0/16). Nearly all peripheral T Cell Lymphomas were BTLA positive (7+/8), whereas no positivity was seen for HVEM. Diffuse Large B Cell Lymphomas were rarely positive (6+/14 for BTLA, 3+/14 for HVEM). In Hodgkin disease, tumour cells were negative for both markers (0/20).

Conclusion: The extinction of BTLA and HVEM expression in normal GC B cells suggests that downregulation of BTLA/HVEM pathway may be involved in the process of antigen-driven B-cell activation. The preferential expression of BTLA and HVEM in SLL and MZL, but not in MCL could be used as a help for the diagnosis of small cell lymphomas.

282 CLINICOPATHOLOGICAL FEATURES OF LYMPHOMA/LEUKEMIA PATIENTS CARRYING BOTH BCL2 AND MYC TRANSLOCATIONS

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Background: Overexpression of Bcl2 protein is known to inhibit cell apoptosis, while the MYC translocation is known to accelerate cell proliferation. Lymphoid malignancy with translocations of both 18q21.3/BCL2 and 8q24/MYC with immunoglobulin genes termed dual hit lymphoma/leukemia (DHL) is rare and has not been studied in detail. This study aimed to clarify the clinicopathological characteristics of DHL.

Material and Methods: We identified DHL cases by nationwide survey in Japan. Translocations of both genes were demonstrated by chromosome analysis. Clinical data of 27 cases and pathology of 20 cases were reviewed.

Results: There were 12 males and 15 females, and their age ranged from 36 to 79 years with a median of 51 years. Twenty-two patients were diagnosed as having DHL at presentation (18 lymphoma and 4 leukemia) and 5 patients at relapse or progression (5 lymphoma). Histological diagnosis of the reviewed cases comprised 2 of follicular lymphoma (FL), 12 of diffuse large B-cell lymphoma, 4 of Burkitt-like lymphoma (BLL), and 2 of composite lymphoma (FL + BLL). Chromosome analysis revealed 13 translocations of t(14;18) and t(8;14), 9 of t(14;18) and t(8;22), 4 of t(14;18) and t(2;8), and 1 of t(2;18) and t(8;14). Twenty-five of the 27 patients had primary extranodal involvement at DHL onset. Progression of the malignancy to the central nervous system (CNS) was highly observed in 15 patients. An overall survival time of 1 year was observed in only 22% of cases from the time diagnosis of DHL.

Conclusion: DHL is a rare but distinct subgroup with extremely poor prognosis characterized by frequent extranodal involvement and CNS progression.

283 CHARACTERIZING BIOLOGICAL FEATURES OF B CELLS CARRYING *BCL2/IGH* TRANSLOCATION

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Introduction: *BCL2/IGH* translocation is a hallmark of follicular lymphoma and diffuse large B-cell lymphoma of germinal center B-cell type. Although being a strong determinant of these histological subtypes, this translocation is considered to be insufficient by itself and further gene alterations are necessary for lymphomagenesis. In this study, we aimed to clarify how *BCL2/IGH* translocation influences on the biological features of B cells *in vivo* and contributes to the development of specific lymphoma subtypes.

Materials and Methods: We generated Eμ-BCL2/CAG-EGFP double transgenic (Tg) mice by crossing Eμ-BCL2 Tg mice with CAG-EGFP Tg mice, and their bone marrow mononuclear cells were syngeneically transplanted to wild-type C57BL/6 mice without myeloablative treatment. Afterward, chimeric splenocytes of the recipient mice were collected and analyzed. Functional analysis of Eμ-BCL2$^+$ B cells was also performed *in vitro*.

Results: Flow cytometry of the recipient splenocytes demonstrated that Eμ-BCL2$^+$ B cells, analyzed by gating on EGFP$^+$B220$^+$ cells, preferentially differentiated into follicular B cells, but exhibited a retarded cell cycle progression compared to endogenous B cells. Interestingly, blastic component of Eμ-BCL2$^+$ B cells increased when they were transferred into wild-type mice but not when transferred into Eμ-BCL2 Tg mice. These results suggest that Eμ-BCL2$^+$ B cells are recurrently stimulated in follicles when they exist in healthy individuals as a minor population, while they are mostly quiescent in Eμ-BCL2 Tg environment. We found cell autonomous upregulation of *BCL6* gene in Eμ-BCL2$^+$ B cells, and notably, Eμ-BCL2$^+$ B cells had reduced terminal differentiation capacity in response to B cell receptor stimulation. These features seemed to be attributed to the modification of transcriptional activities by excessive Bcl-2 protein.

Conclusions: Our data suggest that *BCL2/IGH* translocation not only provides anti-apoptotic property but also leads to biased and incomplete differentiation of B cells. We propose that Bcl-2 deregulated B cells compose a distinctive population that may serve as a reservoir of lymphoma of germinal center B-cell type.

284 GENOME-WIDE ANALYSIS OF 34 AGGRESSIVE NON HODGKIN LYMPHOMAS WITH *MYC* REARRANGEMENT (*MYC*/LNH) USING HIGH-DENSITY SNP ARRAYS REVEALS FREQUENT CRYPTIC ALTERATIONS INCLUDING AMPLIFICATION OF THE MICRORNA *17-92* LOCUS

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Chromosomal translocation of the *MYC* oncogene is a hallmark of Burkitt lymphoma/leukemia (BL) but is also detected in 5-30% of diffuse large B-cell lymphomas (DLBCL).The *MYC* deregulation is a necessary but not sufficient oncogenic event in BL pathogenesis. The role of *MYC* inDLBCL remains unclear: it is supposed to be a secondary event. Additional (epi)genetic alterations should cooperate in BL pathogenesis such as inactivation of the p14arf-MDM2-TP53 pathway or secondary genomic alterations. We showed the independent negative prognostic impact of the heterogeneous 13q alterations 7q in childhood BL. 34 morphologically reviewed *MYC*/LNH (28 BL, 6 DLBCL) were analyzed with 50K Xba SNP arrays (Affymetrix) to characterize additional allelic imbalances and losses of heterozygosity (LOH). Acquired partial uniparental disomies (pUPD) are copy neutral LOH: 39 pUPD (>5 Mb) were found in 13 BL & 5 DLBCL; 16 were telomeric, 5 recurring (n>=2) regions were noticed (1p, 6p, 9p, 17p 75 of these chromosomal imbalances were cryptic at karyotype. A recurring 13q gain was found in 8 patients. The minimal amplified region of 4.35 Mb includes 3 candidate genes: *GPC5*, *GPC6* and *C13orf25* which contains the miR-17-92 cluster. Mature miRNA expression profiles (Taqman low density array) confirmed the overexpression of the 7 miRNAs composing this cluster in presence of the genomic 13q amplification. These results suggest the contribution of the miR-17-92 cluster known to interact with *MYC* oncogenein lymphomagenesis. Additionally, acquired pUPD may be another way of p14arf-MDM2-TP53 inactivation.

285 BCL2, BCL6, MYC, MALT 1 AND BCL10 REARRANGEMENTS IN NODAL DIFFUSE LARGE B-CELL LYMPHOMAS: A MULTICENTER EVALUATION OF A NEW SET OF FISH PROBES AND CORRELATION WITH CLINICAL OUTCOME

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Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma. The highly variable outcome reflects a heterogeneous group of tumours, with different genetic abnormalities and response to therapy. A series of 74 primary nodal DLBCL from four different Swiss and Italian Institutions was investigated using a FISH approach to detect BCL2, BCL6, MYC, BCL10 and MALT1 rearrangements, with the aim to validatea new panel o split signal FISH probes. A gene specific abnormality was identified in 62% of our series and the most involved gene was BCL6 (44.7%). Twenty eight percent of DLBCL showed rearrangements of more than one gene. The results obtained demonstrated that FISH analysis on paraffin embedded sections using the DAKO set probes are useful for a routine definition of a gene rearrangement in DLBCL even if the sensitivity of FISH on histological section may be reduced due to a peculiar feature of the histological sample. Practical strategies for using FISH analysis to detect cytogenetic rearrangements in lymphoma histological sections were pointed out. This study demonstrated chromosome translocations affecting BCL2, BCL6, BCL10 and MYC in a multi-centric series of paraffin embedded samples of DLBCL. The pattern of the observed translocations highlighted that not only DLBCL represent a heterogeneous entity, but that even individual cases may contain different sub-clones bearing different chromosomal translocations. When DLBCL were subdivided into GC and ABC groups, an inverse pattern of BCL2 and BCL6 rearrangements was observed. Of interest the presence of chromosome rearrangements was associated with a worse prognosis.

286 BLOCKADE OF PD-1/PD-LIGAND PATHWAY RESTORES FUNCTION OF INTRATUMORAL EFFECTOR T CELLS IN FOLLICULAR LYMPHOMA

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Background: The inhibitory receptor programmed death 1 (PD1), a negative regulator of activated T cells was recently shown to be upregulated on the surface of HIV-specific CD4+ and CD8+ T cells in humans and was associated with reversible impairment of T-cell function. Here, we examined the role of PD-1 on the function of intratumoral T cells in patients with follicular lymphoma.

Materials and Methods: PD-1 expression was determined on peripheral blood and intratumoral T cells in patients with follicular lymphoma at the time of initial diagnosis by flow cytometry. To test the function, intratumoral T cells were isolated and cultured in the absence or presence of PMA/Ionomycin or autologous tumor cells with or without anti-human PD-1 blocking antibody or isotype control antibody (R& D Systems). Supernatants were harvested after 48 hours and Th1/Th2 cytokine (IL-2, IL-4, IL-5, IL-10, IL-13, GM-CSF, IFNg and TNFa) production was measured by multiplex cytokine assay.

Results: PD-1 expression was significantly upregulated on peripheral blood and intratumoral CD4+ and CD8+ T cells in patients with follicular lymphoma as compared with normal donor PBMC (mean 5% for CD4+ T cells and 7% for CD8+ T cells). Furthermore, PD-1 expression was significantly higher on intratumoral (mean 67% for CD4+ T cells and 47% for CD8+ T cells) compared with peripheral blood (mean 33% for CD4+ T cells and 22% for CD8+ T cells) T cells in follicular lymphoma. PD-1 expression was associated with impaired cytokine production and blockade of the PD-1/PD-ligand pathway with antibodies against PD-1 significantly enhanced intratumoral T-cell Th1 but not Th2 cytokine production in response to polyclonal or autologous tumor cell stimulation.

Conclusions: PD-1 is markedly upregulated on intratumoral and peripheral blood CD4+ and CD8+ T cells of patients with follicular lymphoma, associated with impaired T-cell function, and blocking PD-1 restored the function of the T cells. These data suggest that blockade of PD-1/PD-ligand pathway may be used in combination with other immunomodulatory strategies such as vaccines and adoptive T-cell therapies to enhance their efficacy.

287 CD74 ASSOCIATES WITH FAS AND INHIBITS FAS-MEDIATED APOPTOTIC SIGNALING

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Introduction: Resistance to Fas-mediated apoptosis in hematopoietic cancers interferes with the efficacy of currently available chemotherapy. Our prior research on human herpesvirus 8 oncoprotein K1 showed that K1 binds to Fas and interferes with activation of Fas-mediated apoptotic signaling (Wang, W, et al, Blood 2007; 109:5455-62). Herpesvirus proteins often mimic host proteins or their functions, we thus searched for endogenous host proteins associated with inactive Fas in order to identify potential regulators of Fas signaling. Potential inhibitors of Fas were identified by immunodepletion of cell extracts from B-cell lymphoma-derived BJAB cells with activating anti-Fas antibody and by subsequent coimmunoprecipitation of proteins associated with nonactivated Fas. CD74, the invariant light chain of MHC II, was identifed in complex with inactive Fas by liquid chromatography tandem mass spectroscopy. Interestingly, overexpression of CD74 was previously reported in hematopoietic cancers: in 43 of 66 cases of pediatric non-Hodgkin lymphomas (Miles, R.R., et al., Br J of Haematology. 2007; 138: 64-71), in 8 out of 14 multiple myeloma-derived cell lines and B-cell lymphoma cell lines Raji and Ramos (Burton, J.D., et al., Clin Cancer Res. 2004; 10: 6606-6611), in 11 of 16 cases of leukemia (Kaddu, S., et al., J Am Acad Dermatol. 1999; 40: 966-978), as well as in 9 out of 11 cases of non-small-cell lung carcinoma (Ioachim, H.L., Am J Surg Pathol. 1996; 20: 64-71). CD74 expression in cancers was thus suspicious.

Methods/Results: Through overexpression of CD74 in Fas-positive HEK 293 cells and suppression of CD74 expression in BJAB cell using siRNA technology we have determined that cells overexpressing CD74 are more resistant to agonistic antibody CH-11-induced Fas-mediated apoptosis then their relative controls. We have also mapped the domain of CD74 required for association with Fas to a membrane-proximal region of CD74 by expressing deletion mutants. Transfection of mice with plasmid encoding full length CD74 protected mice from lethal challenge with agonistic anti-Fas antibody Jo2. All 5 of vector transfected mice died within 6 hours from challenge, while 4 out of 5 CD74 transfected mice survived the challenge ($P<0.05$).

Conclusions: Our results support the idea of an endogenous regulatory system of Fas-mediated apoptosis that utilizes transmembrane proteins interacting with Fas. We anticipate that specific blocking of the CD74-Fas interaction will sensitize CD74 overexpressing cancer cells to Fas-mediated apoptosis and thus will increase effectiveness of chemotherapy for hematopoietic cancers.

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289 CHANGES IN SEROLOGICAL BIOMARKERS (BM) OF CELL DEATH DURING CHEMOTHERAPY (CT) IN PATIENTS WITH LYMPHOMA

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Introduction: Early identification of chemoresistant disease and a focus on toxicity is important in patients (pts) with lymphoma where there is the potential for long term survival. The Cell Death Detection kit^{TM} (Roche) detects nucleosomal DNA (nDNA) shed from dying nucleated cells whilst the M65^{TM} assay (Peviva) detects cytokeratin 18 (CK18) products specific to dying epithelium. The utility of these assays as biomarkers of early therapeutic response and toxicity in lymphoma is unknown.

Methods: Serum samples from 22 patients with lymphoma (7 Hodgkin (HL) treated with ABVD, 7 follicular (FL) treated with R-CVP and 8 diffuse large B cell (DLBCL) treated with R-CHOP) were analysed for nDNA and CK18. Samples were taken throughout therapy (days 1, 3, 8, 15 cycle 1, days 1, 3 subsequent cycles). Assays were performed in accordance with manufacturers' instructions. Control samples were taken from healthy volunteers. Toxicity was graded (CTCAE version 3.0). Statistical analysis was non-parametric with significance at $P<0.05$. The area under the curve for CK18 during cycle 1 was estimated using the trapezoid method.

Results: nDNA was significantly higher in patients than in 61 healthy controls (median 1.34 vs 0.30 ($p<0.0001$). Baseline CK18 was not elevated (285 vs 258 U/L (NS)) in contrast to elevations reported in patients with epithelial tumours. There was no significant difference between nDNA in HL, FL or DLBCL, however nDNA was higher in patients with stage II and III disease than stage I (1.58 vs 0.85 $P<0.05$). nDNA levels fell after therapy (median day 8, 0.55 $P<0.05$) and remained low (median day 22, 0.42 $P<0.05$). Administration of chemotherapy provoked significant rises in CK18 by day 3 (median +31 U/L, $p<0.05$) which returned to baseline by day 8 (median -0.1 U/L NS). Persistent elevations in CK18 throughout cycle 1 were seen in patients experiencing the worst epithelial toxicity (Cumulative CTCAE score <3 median increase 1% vs >3 median increase 30% $P<0.05$).

Conclusion: This pilot study reveals the potential of these serological assays of cell death as response and toxicity biomarkers in lymphoma. The high baseline levels of nDNA pre-therapy which fall post therapy suggest nDNA as a biomarker of early tumour response. CK18 release peaking day 3 post therapy reflects epithelial damage. Persistent CK18 elevation in patients reporting more side-effects supports serum CK18 as a useful toxicity biomarker. Integration of these biomarkers into future therapeutic trials in lymphoma is warranted.

290 ARE SERUM ANGIOGENIC (VEGF AND BFGF) AND ANTIANGIOGENIC (ENDOSTATIN) FACTORS PREDICTIVE OF CLINICAL OUTCOME IN PATIENTS WITH LYMPHOMAS?

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Purpose: To evaluate the predictive value of angiogenic and antiangiogenic factors in the clinical outcome.

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Patients and Methods: Frozen samples were obtained from patients treated between March 1987 and September 1988. Thereafter patients entered the study prospectively. Accrual ended at the end of August 2001. Serum bFGF, VEGF and endostatin concentrations were measured using ELISA assays before treatment.

Results: Thirty patients had Hodgkin lymphoma. Fifty four patients and eighty two patients had indolent and aggressive lymphoma respectively. Treatments of lymphoma patients mostly consisted of ACVBP or CHOP-like treatments. Treatments of Hodgkin lymphoma patients were EBVP and MOPP/ABV. The median follow-up is 7.4 years (range 0.1- 15.9 years). The 5-year overall survival for patients with Hodgkin, aggressive non Hodgkin and indolent lymphoma were 100%, 60% and 72% respectively. In multivariate analyses, VEGF, endostatin, the ratio of VEGF/endostatin and performance status were highly significant in terms of progression - free survival and overall survival.

Conclusions: Serum angiogenic and antiangiogenic factors were found to be independent prognostic factors on overall survival and progression-free survival.

291 THE ONCOGENIC EFFECT OF GROWTH FACTOR INDEPENDENCE 1(*GFI1*) IN T-CELL LYMPHOMAS IN A MURINE MODEL

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Background: The retrovirus, SL3-3 Murine Leukemia Virus (MLV), induces T-cell lymphomas when injected into newborn mice of the NMRI inbred strain making it at present, the most reliable model of MLV-induced T-cell lymphomagenesis. The oncogenic effects of MLV are caused by insertional mutagenesis of the host genome and often result in alteration of the product of genes involved in lymphoma development. Integration sites in the NMRI mouse genome thus provide an efficient screening method for identifying genes involved in the progress of murine and potentially also human lymphomas. The proto-oncogene, *Gfi1*, is a frequent target of retroviral integration and its disruption is associated with both murine and human cancers.

Materials and Methods: By high-throughput sequence identification of retroviral integration sites in MLV infected NMRI inbred mice, we have mapped 182 out of 1565 insertions to the genomic locus of *Gfi-1*, making it a hotspot for MLV integration. Forty five tumors harboring integrations in the *gfi1* locus were screened for alternative transcripts by RT-PCR and sequencing. The impact of MLV integration on *Gfi1* gene and protein expression was analyzed by qPCR, Northern and Western Blot.

Results: We have identified four tight SL3-3 integration clusters in the *gfi1* locus. A new SL3-3-specific cluster of 30 integrations was located within a 1kb window in the 3'UTR of the *Gfi1* gene. Three new *Gfi1* transcripts were identified and gene expression was demonstrated to be increased up to 80-fold in tumors possessing SL3-3 integrations in the *gfi1* locus. We have observed major variations in the Gfi1 protein expression and a potential new protein isoform.

Conclusion: We have identified a new proviral insertional cluster in the *Gfi1* gene using the NMRI inbred mouse model and demonstrated that proviral insertions in the *Gfi1* gene result in deregulation of both its gene and protein expression, establishing its oncogenic properties.

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