Genomics

187 DISCOVERY OF *CIITA* GENE FUSIONS IN B CELL LYMPHOMAS BY NEXT GENERATION SEQUENCING

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Introduction: Translocations, particularly those involving immunoglobulin gene loci, are critically involved in the molecular pathogenesis of B cell lymphomas. However, recurrent genomic rearrangements have not been described in primary mediastinal B cell lymphoma (PMBCL) and only rarely in Hodgkin lymphoma (HL), entities that show biological overlap and share many similarities by gene expression profiling.

Methods: To identify novel fusion transcripts resulting from translocations, we investigated HL cell lines KMH2, L428, DEV and 7 lymph node specimens of PMBCL by whole-transcriptome paired-end sequencing (RNA-seq). Candidate predictions were validated by fluorescence *in-situ* hybridization (FISH) and polymerase chain reaction.

Results: We discovered highly expressed gene fusions involving the MHC class II transactivator *CIITA* (16p13.13) in KM-H2 and DEV cells, and 2 out of 7 PMBCL samples. Subsequent evaluation of 288 unrelated B cell lymphoma samples demonstrated that genomic *CIITA* breaks were highly recurrent in PMBCL (38%), mediastinal grey zone lymphomas (GZL, 27%) and classical HL (15%), but were found only in 3% of diffuse large B cell lymphomas. Furthermore, *CIITA* was found as a promiscuous partner of various in-frame gene fusions involving the genes *FLJ27352, CD273, CD274, RUNDC2A, RALGDS,* and *SOCS1*. *CIITA* gene alterations were associated with decreased disease specific survival in 57 PMBCLs (p=0.044). As functional consequences of *CIITA* gene fusions we identified down-regulation of surface HLA class II expression and overexpression of CD274 and CD273 leading to T cell inactivation in vitro.

Conclusions: We discovered novel fusion genes involving the master regulator of MHC class II expression *CIITA* that were highly recurrent in PMBCL, HL and GZL. Our functional studies suggest that escape from immunosurveillance through various mechanisms plays an important role in the pathogenesis of these lymphomas. Thus, recurrent rearrangements of *CIITA* represent a novel genetic mechanism underlying tumor-microenvironment interactions across a spectrum of lymphoid cancers.

188 DISCOVERY OF RECURRENT ONCOGENIC MUTATIONS IN LYMPHOMA BY RNA-SEQ

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The recent development of high throughput mRNA sequencing technologies has allowed major advances in the characterization and quantification of transcriptomes in human cancer. These advances include gene mutation detection, gene fusion discovery, analysis of alternative splicing events, and unbiased gene expression profiling.

To comprehensively discover pathogenic sequence variants in lymphomas, we used Illumina technology to sequence mRNA of 173 lymphoma biopsies and cell lines, including 63 diffuse large B-cell lymphomas (DLBCL) of activated B-cell-like (ABC) subtype, 71 DLBCL of germinal center (GCB) subtype, and 29 Burkitt's lymphomas (BL). Paired-end sequence reads were aligned using the Burrows-Wheeler Alignment Tool yielding an average of 5.5 aligned gigabases per sample and an average coverage of 35-fold. Aligned sequences were further annotated using several genome annotation databases that help determine the pathogenic contribution of identified sequence alterations.

This study revealed several genomic alterations that were verified by Sanger sequencing. Notably, many of these variants occurred more frequently in one of the lymphoma subtypes, indicating functionally important genetic lesions caused by distinct selection pressures in each subtype. Using these methodologies, we discovered highly recurrent, oncogenically active MYD88 mutations in ABC DLBCL, as recently described. We will present further analyses that have revealed additional highly recurrent sequence alterations that are likely to contribute to lymphoma pathogenesis.

189 QUANTITATIVE PHOSPHOPROTEOMIC ANALYSIS OF B-CELL LYMPHOMA CELLS REVEALS DISTINCT PHOSPHOTYROSINE SIGNATURES BETWEEN MANTLE CELL LYMPHOMA, FOLLICULAR LYMPHOMA AND BURKITT LYMPHOMA

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Background: Deregulation of multiple pathways controlled by tyrosine phosphorylation underlies the pathogenesis of many human diseases, especially cancers. Although B-cell non-Hodgkin lymphomas (NHLs) represent the seventh most common cancer in western countries, the extent to which phosphotyrosine deregulation may be involved in their pathogenesis is largely unknown. In this study, we employed a mass spectrometry-based quantitative tyrosine phosphoproteomic strategy to profile phosphotyrosine signatures in 3 B-cell NHL entities.

Material an Methods: Five milligrams of protein from 14 human B-NHL cell lines (4 mantle cell lymphomas (MCL), 3 Burkitt lymphomas (BL) and 7 follicular lymphomas (FL) were digested by trypsin and peptides were subjected to phosphopeptide enrichment using immobilized metal affinity chromatography (IMAC) followed by immunoprecipitation using a cocktail of 3 anti-phosphotyrosine antibodies. Phosphopeptides were subjected to liquid chromatography (LC) and MS/MS. Spectra were searched against the UniProtKB database using X!Tandem with k-score. All proteins with a probability ≥0.8 at false discovery rate of 1% were considered for further analysis. Quantitation of identified peptides was based on spectral counts of the phosphorylated tyrosine peptides.

Results: Quantitative phosphoproteomic analyses revealed a total of 48, 22, and 11 tyrosine phosphopeptides, corresponding to 30, 16 and 11 phosphoproteins in BL, FL and MCL, respectively. While a subset of tyrosine phosphoproteins were identified across all three lymphoma entities, distinctive signatures characterized by differential recovery of tyrosine phosphopeptides from CDK1 and CDK5 were observed in MCL, while differential recovery of phosphopeptides implicated in active B-cell receptor signaling (LCK, LYN, SYK) was observed in BL and FL. Immunoprecipitation and western blot studies were performed to validate the differential phosphorylation of a subset of tyrosine phosphoproteins discriminating between GC-derived lymphoma cell lines from MCL-derived cell lines.

Conclusions: We identify distinct protein phosphorylation signatures between MCL and germinal center derived lymphomas. Our study reveals the utility of unbiased phosphoproteome interrogation of B-NHLs to characterize signaling networks that may provide insights into the pathogenetic mechanisms.

190 GENE EXPRESSION PROFILE OF MARGINAL ZONE LYMPHOMAS AND WALDENSTRÖM MACROGLUBULINEMIA REVEALS SPECIFIC PATTERNS FOR DIFFERENT SUBGROUPS

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Introduction: Biology of splenic and nodal marginal zone lymphomas (SMZL and NMZL) is poorly understood, and they lack specific markers for differential diagnosis between them and with Waldentröm Macroglobulinemia (WM)/lymphoplasmacytic lymphomas (LPL).

Aim: To search for new markers specific for these entities and to better understand transformation mechanisms.

Methods: An Affymetryx transcriptome study was performed on 170 tissue samples including *(i)* SMZLs (typical, CD5+, in progression (SMZL/P), or other (not all SMZL features)), *(ii)* NMZLs (typical, with plasma cell differentiation (NMZL-PCD) or other), *(iii)* disseminated MZLs (tumors with features of NMZL, associated with either splenomegaly and/or extranodal infiltration, *(iv)* WM/LPL (bone marrow disease only (BM-WM) or either with late nodal localization or as nodal primary disease (NLPL/WM) and *(v)* reactive lymphoid tissues from different organs, as well as CLLs, follicular lymphomas, MALT and multiple myeloma as controls.

Results: Among spleens, unsupervised analysis led to identification of 2 specific clusters for CD5+ SMZLs and for SMZL/P, distinct from other SMZLs. A supervised analysis on both lymph nodes and bone marrows evidenced 187 genes, corresponding to 3 specific signatures for typical NMZL, NLPL/WM and BM-WM. Transcriptomic profiles of NMZL-PCD overlapped those of NLPL/WM. Genes of plasma cell differentiation were noted for NMZL-PCD, NLPL/WM and BM-WM.

Conclusion: These results first support the concept that, among SMZLs, CD5+ variant on one hand and cases in progression on the other hand are associated with specific gene expression patterns. Second, these results tend to separate NMZL in 2 groups according to the absence or presence of plasmacytic differentiation, the latter being very close to NLPL/WM, but distinct from BM-WM, which appears as a different entity.

N. Gachard and A. Traverse-Glehen: equal contribution; F. Berger and J. Feuillard: co-corresponding authors

191 CHARACTERIZATION OF GASTROINTESTINAL MARGINAL ZONE B-CELL LYMPHOMAS AND VARIANTS USING HIGH-RESOLUTION SNP-ARRAYS

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Background: So far, gastrointestinal marginal zone B-cell lymphomas have only been analyzed by CGH and little is known about the genomic complexity of its large cell variants. Therefore, we used SNP analysis, a technique with much higher sensitivity, to further characterize this lymphoma entity.

Patients and Methods: We extracted genomic DNA from frozen tissue samples of 28 gastrointestinal marginal zone B-cell lymphomas (n=7) and large cell variants (n=21). We performed SNP analysis using the Affymetrix HGW SNP array 6.0 platform. Results were correlated with FISH and IHC analyses.

Results: While small cell lymphomas have on average 8 aberrations longer than 0.2MB each case, large cell variants have more than 14. Most affected regions are on chromosomes 1 and 11 (large cell lymphomas) and chromosomes 1 and 9 (small cell lymphomas). Both small and large cell lymphomas have losses in regions 1p13 and 6q15 as well as gains on 1p36 and 17q21. Losses on 6q24 (5/21) and gains on 11q23 (8/21) are restricted to the large cell lymphomas. 5/21 large cell and 1/7 small cell lymphomas have losses or deletions of 6q14.1a-c containing *HTR1B, IRAK1BP1, PHIP, HMGN3, LCA5* and *SH3BGRL2*. 6/21 large cell lymphomas have gains or amplifications of 2p16.1a-15d containing *PAPOLG, REL, PUS10* and *PEX13*. Amplification of *REL* was confirmed by FISH in these cases. Immunohistochemical staining for REL was positive in at least 30% of the lymphoma cells. SNP-analysis of one sample with strongly positive cytoplasmatic and nuclear staining revealed a LOH on 2p16.1a whereas the other five samples are heterozygous for the amplified region. Comparing the SNP profiles of two areas of the same tumor both with a t(11;18)*Api2/Malt1* but with slightly different morphology, the analysis revealed additional gains in the more blastic part. Investigating two lymphoma samples from the same patient with an interval of two years, FISH analysis showed a signal pattern pointing to a large deletion in the *IGH* locus exclusively in the later sample. SNP analysis confirmed this result and revealed ten additional aberrations illustrating increasing genomic complexity during lymphoma progression.

Conclusions: Small and large cell variants of gastrointestinal marginal zone B-cell lymphomas have a distinct pattern of genomic aberrations but share some overlapping features. *REL* is frequently amplified in large cell variants. In general, SNP analysis by array is a powerful and sensitive tool for molecular characterization of lymphomas and lymphoma progression.

192 GENOMIC PROFILES OF MALT LYMPHOMAS: VARIABILITY ACROSS ANATOMIC SITES

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Introduction: MALT lymphomas represent the most common type of marginal zone B-cell lymphoma. Important differences exist for the different involved anatomical sites, including distribution of recurrent translocations and association with autoimmune disorders or infectious agents. Besides chromosome translocations, unbalanced DNA changes occur in MALT lymphomas including gains on chromosome 3 and 18, deletions affecting TNFAIP3/A20 (6q23) and gains of the short arm of chromosome 6. Site-specific differences might impact outcome and therapeutic approaches.

Aim: To test the existence of unbalanced DNA alterations specific for different anatomic sites of involvement, genomic profiles of 130 MALT lymphomas were analyzed.

Patients and Methods: All 130 cases were analyzed using the Affymetrix Human Mapping 250k Nsp array directly by the authors in 57 cases or drawn from publicly available raw CEL files in additional 73 cases. The main anatomical sites were: ocular adnexa (41%), stomach (21%), parotid or other salivary glands (9%), thyroid (9/130, 6%), lung (6%).

Results: Trisomy 3 and 18 and del(6q23) occurred at similar frequencies in MALT lymphomas primarily involving stomach, orbital adnexa, thyroid, salivary glands and lung. Gains at 6p appeared significantly more common among MALT lymphomas of the orbital adnexa than those occurring at other sites (+6p, 20% vs 0%; P 0.0001; +6p25.3-p21.32, 20% vs 4%, P 0.0087). Gastric involvement showed a trend for a higher frequency of +8q11-q24 (14% vs 3%, P 0.050). Both +6p and del(6q23) were commonly associated with +3q, although they were observed also as single unbalanced lesions: 6p gains occurred in 7/99 (7%) cases without +3q and in 8/31 (26%) with +3q (P 0.004) while del(6q23) occurred in 17/99 (17%) cases without +3q and in 11/31 (35%) with +3q (P 0.03). Del(6q23) and +6p were concomitantly observed in only 4 cases, while 24/28 (86%) had del(6q23) without +6p and 11/15 (73%) had +6p only (P 0.6).

Conclusions: MALT lymphomas mostly appear to bear a common set of unbalanced genomic copy number alterations independent of the anatomical site of presentation, different from what has been observed for chromosome translocations. Gains at 6p and, possibly, at 8q show a preferential occurrence in MALT lymphomas of the orbital adnexa and of the stomach, respectively, two diseases strongly associated with chronic infectious conditions.

193 MICRORNA DEREGULATION IN GASTRIC MALT LYMPHOMA

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Gastric marginal zone b-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) represents a distinct class of extranodal lymphoma that evolves against a background of chronic inflammation induced by persistent infection with the bacterium helicobacter pylori. To date, MALT lymphoma research has largely focused on altered expression of protein coding genes. However, recent evidence suggests that alterations of non-coding RNA, particularly microRNA (MIRNA), also contribute to tumourigenesis. We have identified mir-203 as an important tumour suppressor MIRNA in malt lymphoma in connection with its known oncogene target ABL1. Pharmacological inhibition of ABL1 activity by imatinib blocked malt lymphoma cell proliferation ex vivo and effectively eradicated tumours in vivo. Collectively, our observations suggest that ABL1 plays an important role in malt lymphoma cell biology and support a novel potential application of imatinib in the treatment of malt lymphoma. Low grade malt lymphomas can eventually undergo high grade transformation to a more aggressive counterpart termed gastric diffuse large b-cell lymphoma (GDLBCL). At this stage the lymphomas grow autonomously and are refractory to helicobacter eradication therapy. Genome-wide MIRNA expression profiling revealed a characteristic set of myc-repressed MIRNAs to be specifically downregulated in human GDLBCL compared to MALT lymphoma and gastritis. Of the myc-repressed MIRNAs downregulated in malignant lymphoma, mir-34a showed the strongest anti-proliferative properties when overexpressed in DLBCL cell lines. We could further attribute the tumour suppressive effects of mir-34a to dysregulation of its target FOXP1. Transient knockdown of FOXP1 in DLBCL cell lines significantly impaired the proliferation of the tumour cells. Taken together, our findings elucidate a novel mechanism linking the aberrant expression of myc and concomitant repression of mir-34a to FOXP1 deregulation in GDLBCL.

194 GENE EXPRESSION IN PARAFFIN-EMBEDDED DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) TREATED WITH CHOP OR RCHOP: AN ECOG AND SWOG STUDY

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Intro/Background: Rituximab (R) modulates the prognostic significance of some biomarkers in DLBCL including BCL6, BCL2 and p21. Based on immunohistochemical staining, we showed that R selectively benefited subsets of DLBCL treated with CHOP,

likely reflecting its differential effects on growth and survival mechanisms. With these same specimens, the quantitative nuclease protection assay (qNPA), a new methodology for measuring mRNA levels in formalin-fixed paraffin embedded tissues (FFPE), was used to investigate gene expression and changes in the prognostic profile associated with the addition of R to CHOP in DLBCL.

Material and Methods: Five micron unstained FFPE sections from 182 eligible and evaluable cases enrolled on E4494 and submitted for prospective immunohistochemical correlative studies more than ten years ago were used for this analysis. Tissue was scraped off slides and a multiplexed qNPA was performed in triplicate using a customized ArrayPlate assay for 43 genes of interest. TBP served as a housekeeping gene. The concordance between immunohistochemical staining and gene expression was measured by Kendall rank correlation. Association between standardized log gene expression and patient FFS/OS was obtained using the Cox proportional hazards model adjusting for IPI score. A weighted analysis was used to eliminate the confounding effect of maintenance rituximab.

Results: In 6 cases, tissue from slides prepared >10 years ago was compared to freshly cut sections from corresponding blocks and showed excellent concordance. On-study characteristics for the 176 cases with analyzable data were representative of the greater study population. Immunostaining for BCL2 and BCL6 correlated with log gene expression levels (p<.001, and p=.026, respectively). Consistent with our previously reported protein expression data, BCL6 mRNA was a favorable prognostic marker for OS in CHOP treated cases (p =.014), but not RCHOP adjusted for the IPI (p=.27). Other genes that lost their adverse prognostic significance with addition of R to CHOP, included PDCD4, CAV1, PIK3CA, and PECAM1. MS4A1 and ROBO4, favorable prognostic markers with CHOP, had no prognostic significance among RCHOP treated patients. BCL2 was associated with a poor outcome in RCHOP treated patients (p=.015) but not CHOP treated cases (p-.48). In the multivariable analysis, in addition to the IPI, only BCL2 gene expression was predictive of both FFS and OS in RCHOP treated patients (HR = 1.79; p=.0037; HR=1.60; p=.0037, respectively). In addition to BCL2, HLA-DRA predicted FFS (HR=.56; p=.0030), while HIF1A predicted OS (HR=.57; p=.0047).

Conclusions: Unstained slides from FFPE tissue stored for many years may be used to investigate gene expression in lymphoma biopsy specimens for which there is mature follow-up. Changes in the prognostic profile associated with the addition of R to CHOP help to identify pathways impacted by rituximab, providing clues to its mechanism of action. BCL2 and HIF1A are powerful predictors of overall survival among RCHOP treated patients.

195 PROGNOSTIC VALUE OF THE 3Q27 AND 18Q21 TRANSLOCATIONS FOR DIFFUSE LARGE B-CELL LYMPHOMA AND FOLLICULAR LYMPHOMA IN THE RITUXIMAB ERA

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Introduction/Background: The 3q27 and 18q21 translocations are major chromosomal alterations in B cell lymphoma. Bcl-6 on chromosome 3q27 encodes B-cell lymphoma (Bcl)-6 protein, which inhibits differentiation to plasma cells. The 18q21 translocation cause the constitutive activation and increased expression of the antiapoptotic Bcl-2 protein. However, these prognostic impact of these translocations in the rituximab (R) era is unclear. We aim to determine the frequency of the 3q27 and 18q21 translocations in diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) and to evaluate their prognostic impact in the R era.

Patients and Methods: The study included 93 patients with DLBCL patients and 98 with FL; they had abnormal karyotypes that had been detected using G-banding at diagnosis. The patients underwent R-CHOP therapy: doxorubicin, cyclophosphamide, vincristine, prednisolone, and R; median follow-up period was 29 months.

Results: The 3q27 and 18q21 translocations were detected in 14 (15%) and 22 (24%), respectively, of the patients with DLBCL patients and in 14 (14%) and 77 (79%), respectively, of those with FL. The 2-year overall survival (OS) rates for patient with DLBCL and for those with FL were 79.0% and 95.1%, respectively; the 2-year progression-free survival (PFS) rates were 70.1% and 62.0%, respectively. The 2-year OS and PFS rates did not significantly differ between the 3q27, 18q21, and other chromosomal abnormality groups for DLBCL (2-year OS, 65.3% vs. 85.7% vs. 79.3%, respectively; 2-year PFS 59.2% vs. 65.4% vs. 73.6%, respectively; not significant) and FL (2-year OS, 85.7% vs. 94.7 vs. 100%, respectively; 2-year PFS, 42.9% vs. 64.0% vs. 60.6%; NS). These rates did not significantly differ between the patients with 3q27 translocation-positive DLBCL and those with 3q27 translocation-positive FL or between the patients with 18q27 translocation-positive DLBCL and those with 18q27 translocation-positive FL.

Conclusions: In the R era, the presence of the 3q27 and 18q21 translocations is not correlated with the clinical outcome of patients with DLBCL or FL.

196 THE DIFFERENT EPIDEMIOLOGIC SUBTYPES OF BURKITT LYMPHOMA SHARE A LARGELY HOMOGENOUS MICRO RNA PROFILE DISTINCT FROM DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction: Sporadic Burkitt Lymphoma (sBL) can be delineated from diffuse large B-cell lymphoma (DLBCL) by a very homogeneous mRNA expression signature. However, it remained unclear whether all three BL variants – sBL, endemic BL (eBL) and immunodeficiency-associated BL (HIV-BL) – represent a uniform biological entity despite their differences in geographical occurrence, association with immunodeficiency and/or incidence of EBV infection. To address this issue, we generated microRNA (miRNA) profiles from 18 eBLs, 31 sBLs and 15 HIV-BLs. In addition, we analyzed the miRNA expression of 86 DLBCLs to determine whether miRNA profiles recapitulate the molecular differences between BL and DLBCL evidenced by mRNA profiling.

Patients and Methods: Archival specimens of DLBCLs and BLs were obtained from the Institute of Pathology, Campus Benjamin Franklin, Charité-Universitätsmedizin Berlin, Germany, the Department of Human Pathology and Oncology, University of Siena, Italy and the Department of Human Pathology, University of Nairobi, Kenya. Samples were hybridized to Ohio State University custom miRNA microarray chips. MiRNA data were validated by qRT-PCR.

Results: A signature of 38 mature miRNAs (FDR<0.05) was obtained between BLs and DLBCLs. Validation was performed with 11 out of 38 miRNAs, chosen due to their involvement in MYC or NFkB signalling and their accordant importance for the biology of BL and DLBCL. The t-test confirmed that hsa-miRs-23a, -26a, -29b, -30d, -146a, -146b-5p, -155 and -221 are statistically significantly higher expressed in DLBCL than in BL samples. Furthermore, ANOVA (FDR<0.05) detected minor miRNA expression differences between BL variants. In particular, 6 miRNA (hsa-miR-191, hsa-miR-374a, hsa-miR-193a-5p, hsa-miR-10b, hsa-miR-216b, hsa-miR-499-3p) showed marginal expression differences between eBL and sBL.

Conclusions: Based on the current WHO diagnostic criteria, BL cannot reliably be distinguished from other types of mature aggressive B-cell lymphoma, especially from diffuse large B-cell lymphoma (DLBCL). We have therefore used miRNA profiling to gain further insights into the molecular pathology of BL with respect to its distinction from DLBCL and with respect to the differences between its epidemiological variants. Our miRNA profiling results confirm that BL and DLBCL represent distinct lymphoma categories and demonstrates that the three BL variants are representatives of the same biological entity with only marginal miRNA expression differences between eBL and sBL.

197 MICRO-RNA SIGNATURES IN BL AND DLBCL. A POTENTIAL DIAGNOSTIC TOOL

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B-cell lymphomas are currently diagnosed on the basis of integrated morphological, immunophenotypic, and molecular findings, thus allowing a more precise definition of different lymphoma types, providing specific molecular markers, and facilitating treatment stratification. In spite of this, some tumor types remain poorly studied, and new molecular markers are still required for some diagnosis. It is the case of BL and DLBCL, two different entities that occasionally are difficult to distinguish, but require a correct diagnosis, since they are treated in a different way.

MicroRNAs (miRNA) are non-coding small RNAs that play a key role in gene expression regulation, contributing to cell differentiation and tumorigenesis. It has been demonstrated that miRNAs have a potential role in cancer diagnosis and specific lymphoma-type recognition. We have recently identified miRNA signatures for the most frequent B-cell lymphoma types, using miRNA microarrays platforms and RT-qPCR. Of special interest was to compare miRNA expression profiles of DLBCL and BL. Thus, the direct comparison of miRNA microarray data between DLBCL and BL identified 43 miRNAs differentially expressed (FDR<0.01). A further RT-qPCR analysis of 28 BL and 43 DLBCL FFPE samples restricted this to a set of 19 miRNAs differentially expressed among these two lymphoma types.

This data generated could contribute to the differential diagnosis of BL and DLBCL. Indeed, while adding 12 more cases to this series, some of these miRNAs, such as miR-

155, miR-146a, or miR-595 and miR-520d are revealing as best differentiators of these two entities.

Then we stepped forward and introduced a series of intermediate BL-DLBCL, a poorly defined group of aggressive B-cell lymphomas that requires further molecular and clinical studies. An unsupervised cluster using the previous selected 19 miRNAs signature divided the group of intermediate cases between BL and DLBCL, expressing miRNAs from the signature of BL, such as miR-520d or miR-595 or from the signature of DLBCL, such as miR-155 or miR146a. Bioinformatic and functional analysis of this series of cases is identifying potential targets for the miRNAs, and elucidating the role of c-myc and other transcription factors in the generation of the miRNA signatures.

198 MICRORNA EXPRESSION PROFILE IDENTIFIES A DIFFERENT SIGNATURE BETWEEN MYC TRANSLOCATION-POSITIVE AND NEGATIVE BURKITT LYMPHOMA CASES

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Introduction: Burkitt lymphoma (BL) is an aggressive B-cell lymphoma with a characteristic clinical presentation, morphology and immunophenotype. Over the past years, the typical MYC translocation and its variants have been considered the molecular hallmark of this tumor. However, BLs with no detectable MYC rearrangement have been identified, though MYC expression was comparable with cases carrying the translocation. Dysregulation of c-MYC expression may be due to additional mechanisms, such as a microRNA (miRNA) imbalance. We previously demonstrated that two miRNAs, hsa-miR-9* and hsa-miR-34b, are differentially expressed between MYC translocation-negative and -positive BLs, and may account for c-MYC over-expression in the absence of translocation. In this study, we have analyzed the complete miRNA expression profile to achieve an overall view into the microRNA dysregulation in such cases.

Patients and Methods: Twenty formalin-fixed and paraffin-embedded (FFPE) BL specimens were collected at the Department of Human Pathology, University of Nairobi, Kenya and at the Department of Human Pathology and Oncology, University of Siena, Italy. For miRNA profiling, we hired an external facility (Exiqon, Copenhagen, Denmark), using the miRCURY^{TM} LNA Array (5th Generation arrays, hsa, mmu and rno, Exiqon). MiRNA data were validated by qRT-PCR.

Results: A two-tailed T-test identified 15 differentially expressed miRNAs out of 1273 with p-values lower than 0.01. MYC translocation-negative BLs showed a trend of miRNA down-regulation in contrast to the MYC translocation-positive ones. qRT-PCR validation was performed on hsa-miR-29c, hsa-miR-30e and hsa-miR-331-5p. Unexpectedly, no significant difference in hsa-miR-34b and hsa-miR-9* expression emerged. One explanation for such a discrepancy could be that microarray and qRT-PCR techniques have different degrees of sensitivity. In fact, microarray results were confirmed by qRT-PCR, and fold changes were higher than those obtained by microarray studies.

Conclusions: Profiling of the miRNome offers a powerful tool to gain better insight into different cancer entities. Using this approach, we showed that MYC translocation-positive and negative BLs differ in terms of miRNA expression, revealing a clear-cut miRNA signature between such cases. This finding give new evidence on c-MYC regulation and may have implications for a broad range of important physiological processes, the dysregulation of which could have an impact on the lymphomagenesis of these cases.

199 NEW INSIGHTS INTO THE PATHOGENESIS OF BURKITT LYMPHOMA: EBV-ENCODED AND HUMAN MICRORNA PROFILING

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Introduction: Burkitt Lymphoma (BL) is an aggressive B-cell lymphoma with a high proliferative index. The World Health Organization (WHO) classification of lymphoid recognizes three clinical forms of BL: endemic (eBL), sporadic (sBL) and immunodeficiency-associated (ID-BL). Each affects different populations and can present in different forms. More than 90% of eBL carry latent Epstein-Barr Virus (EBV) in the form of nuclear extra-chromosomal episomes, whereas only about 20% of sBL are associated with EBV. Recent data suggest a cellular microRNA (miRNA) dysregulation in sBL and eBL cases, which mainly differ in term of EBV association and possibly cell of origin. Furthermore, it should be considered that EBV itself encodes viral miRNAs, which maybe partecipate in BL pathogenesis. In order to gain an overall view into miRNA alterations in EBV-positive and –negative BLs, we profiled human and viral miRNA expression in such cases.

Patients and Methods: Fresh tissues of eighteen cases of BL (six EBV-positive BLs and twelve EBV-negative BLs) collected at the Department of Human Pathology, University of Nairobi, Kenya, at the Department of Human Pathology and Oncology, University of Siena, Italy, and at National Cancer Research Centre, CNIO, Madrid, Spain were used for this study. For miRNA profiling Agilent® miRNA expression microarray technology was used. After array normalization, a t-test (limma) was used to compare EBV-positive and -negative samples.

Results: Firstly, we identified 13 out of 64 viral miRNA expressed in EBV-positive BL cases. Furthermore, miRNA profiling revealed 8 out of 470 human miRNA deregulated in EBV-positive and –negative BLs (FDR<0.05). Bioinformatic prediction on human miRNAs suggests their involvement on cell cycle, proliferation, apoptosis and differentiation. MiRNA validation by qRT-PCR and functional in vitro studies on selected miRNAs will be discussed.

Conclusions: The EBV contribution to the pathogenesis of BL is still matter of debate. Our preliminary results identified a subset of viral miRNA expressed in EBV-positive BL cases. In addition, microarray results revealed a differential expression of human miRNAs among EBV-positive and –negative BL cases. These preliminary finding pave the way to a better understanding into the emerging interplay between cellular miRNA and EBV infection in BL pathogenesis.

200 THE GENETICS OF RICHTER SYNDROME REVEALS DISEASE HETEROGENEITY AND PREDICTS SURVIVAL POST-TRANSFORMATION

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Background: The scarcity of biologic information about Richter syndrome (RS) has hampered the identification of molecular predictors of RS outcome.

Methods: We addressed this issue by performing a comprehensive molecular characterization of 86 pathologically-proven RS.

Results: *TP53* disruption (40/85, 47.1%) was the most frequent genetic lesion. By multivariate analysis and model validation, *TP53* disruption was selected as an independent predictor of RS survival (HR: 2.27; p=.004), along with achievement of CR after RS treatment (HR 0.21; p<.001) and ECOG PS >1 (HR: 4.58; p<.001). By recursive-partitioning analysis, *TP53* status, response to RS treatment and ECOG PS were used to build an algorithm for stratifying RS survival. Patients presenting with ECOG PS >1 had short survival, irrespective of *TP53* status and type of response to RS treatment (median: 7.8 months). Patients presenting with ECOG PS $\leq$1, but harboring *TP53* disruption or not achieving CR after RS treatment, had an intermediate survival (median: 24.6 months). Patients presenting with ECOG PS $\leq$1, no *TP53* disruption, and achieving CR after RS treatment displayed a long survival (70% at 5 years). RS was clonally related to CLL in 50/63 (79.3%) assessable pairs, and clonally unrelated in 13/63 (20.6%).Compared to clonally related RS, clonally unrelated RS harbored less frequently *TP53* disruption (23.1% vs 60.0%; p=.018) and stereotyped VH CDR3 (7.6% vs 50.0%; p=.009), and were characterized by a significantly longer survival (62.5 months; vs 14.2 months; p=.017).

Conclusions: *TP53* disruption is one of the major factors affecting RS survival. This observation provides the rationale for testing pre-transplant induction treatments with agents circumventing *TP53* disruption. Clonally unrelated RS is clinically and biologically distinct from clonally related RS and should be considered as a secondary DLBCL arising *de novo* in the context of CLL. Therefore, the diagnosis of RS should be restricted to clonally related cases.

201 WHAT'S GOING ON AT THE END OF TELOMERES IN SÉZARY SYNDROME?

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Background: Tumorigenesis is a multistep process that requires the accumulation of multiple genetic changes. Although the mechanisms generating aberrant cancer genomes remain incompletely understood, increasing evidence suggests that tumor development can be driven by shortening of telomeres, the nucleoprotein caps that

protect chromosome ends. Telomere status has been evaluated extensively in solid tumours, and on a smaller scale in hematopoietic tumours. Few studies have evaluated the same issue in cutaneous lymphoma and none to date in Sézary syndrome (SS).

Aim: In this context, we are interested to estimate chromosomal disorders and telomere length in SS cell lines (HuT78, SeAX) and in 20 SS patients from the Primary Cutaneous Lymphoma Aquitaine cohort by comparison with 43 healthy donors peripheral blood lymphocytes.

Material and Methods: Using cytogenetic tools (multicolour fluorescence *in situ* hybridisation (mFISH), FISH, array-comparative genomic hybridisation and R bands) we have detected both chromosomal rearrangements and estimated the ploïdy of SS karyotypes cell lines and patients. Quantitative polymerase chain reaction assay and quantitative FISH allowed us to estimate telomere length in SS cell lines and SS patients compared to controls donors.

Results: A significant reduction in telomere length regions, compared with healthy donors (P<0.0.01), was observed in more than 90% of cases (cell lines and patients). Median telomere length was 0.52 for SS patients and 1.01 for healthy donors. Complex karyotypes with 6 to 18 chromosomal rearrangements per metaphase were observed in more than 70% of cases (cell lines and patients). Chromosomes most frequently affected by such alterations (50% to 87.5%) were chromosomes 1, 2, 6, 8, 10, 17 and 19. Interestingly, such complex karyotypes were generally observed in near-diploid tumors. Moreover, such pattern was stable in sequential samples of 4 patients suggesting that after a ''telomere'' crisis, the chromosomal rearrangements and imbalances define a specific cytogenetic signature of the disease.

Conclusion: Our data suggest that telomere shortening is a common feature in SS that may promote chromosome end-to-end fusions and chromosomal breakages at the onset of the disease and that such chromosomal signature remains stable after tumorigenesis.

202 MICRORNAS IN ADVANCED CLASSICAL HODGKIN LYMPHOMA: SIGNATURES WITH PROGNOSTIC SIGNIFICANCE

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Introduction: A significant fraction of patients with advanced classical Hodgkin lymphoma (cHL) are refractory to current standard treatments. MicroRNAs are regulators of important biological processes with a proven role in cancer pathogenesis. Although cHL specific miRNA signatures have been proposed, their potential prognostic role remains unclear.

Methods: Global miRNA expression data from 32 tumor samples of advanced cHL patients and 5 cHL-derived cell lines (L540, L1236, L428, HDLM2 and KMH2) were used to identify profiles from the HRS cells and their non-tumoral microenvironment validating selected miRNAs by laser capture microdissection. Top differentially expressed miRNAs among patients with favorable vs unfavorable outcome were selected to set up a qPCR assay for additional validation in an independent set of 229 cHL (FFPE) samples, randomly split and assigned to either estimation (152) or validation sets (68). A logistic regression predictive model was derived in the estimation series of patients, and confirmed in the validation series. To identify pathogenic roles and potential targets, two of the miRNAs found to be associated with worse outcome (mir21 and mir30d), were studied by oligonucleotide antimiRNA in cell lines.

Results: A cHL signature was identified, with 234 miRNAs differentially expressed (FDR<0.5) between tumoral samples and cHL cell lines, including miRNAs reporting from either theprimary HRS and their reactive background. The final model derived from the qPCR assay by logistic regression analysis included mir21, mir30e, mir30d, and mir92b and was able to classify correctly 70% of cases in the estimation set, and 64% in the validation set. Moreover, the different predicted risk groups showed significant differences in survival probability, with FFS probability of 72% versus 49% in the estimation and 56% versus 35% in the validation set. Oligonucleotide antimiRNAs transfection experiments showed decrease in both, miR-21 and miR-30d levels, and functional analyses could identify variations in cell viability after miRNA silencing.

Conclusion: MicroRNA expression analyses identify specific profiles from the tumor cells and their non-tumoral microenvironment, and clinical outcome can be predicted using regression models that integrate microRNA signatures.