Session 4: lymphoma genomics

055 INTRODUCTORY LECTURE

R. Siebert
Institute of Human Genetics, Christian-Albrechts-University Kiel, Kiel, Germany

Since the first introduction of a genome-wide screening technique, namely cytogenetic analysis of banded metaphase chromosomes, into the analysis of malignant lymphoma in the 1970s the detection of genetic aberrations in neoplastic lymphocytes and their precursors has significantly advanced our understanding of lymphoma biology. Consequently, the identification of recurrent genetic aberrations has fostered biology-based lymphoma classification systems and the detection of such aberrations is increasingly gaining importance in the diagnostic process. The detection of so called primary chromosomal aberrations, which predominately means recurrent chromosomal translocations leading to oncogene activation, is becoming standard in many diagnostic laboratories. Nevertheless, the advent of genome-wide high-resolution array-based screening technologies (like array-CGH, SNP-arrays or array-based gene expression or epigenetic profiling) and the very recent introduction of genome-wide sequencing on the DNA- and RNA-level has yielded high-dimensional data which now pose novel challenges for diagnostics and prognostication. On the one hand these technologies provide exciting insights into novel tumorigenic events, commonly deregulated pathways and potentially druggable targets, on the other the overwhelming amount of data require cautious interpretation and clinical evaluation in trials. Moreover, novel genomic markers need to be considered in the light of well established clinical variables. The future integration of novel findings in lymphoma genomics into routine lymphoma care leaves us with several challenging questions, like: What is the leading marker of lymphoma diagnostics? Which kind of genetic diagnostics is indicated? What do genetic factors add to prognostication? Which new therapies can be developed on genomic findings? This introductory lecture aims at reviewing recent developments in lymphoma genomics and epigenomics and at highlighting the importance of (epi)genomics for lymphoma biology, classification, prognostication and treatment.

056 FUNCTIONAL CHARACTERIZATION OF AN 11Q24.3 GAIN CONTRIBUTING TO THE PATHOGENESIS OF DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) BY BLOCKING B-CELL MATURATION

P. Bonetti[1], M. Testoni[1], M. Scandurra[1], M. Ponzoni[2], R. Piva[3], M. Tibiletti[4], I. Kwee[1], A. Mensah[1], T. Greiner[5], W. Chan[6], G. Gaidano[7], M. Piris[8], E. Zucca[1], G. Inghirami[9], F. Bertoni[1]
[1]Experimental Oncology, IOSI, Bellinzona, Switzerland, [2]HSR, MI, Italy, [3]CeRMS, Turin, Italy, [4]Insubria Univ, Varese, Italy, [5]Nebraska Univ, Omaha, United States, [6]Nebraska Univ, Omaha, United States, [7]A.A. East. Piedmont Univ., Novara, Italy, [8]CNIO, Madrid, Spain, [9]CeRMS, Turin, Italy

Introduction: DLBCL is a heterogeneous disease. Normal germinal center (GC) B-cell differentiation requires a complex transcriptional program and alterations of genes involved in this process (for example, BCL6, BLIMP1/PRDM1) are relevant for DLBCL pathogenesis.

Aim: To study the pathogenetic role of an 11q24.3 recurrent gain in DLBCL patients.

Patients and Methods: Genomic profiles were obtained from 166 Affymetrix 250K SNP arrays and integrated with gene expression data (GeneChip U133 plus 2.0) in 54 cases. Data were validated by PCR and immunohistochemistry. Gene silencing experiments were done with shRNA and run in triplicate.

Results: A recurrent gain was detected at 11q24.3 in 26% of DLBCL and was associated with high expression of the transcription factors ETS1 and FLI1. Gene expression analysis revealed 228 transcripts with a significantly different expression between cases with or without the lesion ($p<0.01$, >2-fold change). Gene set enrichment analysis (GSEA) identified genes up-regulated by the EWSR1/FLI1 fusion protein as the most significant overlap between genes up-regulated in 11q24+DLBCL and GSEA Chemical and Genetic Perturbations catalog, suggesting a biological effect of the observed genomic lesion. No relation with DLBCL cell of origin was observed. To study the biological meaning of the lesions, ETS1 and FLI1 expressions were down-regulated in a DLBCL cell line bearing the same lesion observed in clinical specimens. Preliminary results showed the down-regulation causes cell death. Also, the transcriptional program of GC B-cell terminal differentiation was affected after gene silencing. In particular, down-regulation of ETS1 caused an up-regulation of BLIMP1, the master regulator of plasma cell differentiation. On the other hand, FLI1 silencing caused down-regulation of PAX5, a transcription factor that inhibits plasma cell differentiation.

Conclusions: In DLBCL, a recurrent gain at 11q24.3 determines the over-expression of the transcription factors ETS1 and FLI1. Functional experiments suggest that these genes are essential for viability of DLBCL cells and that they contribute to the developmental arrest during GC B-cell transition towards plasma cells. PB, MT, MS equally contributed.

057 PRDM1 IS A TUMOR SUPPRESSOR GENE IN NATURAL KILLER CELL MALIGNANCIES

C. Kucuk[1], J. Iqbal[1], R. Lu[2], T. Mckeithan[3], P. Gaulard[4], W. C. Chan[1]
[1]Pathology and Microbiology, University of Nebraska Medical Center, Omaha, United States, [2]Cell Biology and Genetics, University of Nebraska Medical Center, Omaha, United States, [3]Internal Medicine, University of Nebraska Medical Center, Omaha, United States, [4]Departement de Pathologie, Faculte de medecine/Universite Paris, Creteil, France

Background: Natural Killer (NK)-cell lymphomas (NKCL) account for 1-2% of all non-Hodgkin lymphomas. Although the incidence of NKCLs is relatively low, the clinical course of these lymphomas is highly aggressive. The genes responsible for the neoplastic transformation of NKCLs have not been identified yet. We observed that *PRDM1* is frequently deleted and methylated and may act as a tumor suppressive gene in NKCLs.

Methods: *PRDM1* gene copy number analysis in NK cell lines and NKCL cases was performed using q-PCR. Biotage pyrosequencing was performed to assess the level of CpG methylation in the CpG island of the *PRDM1* promoter. Mutation analysis was done with Sanger sequencing of the PCR amplified fragments derived from the PRDM1 coding sequence of NKCL cases. PRDM1 was ectopically expressed in KHYG1 and KAI3, two NK cell lines with no PRDM1 expression, using a retroviral construct. Annexin-V and Hoescht staining were applied to assess apoptosis and cell cycle, respectively after PRDM1 reconstitution and when the cell lines were cultured at different levels of IL2. PRDM1 transcript levels were determined with q-RT-PCR on highly purified human peripheral blood NK cells cultured in the presence of IL2.

Results: We observed mono-allelic deletion of *PRDM1* in 6 of 8 (75%) NK cell lines and at least in 7 of 18 (39%) NKCL cases. Methylation analysis revealed a hypermethylated region up-stream of the transcriptional start site (TSS) compared with normal NK cells in 5 of 9 (56%) NK cell lines that correlates well with low transcription of *PRDM1*. A similar hypermethylation pattern was also observed in 12 of 17 (70%) NKCL cases. Mutation analysis did not reveal any deleterious mutations in 18 NKCL cases although they were demonstrated in two cell lines. The reconstitution ofPRDM1 increased apoptosis and led to G2/M cell cycle arrest in KHYG1 and KAI3 cells that was more pronounced when IL2 concentration in the culture was reduced. PRDM1 transduced cells were selectively eliminated in long term culture in KHYG1 cells. IL2 stimulated normal human NK cells isolated from the peripheral blood showed a progressive increase in *PRDM1* mRNA with time.

Conclusion: There is good evidence that *PRDM1* is a tumor suppressor gene that is inactivated by a combination of genetic and epigenetic mechanisms in NKCL. This conclusion is further supported by in vitro studies showing the negative selection pressure exerted upon reconstitution of PRDM1 expression in NK cell lines and the increase in the proportion of cells having cell cycle arrest and apoptosis after PRDM1 transduction. The upregulation of *PRDM1* in normal NK cells on activation with IL2 suggests that this gene may play a role in the regulation of homeostasis of NK cells.

058 IDENTIFICATION OF FOXO3 AND PRDM1 AS TUMOR SUPPRESSOR GENE CANDIDATES IN NK CELL NEOPLASMS BY THE COMBINATION OF GENOMIC AND FUNCTIONAL ANALYSES

K. Karube[1], M. Nakagawa[1], S. Tsuzuki[1], Y. Ko[2], S. Nakamura[3], M. Seto[1]
[1]Molecular Medicine, Aichi Cancer Center Research Institute, Nagoya, Japan, [2]Department of Pathology, Samsung Medical Center, Seoul, Korea, Republic of, [3]Department of Pathology and Clinical Laboratories, Nagoya University Hospital, Nagoya, Japan

Introduction: The molecular pathogenesis of NK cell neoplasms is still remained unknown. We performed oligo-array CGH analyses, gene expression profiling analyses and in vitro functional assays of NK cell neoplasms attempting to clarify their molecular pathogenesis.

Materials and Methods: Oligo array CGH analyses and comprehensive gene expression profiling analyses were performed on 35 cases of NK cell neoplasms and seven NK cell lines. Subsequently, we established Tet-OFF NKL, one of NK cell lines, for functional analyses.

Results: The narrow region of 6q21 contained seven known genes, POPDC3, PREP, PRDM1, ATG5, AIM1, LACE1 and FOXO3, were most frequently deleted (36%) (table). Moreover, the genomic regions including A20 and HACE1, famous tumor suppressor genes located in 6q, were deleted in 28% or 31% of the analyzed cases, respectively (table). Among these genes, expression levels of A20, PRDM1, HACE1 and FOXO3 were strongly down-regulated in neoplastic samples (table). However, only re-expression of FOXO3 and PRDM1 suppressed the Tet-OFF NKL proliferation while that of other genes did not (table). FOXO3 and PRDM1 were considered to be strong candidate genes and further analyzed. Mutation analysis revealed nonsense mutations

of PRDM1 in two cases (one cell line and one clinical sample) and missense mutations of FOXO3 in three clinical cases. Furthermore, phosphorylation of FOXO3, inactive form of FOXO3 translocating to the cytoplasm, was detected by the Western blotting analyses of NK cell lines.

Conclusion: The inactivation and the down-regulation of PRDM1 and FOXO3 were considered to play an important role in the pathogenesis of NK cell neoplasms.

Gene name	Frequency of genomic loss	Expression level*	Growth suppression rate**
PRDM1	36%	-0.41	0.35
FOXO3	36%	-0.74	0.13
A20	28%	-1.50	1.10
HACE1	31%	-0.45	1.17
ATG5	36%	-0.27	1.05
PREP	36%	-0.16	1.07
LACE1	36%	-0.11	0.95
AIM1	36%	-0.03	-
POPDC3	36%	NA***	-
GFP	-	-	1.06

* Average $\log_2$ ratio of expression levels between neoplastic samples and normal NK cells.
** Average ratio of the cultured cell numbers between those without Doxycyclin and those with Doxycyclin.
***not applicable because of the very low expression level in normal NK cells.

059 IDENTIFICATION OF A "DELETION BLOCK" AT 13Q14 THAT PERTURBS MULTIPLE SIGNALING AND SURVIVAL PATHWAYS IN PRIMARY CLASSICAL HODGKIN LYMPHOMA

B. Chapuy[1], M. Green[1], J. Lu[2], S. Monti[2], Y. Hao[1], K. Yeda[1], S. Rodig[3], T. Curie[3], K. Takeyama[1], T. Golub[2], J. Kutok[3], M. Shipp[1]
[1]Medical Oncology, Dana-Farber Cancer Institute, Boston, United States, [2]Cancer Program, Broad Institute, Cambridge, United States, [3]Department of Pathology, Brigham & Women's Hospital, Boston, United States

Classical Hodgkin Lymphoma (cHL) Reed-Sternberg (RS) cells have crippling mutations of their rearranged Ig genes, lack B-cell receptor-mediated survival signals and rely on alternative survival pathways including Pi3K, NFkB and AP-1. Although cHL RS cells exhibit constitutive activation of AP-1, the underlying mechanism remains undefined. In a screen for genetic abnormalities in cHL, we integrated microRNA (miR) expression profiles with high-resolution copy number data and identified single copy loss of the miR-15a/16-1 locus (chromosome 13q14) and decreased miR-15a/16 expression in 75% of cHL cell lines. In laser-capture microdissected primary Hodgkin RS cells, we confirmed single copy loss of the miR-15a/16-1 locus by qPCR and associated decreased miR-15a/16 abundance by RT-PCR in 5/7(71%) of mixed cellularity and 4/12(33%) of nodular sclerosis cHL(9/19 [47%] total). We reconstituted miR-15a/16 expression with retroviral vectors in cHL cell lines with single copy loss of chromosome 13q14. Reconstitution of miR-15a/16 was associated with cell cycle arrest, significantly decreased cellular proliferation and induction of apoptosis (88% apoptotic cells at 48 hours), and significant downregulation of multiple survival pathway components and known miR-15a/16 targets including panAKT, BCL2 and cyclin D1. Using a stand-alone miRanda algorithm with direct sequence entry, we also identified candidate miR-15a/16 binding sites in the AP-1 component, cJun, and confirmed miR-15a/16-dependent expression using luciferase reporters. In multiple cHL cell lines, miR-15a/16 reconstitution decreased the expression of cJun and additional known AP-1 targets such as galectin-1. Because the 13q14 deletion in HL extends beyond the microRNA locus, we hypothesized that this "deletion block" contained additional independent tumor suppressors. Of interest, the recently described negative regulator of NFkB signaling, Dleu7, was included in the cHL 13q14 deletion. Using a quantitative IHC approach in primary cHL specimens, we confirmed lower Dleu7 protein abundance in the cases with miR-15a/16-1 loss. Functional reconstitution of Dleu7 markedly decreased cellular proliferation in informative HL cell lines. In summary, we have identified a frequent 13q14 "deletion block" in cHL cell lines and primary tumors that includes cooperating tumor suppressors, miR-15a/16-1 and Dleu7, which modulate multiple critical survival pathways.

060 NEXT GENERATION SEQUENCING REVEALS GENES INVOLVED IN HISTONE MODIFICATION ARE FREQUENTLY MUTATED IN NON-HODGKIN LYMPHOMA

R. D. Gascoyne[1], R. Morin[2], M. Mendez-Lago[2], A. Mungall[2], N. Johnson[1], D. Scott[1], R. Moore[2], J. Connors[3], M. Hirst[2], R. Goya[2], L. Rimsza[4], S. Jones[2], D. Horsman[1], K. Mungall[2], M. Marra[2].
[1]Pathology, British Columbia Cancer Agency, Vancouver, Canada, [2]Genome Sciences Centre, BC Cancer Agency, Vancouver, Canada, [3]Medical Oncology, BC Cancer Agency, Vancouver, Canada, [4]Pathology, University of Arizona, Tucson, United States

Introduction: Follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) are the two most common types of non-Hodgkin lymphoma (NHL) and together represent 60% of NHL diagnoses each year in North America and Europe.

Methods: To identify genes and mutations broadly relevant to lymphomagenesis, we sequenced tumour and matched normal DNA from a single FL and three DLBCL cases. To explore the frequency of somatic mutations in the genes identified, we then sequenced the mRNA from an extension set composed of an additional 119 NHL samples, including 10 DLBCL cell lines, and 8 normal centroblast controls.

Results: This analysis revealed candidate somatic mutations in 137 genes in at least three of the extension set cases and significant evidence for positive selection acting on 25 of these. Among these candidate mutations, we confirmed additional somatic mutations in 41 of these genes, most of which had no previously known role in lymphoma. Among the recurrently mutated genes, we observed many previously implicated in altering chromatin structure and function. For example, 32% of DLBCL and 89% of FL cases had nonsense or frame-shifting somatic mutations in *MLL2*, which encodes a histone methyltransferase whose function is associated with transcriptional activation; 27.5% of DLBCL cases had somatic mutations in either of *CREBBP* or *EP300*, a pair of functionally related histone acetyltransferases and 12.7% of DLBCL and 15.3% of FL cases had somatic mutations in *MEF2B*, a calcium-regulated gene that cooperates with CREBBP and EP300 in acetylating histones. Some recurrent mutations were cell-of-origin (GCB *vs* ABC) specific.

Conclusions: These data provide a comprehensive overview of the mutational landscape of DLBCL and give some insight into recurrent mutational events in FL. Importantly; these data implicate several novel genes in the malignant transformation of B cells and specifically indicate a previously unappreciated role of perturbations to maintenance of epigenetic histone marks in lymphomagenesis.