Detection of genomic imbalances in microdissected Hodgkin and Reed-Sternberg cells of classical Hodgkin's lymphoma by array-based comparative genomic hybridization

Sylvia Hartmann,[1] José I. Martin-Subero,[2] Stefan Gesk,[2] Julia Hüsken,[1] Maciej Giefing,[2,3] Inga Nagel,[2] Jennifer Riemke,[2] Andreas Chott,[4] Wolfram Klapper,[5] Marie Parrens,[6] Jean-Philippe Merlio,[6] Ralf Küppers,[7] Andreas Bräuninger,[1] Reiner Siebert,[2] and Martin-Leo Hansmann[1]

[1]Senckenberg Institute of Pathology, University of Frankfurt, Frankfurt, Germany; [2]Institute of Human Genetics, Christian-Albrechts-University Kiel, University Hospital Schleswig- Holstein, Campus Kiel, Kiel, Germany; [3]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland: [4]Dept. of Pathology, Medical University of Vienna, Vienna, Austria; [5]Dept. of Pathology, Christian-Albrechts-University Kiel, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany; [6]Dept. of Pathology and Molecular Biology, CHU and University of Bordeaux 2, Bordeaux, France; [7]Institute for Cell Biology (Tumor Research), University of Duisburg-Essen, Medical School, Essen, Germany

Supplementary Data

Quantitative PCR with genomic DNA for confirmation of CDKN2B *deletion*

Sets of 100 microdissected CD30-positive HRS cells and reactive lymphocytes from the same case underwent proteinase K-digestion (Roche, Mannheim, Germany) for 8 h at 55°C with 10 min inactivation at 95°C. *TIE1* was selected as the control gene since no genomic aberrations of *TIE1* were observed in CGH array data. The first round of PCR was conducted for each amplicon of *CDKN2B* as a multiplex reaction with *TIE1* as the control gene according to the following thermal profile: 95°C for 5 min, 61°C for 1 min, 72°C for 45 sec and then 25 cycles of 95°C for 30 sec, 61°C for 30 sec, and 72°C for 45 sec. The following first round primers were used: *CDKN2B*-Exon1-US1: 5´-TGGGAAGAAGGGAAGAGTGTCG-3´, *CDKN2B*-Exon1-LS: 5´-TGTCGCACCTTCTCCACTAGTCC-3´, *CDKN2B*-Exon2-US1: 5´-CATGCGTAAACGACACTCTCTGG-3´, *CDKN2B*-Exon2-LS: 5´-CTCATCCTGTGTAGTCTGCCTGC-3´, *TIE1*-Exon14B-US: 5´-AGCCCTTGCCAGCCCTTTCTCC-3´, *TIE1*-Exon14B-LS1: 5´-CCCTGGCAAGCTACTCATGTGG-3´.

The second round of PCR for *CDKN2B* and *TIE1* was performed as quantitative realtime-PCR in duplicates using Roche FastStart Universal SYBR Green Master (ROX) on an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Darmstadt, Germany) with the software SDS 2.2.1. For the second round of PCR the following primers were used:

CDKN2B-Exon1-US2: ACGGCCAACGGTGGATTATCCG-3´, *CDKN2B*-Exon1-LS: 5´-TGTCGCACCTTCTCCACTAGTCC-3´, *CDKN2B*-Exon2-US2: 5´- TCGCCCAACTCCACCAGATAGC-3´, *CDKN2B*-Exon2-LS: 5´-CTCATCCTGTGTAGTCTGCCTGC-3´, *TIE1*-Exon14B-US: 5´-AGCCCTTGCCAGCCCTTTCTCC-3´, *TIE1*-Exon14B-LS2: 5´-TACTCATGTGGTGGAGGTGATGG-3´.

CDKN2B-Exon1-PCR, *CDKN2B*-Exon2-PCR and *TIE1*-PCR yielding amplicons of 144 bp, 175 bp and 240 bp, respectively.

Supplementary Table S1. Immunophenotypes of cHL cases used for array CGH.

Cases	*Subtype*	*CD30*	*ALK-1*	*EBER*	*CD15*	*PAX5*
case 1	LD	+	-	+	-	+
case 2	NS	+	-	-	+	-
case 3	NS	+	-	-	-	+
case 4	LD	+	-	-	+	-
case 5	LD	+	-	-	+	+
case 6	LD	+	-	-	-	+
case 7	NS	+	-	-	-	+
case 8	NS	+	-	-	+	-
case 9	LD	+	-	-	-	+
case 10	NS	+	-	-	+	+
case 11	LD	+	-	-	+	+
case 12	LD	+	-	-	-	+

LD: lymphocyte depleted subtype of cHL, NS: nodular sclerosing subtype of cHL.

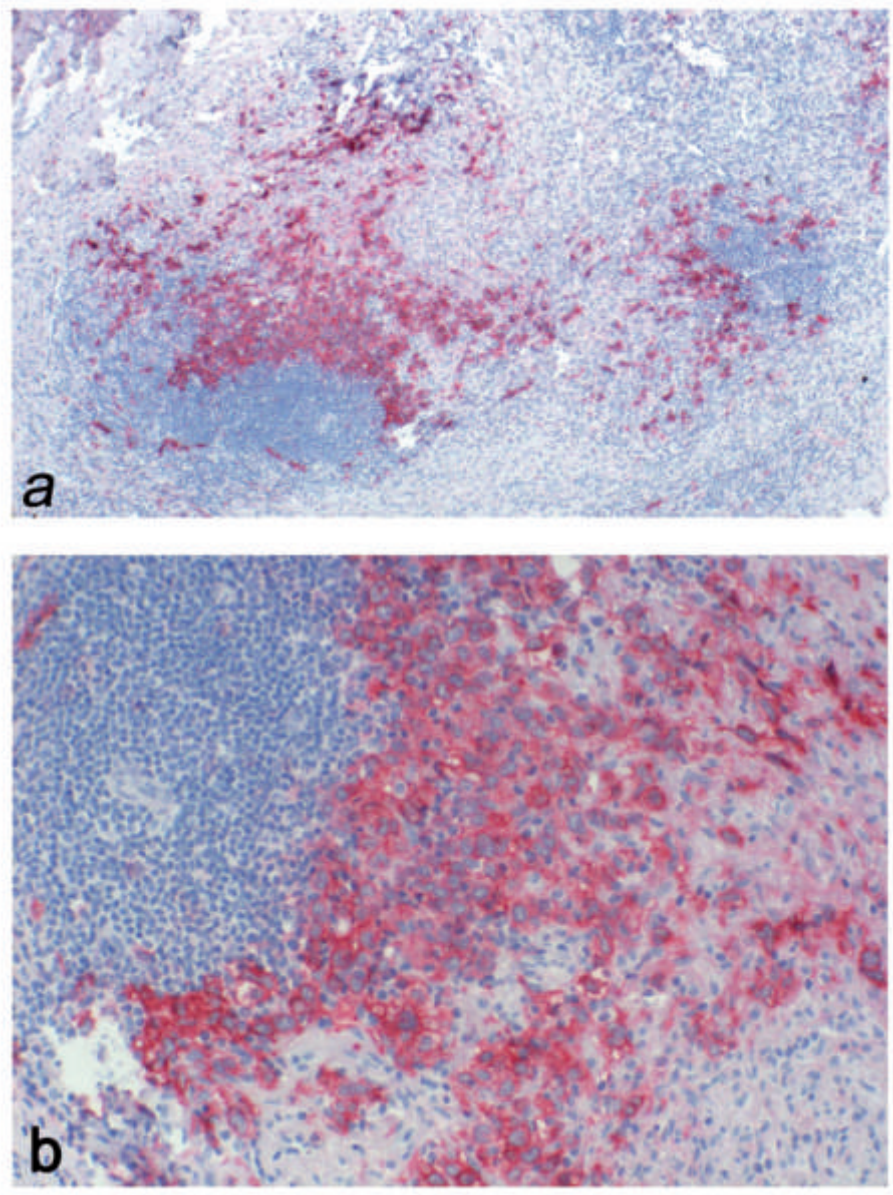
Supplementary Table S2. BAC clones used for FISH and FICTION experiments.

Gene (chromosomal band)	*BAC clones*
REL (2p16.1)	RP11-373L24, RP11-49805
CHD1 (5q21.1)	RP11-58M12,
TNFAIP3/A20 (6q23.3)	RP11-703G8, RP11-783B20
TEK (9p21.2)	RP11-702A14
NOTCH1 (9q34.3)	RP11-251M1, RP11-83N9
STAT6 (12q13.3)	RP11-145A4, RP11-17605
CDK4 (12q14.1)	RP11-93617, RP11-1077C21
JUNB (19p13)	CTD-2659N19, RP11-151022

Supplementary Table S3. Distinct amplifications in HRS cells from ten primary cHL cases.

Chromosome	*Band*	*Linear position (Mb)*[a]	*Case 1*	*Case 3*	*Case 4*	*Case 5*	*Case 6*	*Case 7*	*Case 11*	*Candidate genes*[b]
1	q31-q32	190-211	0.8							
2	p13-16	44-73					0.8			REL
2	q32	189.9-194				1.5				STAT1, STAT4, PMS1
5	q12	50-53.3						0.8		
7	q21-22	102.9-107.6				1.5				
8	q13	67.1-67.2		0.8						
8	q24	143.3-146.2						0.8		
9	p13-24	0-38		0.8						PAX5
9	q22	90.6-90.7			0.9					SYK
10	q24.32	103.8-104.1		0.9						NFKB2
15	q21	48.1-50				1.1				
17	q25	77.4-77.5							1.0	
19	p13.2	8.2-15.1		0.9						TYK2, JUNB
20	q11.22	28.1-31.8				0.8				E2F1

Average log2-ratios of amplified regions in ten cHL cases on 105 K Agilent oligonucleotide arrays. The statistical algorithm applied is ADM-2 with a threshold of 6.0. [a]Linear position on the chromosome in Mb (UCSC Genome Browser on Human - May 2004 Assembly). [b]Candidate genes were selected on the basis of a database of known cancer genes.[26] Cases 2, 8 and 12 did not show any distinct amplifications.



Supplementary Figure S1. cHL case 3 (Nodular sclerosis II), CD30-immunostaining. (A) 4x magnification, (B) 20x magnification.