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Classical Hodgkin lymphoma shows epigenetic features of an abortive plasma cellular differentiation

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Abstract

Background
Epigenetic changes are involved in the extinction of the B-cell gene expression program of classical Hodgkin lymphoma. However, little is known regarding epigenetic similarities between classical Hodgkin lymphoma and plasma cell myeloma cells both of which share an extinction of the gene expression program of mature B-cells.

Design and methods
Global histone H3 acetylation patterns were determined in cell lines derived from classical Hodgkin lymphoma, plasma cell myeloma and B-cell lymphoma by chromatin immunoprecipitation and subsequent hybridization onto promoter tiling arrays. H3K27 trimethylation was analyzed by chromatin immunoprecipitation and real-time DNA-PCR for selected genes. Epigenetic modifications were compared to gene expression data.

Results
B-cell characteristic genes were hypoacetylated in classical Hodgkin lymphoma and plasma cell myeloma cell lines as demonstrated by comparison of their histone H3 acetylation patterns to those of B-cell lines. However, the number of genes jointly hyperacetylated and expressed in classical Hodgkin lymphoma and plasma cell myeloma cell lines such as IFR4/MUM1 and RYBP is limited. Moreover, H3K27 trimethylation for selected B-cell characteristic genes revealed that this additional epigenetic silencing is much more prevalent in classical Hodgkin lymphoma as compared to plasma cell myeloma.

Conclusion
Our epigenetic data support the view that classical Hodgkin lymphoma is characterised by an abortive plasma cell differentiation with a down-regulation of B-cell characteristic genes but without activation of most plasma cell typical genes.
Introduction

Disruption of epigenetic patterns is recognized as one of the hallmarks of cancer and plays a central role in determining the tumor phenotype.\textsuperscript{1} Furthermore epigenetic analyses are of interest since epigenetic therapy is emerging as a valuable and effective treatment approach for peripheral T-cell lymphoma and is also envisaged for B-cell and Hodgkin lymphoma.\textsuperscript{2-5}

There are different sets of epigenetic instructions which are involved in the regulation of gene expression.\textsuperscript{1} DNA methylation of gene promoters significantly contributes to gene silencing whereas histone modifications such as acetylation of lysine 9 and 14 of histone H3 (H3K9/14 acetylation) is positively correlated with gene activation.\textsuperscript{1} Furthermore epigenetic silencing of genes can be mediated by Polycomb complex (PcG) proteins.\textsuperscript{6} PcG proteins were first identified in Drosophila as regulators of the expression of \textit{Hox} genes.\textsuperscript{7} In humans the PcG proteins comprise two functionally and biochemically distinct multimeric Polycomb repressive complexes (PRCs), called PRC1 and PRC2. According to a currently proposed model PRC2 initiates transcriptional repression through trimethylation of histone H3 lysine 27 (H3K27) whereas PRC1 maintains this repressive condition.\textsuperscript{6} PcG complexes play an important role in the B-cell development and the germinal center reaction, and increased or decreased activity of PcG proteins is thought to contribute to lymphomagenesis.\textsuperscript{8-10} The precise interplay between the various epigenetic mechanisms is very complex and not fully understood.\textsuperscript{1,11,12}

Classical Hodgkin lymphoma (cHL) is a monoclonal lymphoid neoplasm derived from (post-) germinal center B-cells in almost all instances.\textsuperscript{13-15} Morphologically, cHL is composed of a usually small number of mononuclear Hodgkin (H) cells and multinucleated Reed-Sternberg (RS) cells residing in an extensive inflammatory background.\textsuperscript{15} A key feature, that distinguishes cHL from other B-cell lymphomas, is the almost complete absence of B-cell markers from the HRS cells (e.g. CD19, CD20 and CD79b) and the up-regulation of B-cell lineage inappropriate genes.\textsuperscript{15,16}

Recently it has been proposed that the initial events finally leading to cHL are caused by epigenetic modifications which have the capacity to induce a transcriptional avalanche effect including an up-regulation of cHL-characteristic but B-cell lineage inappropriate genes and an extensive down-regulation of the B-cell expression program.\textsuperscript{17}
A down-regulation of genes typically expressed in mature B-cells also occurs in the course of plasma cellular differentiation, suggesting that the HRS cells might be related to plasma cells.\textsuperscript{18,19} However, immunoglobulin gene expression - which is extremely high in plasma cells - is completely absent from HRS cells. In addition most other markers typically up-regulated in plasma cell are not produced by the HRS cells.\textsuperscript{18}

To elucidate the extent of the relationship between HRS cells and plasma cells from an epigenetic perspective, we analyzed their genome-wide histone H3 acetylation pattern in corresponding cell lines and compared the results with those obtained from B-cell lines. For this genome-wide analysis high density promoter tiling arrays were used in combination with chromatin immunoprecipitation (ChIP-on-chip) employing H3K9/14ac antibodies. Although \textit{in-vitro} cultured cancer cells might contain some artificial epigenetic modifications, we employed established cHL, B-cell lymphoma and plasma cell myeloma (PCM) cell lines since they have retained their cell type specific phenotype and are thus useful models reflecting the \textit{in-vivo} situation in many aspects.\textsuperscript{20} Our results provide evidence that cHL is derived from cells with an incomplete plasma cellular differentiation that show an extensive down-regulation of B-cell antigens but no activation of most plasma cell typical genes. Furthermore, additional suppressive H3K27 trimethylation of B-cell characteristic genes is more extensively found in cHL as compared to PCM cell lines.

\textbf{Design and Methods}

\textit{Cell culture}

Hodgkin lymphoma (cHL) cell lines (L1236, KM-H2, L428 (referred to as cHL cell lines), three plasma cell myeloma (PCM) cell lines (L363, U266, LP-1 (referred to as PCM cell lines) and four B-cell lymphoma cell lines (SU-DHL4, SU-DHL6, HT [diffuse large B-cell lymphoma; DLBCL] and Namalwa [Burkitt lymphoma; BL] (referred to as B-cell lines) were cultured with 5% CO\textsubscript{2} in RPMI 1640 (PAA, Pasching, Austria) and supplemented with 10% fetal bovine serum (PAA) at 37°C.

\textit{Chromatin immunoprecipitation (ChIP)}

B-cell lines (SU-DHL4, SU-DHL6, Namalwa), cHL cell lines (L1236, KM-H2, L428) and PCM cell lines (L363, U266, LP-1) were used for ChIP following the protocol developed by the group of Young with minor modifications.\textsuperscript{21} For each ChIP experiment 10 \textmu g of anti-acetyl-histone H3 (Lys9 + Lys14) antibody (06-599; Millipore, Temecula, CA, USA) was
employed. In addition, H3K27 trimethylation was analyzed by ChIP for selected genes in cell lines derived from cHL (L428, L1236, KM-H2), PCM (U266, L363) and B-cell lymphomas (Namalwa, SU-DHL4) using 15-20 µg of an anti-H3K27 trimethylation antibody (07-499, Millipore). The specificity and suitability of these antibodies for ChIP has been shown by previously published work. A detailed protocol is provided as supplementary information (supplementary Protocol 1).

Successful enrichment of ChIP DNA-fragments was confirmed by quantitative real-time PCR employing SYBR Green PCR-Master Mix (Applied Biosystems, Foster City, CA, USA) on a GeneAmp7900HT Fast Real-Time PCR system (Applied Biosystems). Primer sequences are available from the supplementary Table S1.

**Histone H3 acetylation ChIP-on-chip hybridization and data analysis**

The workflow of the entire ChIP-on-chip approach is depicted in Figure 1. The GeneChip® Human Promoter 1.0R Tiling Array (Affymetrix, Santa Clara, CA, USA) allows the determination of protein/DNA interactions for over 25,500 human promoters which are densely covered by oligonucleotides covering approximately 7.5 kbp upstream and 2.45 kbp downstream of 5′ transcription start sites. For over 1,300 cancer-associated genes, the upstream promoter coverage is extended to 10 kbp.

Approximately 250 ng of ChIP-DNA was used as template for ligation mediated linear amplification according to the protocol of Young et al. to obtain a sufficient amount of labeled DNA for chip hybridization. The GeneChip WT double-stranded DNA terminal labeling kit (Affymetrix) was used for DNA-fragmentation and labeling according to the manufacturer’s instructions. Reaction mixtures were hybridized to the chips for 16 h at 45°C at 60 rpm and stained with streptavidin/phycoerythrin, followed by a biotin-conjugated anti-streptavidin antibody and a second streptavidin/phycoerythrin staining. All liquid handling was carried out by a GeneChip Fluidics Station 450. GeneChips were scanned with the Affymetrix GeneChip Scanner 3000 (Affymetrix) and CEL files were generated with the GCOS 1.3 software (Affymetrix). All experiments were performed as triplicates (cHL and B-cell lines) or as duplicates (PCM cell lines). The CEL files of all experiments are available via the Gene Expression Omnibus (GEO) of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/geo/) under the accession number GSE21254.

Resulting CEL files were analyzed using the Model-based Analysis of Tiling Arrays (MAT) algorithm. We used the default adjustments except for the MaxGap parameter which was reduced to 50 bp and the p-value which was set to 0.005 to find acetylated regions at
higher resolution. Instead of working with the original Affymetrix BPMAP files, we used the microarray probe mapping to NCBI build 36 provided by the MAT homepage (http://liulab.dfci.harvard.edu/MAT/). This analysis resulted in a list of significantly acetylated regions for each of the nine cell lines. The most significantly acetylated 11,000 regions per cell line were selected for further analysis.

Our analysis was primarily focused on the identification of acetylated genes. Therefore we removed from the full list of 46,875 Entrez genes those that lack a validated or reviewed entry in RefSeq, leading to a list of 18,546 Entrez genes associated to 24,885 RefSeq entries. The RefSeq collection includes alternatively spliced transcripts and information about usage of different transcription start sites (TSS).

We calculated a cell line-specific acetylation score for each RefSeq entry as follows: All acetylated regions in a genomic window between 10 kbp upstream of the TSS and 2.45 kbp downstream of the TSS were determined. This window size was set according to the coverage of the promoter tiling array. All acetylation signals within this region were aggregated to an overall score. Therefore longer acetylated regions got a higher score than shorter regions. Furthermore, since acetylated regions inside the core promoter region are expected to have higher relevance for gene expression, we twofold overweighed such regions. Core promoters were defined as 500/100 bp up/down-stream of the TSS, referring to models employed by the Genomatix software tool (Munich, Germany).

For the detection of genes specifically acetylated in cHL and PCM cell lines as compared to B-cell lines, we calculated a p-value for each RefSeq region using a moderated t-test. This p-value is a measure for the probability that the observed acetylation difference between two cell line types occurs by chance. For all genes with more than one RefSeq sequence, we retained the RefSeq sequence with the best p-value. We call a gene specifically acetylated if its p-value is <0.05. We also computed an acetylation fold change. Further details are given as supplementary information (Bioinformatic Algorithm S1).
Enrichment analysis of biological annotations

The Entrez gene identifiers of differentially acetylated genes were uploaded to DAVID (database for annotation visualization and integrated discovery, http://david.abcc.ncifcrf.gov/).\textsuperscript{28,29} We calculated the most overrepresented (enriched) biological annotations employing DAVID default parameters and using the 18,546 Entrez gene identifiers covered by the ChIP-on-chip analysis (supplementary Table S2) as background distribution.

Combined 5-aza-2’-deoxycytidine (AZA) and Trichostatin A (TSA) treatment

The DLBCL-derived cell lines SU-DHL4, SU-DHL6 and HT were demethylated and acetylated by treatment with 5-aza-2’-deoxycytidine (AZA; Sigma-Aldrich, St. Louis, MO, USA) and Trichostatin A (TSA; Sigma, Steinheim, Germany) according to established protocols.\textsuperscript{17} AZA was applied at a concentration of 3 µM for 6 days with replacement at days 2 and 5. At day 5, cells were additionally incubated for 24 h with 625 nM TSA and harvested at day 6. Data analysis of these cell lines was performed in combination with data already available for AZA/TSA-treated BL-derived cell lines Raji, Daudi and Namalwa derived from our previous study (GEO accession number GSE8388).\textsuperscript{17}

Generation and analysis of gene expression data

Gene expression analysis was performed for each of the three PCM cell lines. Furthermore gene expression data was generated in duplicates for the B-cell lines SU-DHL4, SU-DHL6 and HT, which were treated with AZA and TSA as described above. For this purpose, RNA was isolated according to standard protocols (Qiagen, Hilden, Germany) and GeneChip hybridization was carried out with Affymetrix GeneChips HG-U133A using 5 µg high quality total RNA according to the manufacturer's recommendations.

In addition publically available expression data from our group was used (cHL cell lines [KM-H2, L1236, L428] and B-cell lines [Raji, Namalwa, Daudi]; GEO accession number GSE8388)\textsuperscript{17}. Gene expression data were analyzed using the statistical programming environment R and Bioconductor after normalization using Robust Multichip Average (RMA). Fold changes were determined\textsuperscript{27} and p-values were calculated using moderated t-test with subsequent Benjamini-Hochberg (BH) correction.\textsuperscript{30} We defined a probe set as significantly differentially expressed if (i) at least a twofold change was observed and (ii) the
BH-corrected p-value was below 0.05. Probe sets were mapped to Entrez gene identifiers using the BioMart service and library.\textsuperscript{31,32} If more than one probe set was associated with a specific gene, only the probe set with the lowest p-value was used.

Based on the Entrez gene identifiers, the results of the differential acetylation analysis and the expression analysis were merged. For comparison of the ChIP-on-chip data with the results obtained from the gene expression arrays, we generated Venn diagrams. Furthermore published gene expression data from microdissected tumor cells of 12 cHL cases and 21 B-cell lymphoma cases (11 DLBCL, 5 BL and 5 Follicular Lymphoma (FL)) cases (available through GEO Accession Nr. GSE12453)\textsuperscript{33} were used to generate Venn diagrams for independent comparison with the differentially acetylated genes in cHL and B-cell lines. A single sided Fisher's exact test was applied to calculate significance of the intersections in the Venn diagrams. We also generated a heat map using GeneChip HG-U133A expression data of cHL, PCM and B-cell lines employing a list of 158 differentially acetylated genes in cHL and PCM cell lines as compared to B-cell lines.

**Results**

*Analysis of histone H3 acetylated genomic intervals in cHL, PCM and B-cell lines*

Chromatin immunoprecipitation (ChIP) employing a H3K9/14ac-specific antibody and subsequent hybridization of the DNA-fragments to promoter tiling arrays (ChIP-on-chip) was able to identify numerous acetylated genomic intervals in each cell line. Real-time DNA-PCR for selected genes revealed an excellent correlation with the data derived from the ChIP-on-chip experiments (supplementary Figure S1). The boxplot for the lengths of the selected genomic ChIP-on-chip intervals for each cell line (11,000 most significant intervals per cell line) showed comparable results (supplementary Figure S2).

In order to generate cell type specific acetylation patterns we compared the ranked list of acetylated genes obtained in B-cell lines with those derived from cHL and PCM cell lines (supplementary Table S2). The comparison of cHL and B-cell lines revealed that 211 genes were specifically acetylated in cHL (\textit{cHL}_\text{Ac}) and 327 (\textit{B1}_\text{Ac}) in B-cell lines (Figure 2). The consideration of B-cell and PCM cell lines led to the identification of a higher number of genes specifically acetylated in B-cells (n=591; \textit{B2}_\text{Ac}) and 143 genes were specifically acetylated in PCM cell lines (\textit{PCM}_\text{Ac}) (Figure 2).
As expected, the overlap of two sets of genes specifically acetylated in B-cell lines (B1_{Ac} and B2_{Ac}) was high (n=141) and comprises well known B-cell typical genes (e.g. CD19, CD79a/b, BLNK). Vice versa, these 141 genes can be regarded as being hypoacetylated in cHL as well as in PCM cell lines. In contrast, the intersection of the specifically acetylated genes in cHL and PCM cell lines revealed a much lower but highly significant number of genes (n=17). Interestingly within these 17 genes IRF4/MUM1, which is known to be consistently expressed in cHL and PCM cells, is present.34,35 In order to generate a list of genes highly specifically acetylated exclusively in cHL cell lines we removed these 17 genes from cHL_{Ac} which resulted in 194 genes. The color code corresponding to the various intersections in Figure 2 was also applied for the respective genes in the supplementary Table S3.

**Enrichment analysis of biological annotations**

To gain more insight into the biological function of the significantly acetylated or hypoacetylated genes in cHL and PCM cell lines we calculated the most overrepresented (enriched) annotations using the DAVID tools.28,29 The 211 genes acetylated in cHL cell lines were enriched for genes involved in the regulation of apoptosis and cell death, myeloid differentiation and the Toll-like receptor pathway whereas the 143 genes specifically acetylated in PCM cell lines were enriched for cAMP mediated signaling and genes related to transcriptional repression. The only term enriched in the 17 genes specifically acetylated in both, cHL and PCM cell lines, was “ATP binding” (supplementary Table S4). Finally, the 141 genes present in both B-cell lists (B1_{Ac} and B2_{Ac}) were dominated – as expected - by terms related to the B-cell receptor signaling and immune response (supplementary Table S4).

**Comparison of gene expression and histone H3 acetylation status**

To verify the functional impact of the epigenetic profiles assessed by H3K9/14ac ChIP-on-chip, differentially acetylated genes were analyzed for their transcriptional activity. The linkage of Entrez gene identifiers of the acetylated genes to gene expression (Affymterix HG-U133A) revealed a highly significant positive correlation between gene expression and histone H3 acetylation (supplementary Figure S3). The strong positive correlation between histone H3 acetylation and gene expression is demonstrated in a heat map based on 158 genes differentially acetylated in cHL and PCM cell lines (17 hyperacetylated and 141 hypoacetylated genes; Figure 3). This clear positive correlation is also exemplarily shown
CD30, CCR7, IRF4 and PRDM1/BLIMP1, CD20 (MS4A1), CD79a, Bob1 (POU2AF1)) in supplementary Figure S4.

To estimate the relevance of our epigenetic study in cell lines for the in-vivo situation, differentially acetylated genes in cHL and B-cell lines were additionally compared to published gene expression data derived from micro-dissected tumor cells of 11 cHL cases and 21 B-cell lymphoma cases (11 DLBCL, 5 BL and 5 FL). Remarkably, the comparison of these completely independent data sets revealed also highly significant overlaps. 31 genes up-regulated in primary HRS cells were also acetylated in cHL cell lines (p=1.950429e-24) and 65 genes found to be expressed in primary DLBCL, BL and FL cells (p=8.810192e-37) displayed an acetylation in the B-cell lines (supplementary Figure S5 and supplementary Table S5). No overlapping genes were observed in all other intersections.

Comparison of AZA/TSA induced differential gene expression and histone H3 acetylation

Previous work of our group has demonstrated that the epigenetic treatment of B-cell lines with AZA (DNA demethylation) and TSA (histone acetylation) led to an almost complete extinction of their B-cell identity and an up-regulation of B-cell inappropriate cHL-characteristic genes. Therefore, we were interested to correlate the ChIP-on-chip acetylation data to the gene expression induced by AZA and TSA treatment of B-cell lines. For this purpose our previously published data were supplemented with the results from additional AZA/TSA-treated DLBCL cell lines (SU-DHL4, SU-DHL6, HT) giving rise to a final data set of 6 different B-cell lines. 2230 probe sets were shown to be significantly affected by the treatment when all treated and untreated cell lines were compared. The majority of these transcripts (1671) were down-regulated whereas only 559 were up-regulated by the treatment. Since several probe sets cover the same gene, we mapped all probe sets to the corresponding Entrez gene identifiers leading to 1236 down-regulated and 472 up-regulated genes. From these, 1194 down-regulated and 435 up-regulated genes were present on the promoter tiling arrays.

Subsequently, the data derived from AZA/TSA-induced differential gene expression in B-cell lines was compared to the data obtained from the ChIP-on-chip acetylation analysis (supplementary Figure S6). Genes specifically acetylated in B-cell lines and down-regulated by AZA/TSA treatment of B-cell lines displayed an overlap of 67 genes (p=6.047922e-16) including characteristic B-cell transcripts such as CD19, CD20 and CD79a/b (supplementary Table S6). The comparison of the genes specifically acetylated in HRS cells and the genes up-
regulated upon epigenetic treatment of B-cell lines identified 22 genes (p=1.452767e-07) in common (supplementary Table S6). The remaining comparisons were not statistically significant (supplementary Figure S6).

Analysis of PcG mediated H3K27 trimethylation for selected genes

Quantitative real-time PCR with DNA obtained after ChIP with antibodies against H3K27 trimethylation revealed that the promoters of 10 selected B-cell characteristic genes (CD19, CD20 (MS4A1), CD79b, BOB1 (POU2AF1), PU.1, SYK, LCK, TCL1A, BCMA, PAX5) were predominantly enriched in the cHL cell lines (Figure 4). In contrast, promoters of genes usually highly expressed in cHL cell lines (CD30, CCR7, TRAF1, SEMA4C, IL6) showed no enrichment after ChIP for H3K27 trimethylation with the exception of CD30 which is known to be expressed in the cHL cell line L1236 at very low level. An inverse H3K27 pattern was found in the B-cell lines confirming the gene silencing function of this histone trimethylation. Interestingly, H3K27 trimethylation and H3K9/14 hypoacetylation was detectable in the CD20 promoter of Namalwa cells which is, however, well in line with its strongly reduced CD20 mRNA expression (supplementary Figure S4). Finally and most strikingly, only PAX5 and three other B-cell genes (TCL1A, CD20, SYK) were additionally silenced by means of H3K27 trimethylation in PCM cell lines. In contrast, the majority of B-cell genes in cHL cell lines showed a repressive H3K27 trimethylation and a H3 hypoacetylation (Figure 4).

Discussion

The biological events that initiate the almost complete extinction of the B-cell phenotype and the transformation classical Hodgkin lymphoma (cHL) are still unknown. Several reasons have been attributed to this phenomenon including a defective transcription factor machinery responsible for the down-regulation of the respective B-cell antigens. One further possible mechanism, namely a down-regulation of the B-cell phenotype as in plasma cells, was considered only rarely although there are several arguments that support this scenario (e.g. immunohistological expression of IRF4 or late class switch recombination events in cHL cell lines).

To explore the relation of cHL and plasma cells at the epigenetic level, we investigated the global histone H3 acetylation pattern in cHL and plasma cell myeloma (PCM) cell lines and for comparison in several B-cell lines. These acetylation data were supplemented with the
H3K27 trimethylation status for selected genes and the functional impact of these epigenetic patterns on the transcriptome was determined.

**Analysis of acetylated regions in cHL, PCM and B-cell lines**

Chromatin immunoprecipitation (ChIP) with subsequent hybridization of the precipitated DNA-fragments to promoter tiling arrays (chip) was performed to identify the global acetylation pattern of cHL, PCM and B-cell lines. After determination of genes specifically acetylated in each cell line group, we compared these cell type characteristic patterns to each other. This led to the identification of 141 genes which were commonly hypoacetylated in cHL and PCM cell lines and hyperacetylated in B-cell lines (Figure 2). Not surprisingly, 23 of these genes were related to the B-cell receptor signaling or immune response (supplementary Table S4) which is compatible with their consistent down-regulation in cHL and PCM. In contrast, the number of genes acetylated in both, cHL and PCM cell lines, but not in B-cell lines is much smaller (n=17). Moreover, genes specifically acetylated only in cHL cell lines (n=194) were frequently involved in cell death, Toll-like receptor (TLR) signaling pathway and myeloid differentiation. This fits very nicely to the observation that (i) HRS cells are prone to apoptosis\(^{20}\), that (ii) the TLR pathway might be associated with cHL pathogenesis\(^{39}\) and that (iii) HRS cells frequently express myeloid markers.\(^{20}\)

These epigenetic patterns disclose that cHL and PCM cell lines are very similar in respect to the hypoacetylation of B-cell characteristic genes, a finding which might explain the extinction of their B-cell identity. However, the cHL and PCM cell lines only share a very limited number of hyperacetylated genes and genes essential for complete plasma cell differentiation. Although IRF4, a known important transcription factor for plasma cellular differentiation, is present among these 17 genes, one further genes essential for plasma cell development (*PRDM1*/*BLIMP1*) was hyperacetylated only in the PCM cell lines but not in the cHL cell lines (Figure 2).\(^{19}\) Especially the latter finding is compatible with the observation that HRS cells are unable to express genes characteristic for plasma cells.\(^{18}\)

The impact of our epigenetic patterns to the transcriptome was estimated by correlation of the acetylated genes with their corresponding gene expression as assessed by Affymetrix GeneChip analysis. This revealed a strong positive correlation and clearly
demonstrates that histone H3 acetylation affects gene expression to a large extent. However, acetylation as well as other epigenetic mechanisms (e.g. DNA methylation or H3K27 trimethylation) do not reflect an on/off situation but represent modulators able to quantitatively control gene expression. In addition, other non-epigenetic mechanisms (e.g. transcription factor activity) are necessary to induce and to maintain gene expression. Therefore it is not surprising that a significant number of acetylated genes are - despite open chromatin - not concurrently up-regulated in their gene expression.

**H3K9/14 histone acetylation negatively correlates with the DNA-methylation status in B-cell characteristic genes**

It is well known that there is a cross-talk between the different epigenetic mechanisms. To elucidate this aspect for B-cell characteristic genes in cHL we analyzed the H3K9/14 acetylation pattern in respect of DNA methylation. For this purpose we selected nine B-cell specific promoters (CD19, CD20 (MS4A1), CD79b, POU2AF1(Bob1), PU.1 (SPI1), SYK, LCK, TCL1A, BCMA (TNFRSF17) known to be silenced by DNA hypermethylation in cHL based on published data. With the exception of TCL1A, CD20 and BCMA, all promoters investigated displayed an additional histone deacetylation in cHL cell lines thus preventing the binding of transcription factors and, in consequence, the expression of the respective genes. The promoters of CD19, CD79b, PU.1 and SYK were found to be also hypoacetylated in PCM cell lines in line with the absent expression of these genes (supplementary Figure S7). Strikingly and expected, Bob1 (POU2AF1), a transcription factor essential for immunoglobulin gene expression was significantly acetylated in PCM cell lines but not in cHL cell lines (supplementary Figure S4).

Our findings suggest that the cooperative interplay between acetylation and methylation is also effective in the silencing of genes in cHL and PCM. The results of previous studies describing a reactivation of B-cell genes in cHL cell lines exclusively by simple demethylation are in conflict with this concept. However, thorough reinvestigation of these reported demethylation effects by real-time RT-PCR revealed merely a very weak induction of gene expression thus questioning the biological significance of these previous findings. Interestingly, combined demethylation and acetylation is also unable to push the cHL cell lines towards a B-cell phenotype. This clearly demonstrates that histone acetylation and DNA demethylation alone are not sufficient to restore the B-cell phenotype in cHL cell
lines. In addition other mechanisms such as inhibition of B-cell specific transcription factors have to be involved.42

**Epigenetic networking in cHL**

As previously demonstrated, genes atypically up-regulated in cHL are especially important for subsequent extinction of the B-cell phenotype and – potentially – for the pathogenesis of cHL.42 To identify the most relevant genes important for a better understanding of the cHL biology under an epigenetic perspective we compared (i) genes up-regulated by AZA/TSA treatment of B-cell lines and (ii) genes specifically acetylated in cHL cell lines (supplementary Figure S6). 22 genes fulfill these criteria. Strikingly, several of these genes, such as *ATF3, JUN, IRF4, ID2, FSCN1, CCR7* and *RYBP* have already been recognized by previous completely independent studies and modifications of these genes are suggested to play a role in the pathogenesis of cHL.20;42-45

Our special attention was attracted by the Ring1 and YY1 binding protein (RYBP) due to its importance as an interacting partner for the Polycomb group protein Ring1A.46 Polycomb group (PcG) proteins act as transcriptional repressors by means of histone modification and are involved in the regulation of organogenesis and cell lineage fidelity.6

RYBP was shown to be over-expressed in primary cHL cases and to have prognostic relevance whereas it is not detectable in normal B-cells of the lymphoid tissue.45;47 Although the precise mechanism of the RYBP activity is not known it is likely that its up-regulation in HRS cells significantly contributes to an epigenetic silencing of the B-cell phenotype. This view is supported by the fact that not only RYBP was found to be up-regulated in HRS cells but also other components of the multimeric polycomb repressive complexes (PRC) such as RING1, BMI1, MEL-18, EED and EZH2.48 These findings together with the results obtained from our global acetylation analysis, led us to conclude that a derailed epigenetic network is involved in the extinction of the B-cell program and in the malignant transformation of cHL.

This view is additionally supported by our H3K27 trimethylation analysis of ten B-cell promoters (*CD19, CD20, CD79b, BOB1, PU.1, SYK, LCK, TCL1A, BCMA, PAX5*) which demonstrates an almost inverse pattern in the cHL cell lines as compared to the B-cell lines. (Figure 4). Interestingly, the H3K27 trimethylation patterns of PCM cell lines differ to some extent from those of the cHL cell lines. Whereas B-cell characteristic genes such as *CD19* and *CD79a* showed a suppressive H3K27 trimethylation in cHL, but not in PCM cell lines, *PAX5*, a master regulator of the B-cell expression program, was H3K27 trimethylated in both cell
line groups. This shared epigenetic modification of the \textit{PAX5} gene reinforces the hypothesis that PCM and cHL cell lines harbor a common molecular basis for the silencing of the B-cell expression program which is more pronounced in cHL than in PCM. Thus PcG-mediated H3K27 trimethylation can be regarded as a kind of failsafe mechanism to ensure that gene silencing by deacetylation and promoter DNA methylation is additionally stabilized in cHL. Furthermore, for permanent silencing of the B-cell phenotype in cHL, the additional trimethylation of H3K27 appears to be of special interest since this epigenetic modification is not affected by AZA/TSA treatment.

Taken together, our results demonstrate on a global scale that histone H3 deacetylation significantly contributes to the almost complete extinction of the B-cell expression program of cHL. This acetylation-mediated silencing is further reinforced by H3K27 trimethylation (this study) and DNA promoter methylation (published data). PCM cell lines resemble in respect to the hypoacetylation-mediated down-regulation of the B-cell expression program very much cHL cell lines. However, PCM and cHL cell lines differ in their hyperacetylation and H3K27 trimethylation pattern, a fact in line with the fundamental difference of genes expressed in both entities. Thus, our epigenetic data support the view that cHL is characterised by an abortive plasma cell phenotype with a down-regulation of B-cell characteristic genes but without activation of most plasma cell typical genes.

\textit{Authorship and Disclosures}

VS, PT, AE, DL, UL, HS, MH designed research, AS, UP, EO performed research, VS, AE, UP, AS, EO, MJ collected data, VS, PT, KZ, AE, AS, EO, DL, LD, UL, MH analyzed and interpreted data, VS, PT, KZ, DL performed statistical analyses and V.S., PT, KZ, AE, MJ, DL, LD, UL, HS, MH wrote the paper. The authors declare no conflict of interest.
Reference List


Figure Legends

**Figure 1.** Workflow for the analysis of acetylation patterns and gene expression in cHL, PCM and B-cell lines.

**Figure 2.** The genes acetylated in cHL, PCM and B-cell lines were compared to each other in order to identify genes differentially acetylated in the respective group. Since the comparison of genes acetylated in B-cell lines and cHL or PCM cell lines, respectively, represents two different intersections, two different lists of differentially B-cell genes (B1Ac and B2Ac) were generated. Some cell type characteristic genes are exemplarily mentioned in the intersections. The corresponding gene lists are given in supplementary Tables S3.

**Figure 3.** The genes used for hierarchical cluster analysis were selected exclusively based on their acetylation pattern. For this purpose, genes acetylated in cHL and PCM cell lines (n=17) and the 141 significantly acetylated genes derived from the B-cell lists B1Ac and B2Ac were selected (Figure 2). To assess their gene expression, the list of acetylated genes was linked to gene expression data derived from completely independent Affymetrix gene chip (U133A) analyses of the corresponding cHL, PCM and B-cell lines.
**Figure 4.** Promoter H3K27 trimethylation status of B-cell- and cHL-characteristic genes after ChIP and quantification by real-time DNA-PCR in cHL, PCM and B-cell lines. β-actin was chosen as endogenous reference and the enrichment was calculated relative to the ChIP input control. ChIP: chromatin immunoprecipitation.
Fig. 1

Chromatin immunoprecipitation (ChiP) (3 cHL, 3 PCM and 3 B-cell lines) → Amplification and labeling of immunoprecipitated DNA-fragments → Hybridization on human promoter tiling arrays → Identification of significantly acetylated genes → Comparison to gene expression data (cell line and lymphoma data) and Comparison to gene expression data from 6 B-cell lines after AZA/TSA treatment → Enrichment analysis of biological annotations
Fig. 2

Comparison of cHL and B-cell lines

\[ \text{cHL}_{\text{c}} : 211 \]

\[ \text{B1}_{\text{c}} : 327 \]

Comparison of PCM and B-cell lines

\[ \text{PCM}_{\text{c}} : 143 \]

\[ \text{B2}_{\text{c}} : 591 \]

N\text{=194} e.g. JUN,CCRF,\ldots

N\text{=17} e.g. IRF4,MYC,\ldots

N\text{=126} e.g. PRDM1,Blimp1,\ldots

N\text{=141} e.g. CD19,CD79a/\ldots

BLNK,\ldots
Supplementary protocol S1

ChIP with cultured suspension cells

I. Formaldehyde cross-linking of cells
- 1x10^6 cells were used for each immunoprecipitation
- Cells were fixed with 4% formaldehyde solution for 20 minutes at room temperature. Fixation was stopped with 0.25 M glycine
- Cells were washed three times with PBS (4°C) and cell pellets were stored at -80°C in polypropylene tubes.

II. Preblock and binding of antibody to magnetic beads
- 50 µl Dynal magnetic beads coated with Protein A and 50 µl Dynal magnetic beads coated with Protein G were mixed and washed three times with 1 ml blocking solution (PBS containing 0.5% BSA)
- Beads were mixed with 10 µg of Anti-acetyl Histone H3 (Lys9 + Lys14) (06-599) (Upstate) in 250 µl block solution
- Beads and antibody were incubated overnight under rotation at 4°C.
- Beads-antibody-complexes were washed three times with blocking solution and resuspended in 100 µl blocking solution

III. Cell lysis and sonication
- Cell pellets were resuspended in 5 ml lysis buffer 1 (50 mM Heps-KOH, pH 7.5; 140 mM NaCl; 1 mM EDTA; 10% glycerol; 0.5% NP-40; 0.25% TritonX-100) containing freshly added protease inhibitors (Complete, Roche), incubated at 4°C for 10 min and centrifuged at 1800 rpm for 5 minutes at 4°C.
- Pellets were resuspended in 5 ml lysis buffer 2 (10 mM Tris-Cl, pH 8.0; 200 mM NaCl; 1 mM EDTA; 0.5 mM EGTA) containing freshly added protease inhibitors (Complete, Roche), incubated at room temperature for 10 min and spin down at 1800 rpm for 5 minutes at 4°C.
- Pellets were resuspended in 3 ml lysis buffer 3 (10 mM Tris-Cl, pH 8.0; 100 mM NaCl; 1 mM EDTA; 0.5 mM EGTA; 0.1% Na-Deoxycholate; 0.5% N-lauroylsarcosine) containing freshly added protease inhibitors (Complete, Roche).
- 2x 1.5 ml of the samples were separated into 2x 15 ml polypropylene tubes and sonicated with the Bioruptor employing Titan sticks (Diagenode, Liége, Belgium) for 3 x 15 minutes (changing the ice after each sonication round) at high power in pulsed modus (30 sec on, 30 sec of).
- 300 µl of 10% Triton X-100 was added to the pooled 2 x 1.5 ml sample.
- Centrifugation at 13000 rpm for 10 minutes at 4°C to pellet debris.
- 50 µl from the supernatant of each sample was saved as input DNA (negative control) and stored at -20°C. Furthermore, 50 µl from the supernatant were used to analyze the success of DNA sonication by gel analysis according to the Young protocol using 3 µg DNA.¹
- Remaining supernatant was used for immunoprecipitation.

IV. Chromatin immunoprecipitation

- Beads-antibody-complexes were mixed with the supernatant samples and incubated overnight under rotation at 4°C.

V. Wash, elution, and cross-link reversal

- Beads were separated from the supernatant by magnetic force.
- Beads were washed with RIPA buffer (50 mM Hepes-KOH, pH 7.6; 500 mM LiCl; 1 mM EDTA; 1% NP-40; 0.7% Na-Deoxycholate) containing freshly added protease inhibitors (Complete, Roche) for four times.
- Beads were washed with TE containing 50 mM NaCl and added freshly protease inhibitors (Complete, Roche).
- Beads were incubated with 210 µl elution buffer (50 mM Tris-HCl, pH 8.0; 10 mM EDTA; 1% SDS) at 65°C for 15 minutes and thereby resuspended by vortexing every 2 minutes.
- Beads were spin down for 1 minute at room temperature and supernatant with DNA was incubated at 65°C overnight to reverse the crosslink of the DNA. The 50 µl input DNA (negative control) was also dissolved in 150 µl elution buffer and incubated at 65°C overnight.

VI. Digestion of cellular protein and RNA

- Samples were incubated with 200 µl 1 x TE buffer with freshly added 0.25 µg/ml RNaseA at 37°C for 2 hours.
- Samples were incubated with proteinase K (0.2 µg/ml) at 55°C for 2 hours.
- DNA was purified by phenol/chloroform extraction and precipitated by ethanol
- Pellets were resuspended in 70 µl of 10 mM Tris-HCl, pH 8.0.

“Small scale” protocol for H3K27 trimethylation ChiP analysis

H3K27 trimethylation was analyzed by ChIP as described above, for selected genes in the three Hodgkin cell lines and one B-cell lymphoma cell line (Namalwa), employing anti-H3K27 trimethylation antibody (07-499, Millipore). However, instead of performing the ChIP in a 3ml volume we worked with 3 volumes of 1ml in a small scale approach. We used for each tube with 1ml approximately the amount of antibody coated G-beads originally recommended in the Young-protocol for the large scale approach. Therefore 100 µl beads were covalently linked overnight with 15 -20 µg antibody. After the washing steps the antibody coated beads were resolved in 100 µl blocking solution.
References


Description of the Supplementary Data

Supplementary Tables NOT enclosed in this file; please refers to related .xls files

Supplementary Table S1. Primers used for ChIP DNA analyses.

Supplementary Table S2a. 18546 Entrez gene identifiers covered by the ChIP-on-chip analysis (B-cell line in comparison to classical Hodgkin lymphoma (cHL) cell lines) are shown.

The columns can be divided in five sections.
1) Columns A to E describe different identifiers like Entrez Gene, HUGO gene name and RefSeq.
2) Columns F to O cover the results of the ChIP-on-chip analysis.
3) Columns P to R cover the results for the expression analysis between cHL and B-cell lines.
4) Columns S to U cover results from the expression analysis between AZA/TSA treated normal B-cell lines.
5) Columns V to Y cover information about the genomic location of a RefSeq transcript. Each row corresponds to a gene with at least one validated or reviewed RefSeq entry.

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**Supplementary Table S2b.** 18546 Entrez gene identifiers covered by the ChIP-on-chip analysis (B-cell lines in comparison to plasma cell myeloma (PCM) cell lines) are shown. The columns can be divided in five sections.

1) Columns A to E describe different identifiers like Entrez Gene, HUGO gene name and RefSeq.

2) Columns F to O cover the results of the ChIP-on-chip analysis.

3) Columns P to R cover the results for the expression analysis between PCM and B-cell lines.

4) Columns S to V cover information about the genomic location of a RefSeq transcript. Each row corresponds to a gene with at least one validated or reviewed RefSeq entry.

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Supplementary Table S3. The colors in this table match the colored gene groups in Figure 2. Table sheet A: Specifically acetylated genes in cHL (n=211) and B-cell lines (B1_AC in Figure 2: n=327) determined by the cHL versus B-cell line comparison. Table sheet B: The specifically acetylated genes in PCM cell lines (n=143) and B-cell lines (B2_AC in Figure 2: n=591) determined by the comparison of PCM and B-cell lines is shown. The 17 genes which were collectively and significantly acetylated in the cHL and PCM cell lines are marked in red in the cHL and PCM specific gene lists. Furthermore the 141 genes which were found in the intersection of the two B-cell groups (which are therefore jointly hypoacetylated in cHL and PCM cell lines) are marked in blue in the two B-cell line gene lists.

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Supplementary Table S4. Gene Ontology (GO) results. Interesting terms specifically enriched in one group are highlighted in red. The gene numbers of the different table sheets identify the groups shown in Figure 2.
**Supplementary Table S5.** Table sheet A) Genes up-regulated in DLBCL, BL and FL cases (available through GEO Accession Nr. GSE12453) as well as specifically acetylated in B-cell lines (n=65). Table sheet B) Genes up-regulated in microdissected HRS cells of 11 cHL cases (GEO Accession Nr. GSE12453) as well as specifically acetylated in cHL cell lines (n=31).

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**Supplementary Table S6.** Table sheet A) Specifically acetylated genes in cHL cell lines which are also up-regulated in B-cell lines upon AZA/TSA treatment (n=22). Table sheet B) Furthermore, the genes specifically acetylated in B-cell lines and down-regulated in B-cell lines upon AZA/TSA treatment are listed (n=67).

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**Supplementary Protocol S1.** Detailed ChIP protocol.

**Supplementary Figure legends**

**Supplementary Figure S1.** Length of most significant acetylated regions used for the analysis depicted as boxplot for each cell line after analysis employing the MAT algorithm.
The y-axis shows the length of acetylated regions in base pairs (bp). The interquartile range of acetylated region length is similar between the cell lines.

**Supplementary Figure S2.** Venn diagrams for comparison of acetylation (black circle) and gene expression (grey circles) data. A and B: Comparison of classical Hodgkin lymphoma (cHL) and B-cell lines. C and D: Comparison of plasma cell myeloma (PCM) and B-cell lines.

A) An overlap of 102 genes (p=1.295205e-58) was found when the 431 genes up-regulated in B-cell lines and the 241 genes specifically acetylated in B-cell lines were compared. None of the 349 genes up-regulated in cHL cell lines were acetylated in B-cell lines.

B) An overlap of 64 genes (p=6.881182e-42) was found when the 349 genes up-regulated in cHL cell lines and the 162 genes specifically acetylated in cHL cell lines were compared. None of the 431 genes up-regulated in B-cell lines were acetylated in cHL cell lines.

Note that only 241 of the 327 differentially acetylated B-cell genes (B1Ac) and 162 of the 211 differentially acetylated cHL genes (Figure 2) were covered by the U133A Affymetrix GeneChips and thus used for comparison. Furthermore all 431 genes being significantly expressed in B-cell lines and all 349 genes being significantly expressed in cHL cell lines were covered by GeneChip® Human Promoter 1.0R Tiling Array.

C) An overlap of 100 genes (p=1.789886e-38) was found when the 419 genes up-regulated in B-cell lines and the 437 genes specifically acetylated in B-cell lines were compared. None of the 280 genes up-regulated in PCM cell lines were acetylated in B-cell lines.

D) An overlap of 44 genes (p=2.434177e-33) was found when the 280 genes up-regulated in PCM cell lines and the 112 genes specifically acetylated in PCM cells were compared. None of the 419 genes up-regulated in B-cell lines were acetylated in PCM cell lines.

Note that only 437 of the 591 differentially acetylated B-cell genes (B2Ac) and 112 of the 143 differentially acetylated genes in PCM cell lines (Figure 2) were covered by the U133A Affymetrix GeneChips and thus used for comparison. Furthermore all 419 genes being significantly expressed in B-cell lines and all 280 genes being significantly expressed in PCM cell lines were covered by GeneChip® Human Promoter 1.0R Tiling Array.

**Supplementary Figure S3.** Mapping of the acetylation pattern of selected genes typical for cHL, PCM and B-cell lines (**CCR7**, **CD30**, **CD20**, **CD79a**, **IRF4**, **PRDM1**) using the Integrated Genomic Browser (IGB) and their gene expression as determined by GeneChip U133A gene expression analysis (insert).

**Supplementary Figure S4.** Venn diagrams for comparison of acetylation (black circle) and gene expression (grey circles) data.

A) An overlap of 65 genes (p=8.810192e-37) was found when the 485 genes up-regulated in the primary tumor cells of 21 published DLBCL, BL and FL cases (available through GEO Accession Nr. GSE12453) and the 315 genes specifically
acetylated in B-cell lines (B1Ac Figure 2) were compared. None of the 219 genes up-regulated in the HRS cells of cHL cases was found to be acetylated in B-cell lines.

B) An overlap of 31 genes (p=1.950429e-24) was found when the 219 genes up-regulated in the primary HRS cell of 11 published cHL cases (GEO Accession Nr. GSE12453) and the 200 genes specifically acetylated in cHL cell lines were compared. None of the 485 genes up-regulated in the primary tumor cells of the 21 published cases of diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma (BL) and follicular lymphoma (FL) was found to be acetylated in cHL cell lines.

Note that only 315 of the 327 differentially acetylated B-cell genes and 211 of the 219 differentially acetylated cHL genes were covered by the U133 Plus 2.0 Affymetrix GeneChips. All 485 genes being significantly expressed in the 21 published DLBCL, BL and FL cases and all 219 genes being significantly expressed in primary HRS cells were covered by the GeneChip® Human Promoter 1.0R Tiling Array.

**Supplementary Figure S5.** Venn diagrams comparing specifically acetylated genes in cHL and B-cell lines (black circle) with genes up- and down-regulated in B-cell lines after epigenetic treatment with AZA/TSA (grey circles).

A) An overlap of 67 genes was identified when the 243 specifically acetylated genes in B-cell lines were compared to the 1194 genes down-regulated by AZA/TSA treatment of B-cell lines (p=6.047922e-16). In contrast only 3 genes were commonly specifically acetylated B-cell lines and up-regulated in the AZA/TSA treated B-cell lines (n.s.; p=0.9920506).

B) An overlap of 22 genes was identified when the 172 specifically acetylated genes in cHL cell lines were compared with the 435 genes up-regulated by AZA/TSA treatment of B-cell lines (p=1.452767e-07). Only 4 genes were commonly specifically acetylated in cHL cell lines and down-regulated in the AZA/TSA treated B-cell lines (n.s.; p=0.9999694).

Note that only 243 of the 327 differentially acetylated B-cell genes (B1Ac) and 172 of the 211 differentially acetylated cHL genes (Figure 2) were covered by the U133A Affymetrix GeneChips and thus used for comparison. Furthermore only 1194 of 1236 genes down-regulated in AZA/TSA treated B-cell lines and 435 of 472 genes being up-regulated in AZA/TSA treated B-cell lines were covered by the GeneChip® Human Promoter 1.0R Tiling Array.

**Supplementary Figure S6.** Mapping of the acetylation pattern of several genes expressed in cHL, PCM and B-cell lines using the Integrated Genomic Browser (IGB) and their gene expression as determined by Affymetrix U133A GeneChip hybridization.
Supplementary Fig. S3

A specifically acetylated genes in B-cell lines (241) up-regulated genes in cHL cell lines (349)

139 0 349
102 0 0
329

up-regulated genes in B-cell lines (431)

B specifically acetylated genes in cHL cell lines (162) up-regulated genes in cHL cell lines (349)

98 64 285
0 0 0
431

up-regulated genes in B-cell lines (431)

C specifically acetylated genes in B-cell lines (437) up-regulated genes in PCM cell lines (280)

337 0 280
100 0 0
319

up-regulated genes in B-cell lines (419)

D specifically acetylated genes in PCM cell lines (112) up-regulated genes in PCM cell lines (280)

68 44 236
0 0 0
419

up-regulated genes in B-cell lines (419)
Supplementary Fig. S4 Comparison of the acetylation pattern for selected genes with their gene expression (Affymetrix U133A)
Supplementary Fig. S5

A specifically acetylated genes in B-cell lines (315) upregulated genes in DLBCL, BL and FL specimen (485)

250 65 0 0 420
0 0 219

up-regulated genes in in vivo HRS cells (219)

B specifically acetylated genes in Hodgkin cell lines (200) upregulated genes in DLBCL, BL and FL specimen (485)

169 0 0 485
31 0 188

up-regulated genes in in vivo HRS cells (219)
Supplementary Fig. S6

A
specifically acetylated genes in B-cell lines (243)

173
3
67
0
0
1127

AZA/TSA upregulated genes in B-cell lines (435)

B
specifically acetylated genes in Hodgkin cell lines (172)

146
4
1190

AZA/TSA upregulated genes in B-cell lines (435)

AZA/TSA down-regulated genes in B-cell lines (1194)
Bioinformatic Algorithm S1. Our primary aim was the comparison of acetylation patterns of cHL, PCM and B-cell lines to assess the extent of their epigenetic alterations. Therefore, we established a bioinformatic workflow, which avoids binary decisions in early steps of the analysis. The analysis of the ChIP-on-chip data was performed using the MAT algorithm resulting in a list of acetylated regions for each cell line. For all genes we calculated an acetylation score for each gene applying the formula in the insert. Using a moderated t-test the significance between the histone H3 acetylation of cHL and B-cell lines or PCM and B-cell lines was calculated. This resulted in a list of differentially acetylated genes which was used to perform enrichment analysis. The results from the ChIP-on-chip analysis were also compared to gene expression data obtained from the same cHL, PCM and B-cell lines. This figure exemplarily shows the comparison of three cHL cell lines (cHL1-3) and three B-cell lines (B1-3). The analysis pipeline was written in R and the code is available on personal request from PT.

References
