CHAPTER 2

Global correlation of genome and transcriptome changes in classical Hodgkin lymphoma

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Abstract

Hodgkin lymphoma (HL) tumor cells are derived from germinal center (GC) B cells. The molecular mechanisms that cause the transformation of GC B cells are still poorly understood. To identify candidate genes responsible for malignant transformation, we performed serial analysis of gene expression (SAGE). In addition, global changes in DNA copy number (CN) were identified by array-based comparative genomic hybridization (aCGH) to analyze a possible association with up- or downregulated expression levels. Aberrantly expressed genes were identified by comparison of SAGE libraries of 2 classical HL (cHL) and 1 nodular lymphocyte predominant HL (NLPHL) cell line with a GC B cell SAGE library. Comparisons between all three HL or the two cHL cell lines and GC B cells revealed that only 7 and 14 genes, respectively, were commonly overexpressed. In contrast, 125 and 141 genes were consistently downregulated in HL and cHL, respectively. Array-CGH revealed increased CN of chromosomal regions on 2p, 7p, 9p, 11q and Xq in at least 3/4 cHL cell lines and decreased CN of regions on 4q and 11q in 2 cHL cell lines. Combination of gene expression data and aCGH profiles per cell line revealed that 7-18% of the differentially expressed genes mapped to regions with an abnormal CN. These genes showed a good correlation between underexpression and loss of DNA or overexpression and gain of DNA. Despite the fact that we only observed 14 commonly upregulated genes in cHL, FSCN1 on 7p and IRAK1 on Xq both mapped at genomic loci that frequently showed CN gain in cHL. Our results confirm the previously reported “loss of B cell phenotype” in cHL and show that this phenotype may be shared to some extent with NLPHL. The identification of FSCN1 and IRAK1 as genes that are frequently overexpressed perhaps due to increased CN provides evidence for an important role for these genes in cHL pathogenesis.
Introduction

Hodgkin lymphoma (HL) is an unusual malignancy based on the presence of only a minority of tumor cells scattered among a large reactive background. Two main HL subtypes are recognized, one is classical HL (cHL) with malignant cells known as mononucleated Hodgkin- and multinucleated Reed-Sternberg (HRS) cells. The other is nodular lymphocyte predominant (NLP) HL with tumor cells called lymphocytic and histiocytic (L&H) cells. Due to the scarcity of tumor cells in HL the origin of the tumor cells has been uncertain for a long time. The combination of sensitive PCR techniques and single cell microdissection has revealed that both HRS and L&H cells have clonal immunoglobulin (Ig) gene rearrangements and are therefore considered to be derived from germinal center B cells. L&H cells are characterized by ongoing mutations and functional Ig expression, whereas HRS cells lack an active hypermutation process and display crippling Ig mutations in ~25% of cases.

Several genetic approaches like classical cytogenetics, loss of heterozygosity (LOH) and comparative genomic hybridization (CGH) have been used in the past to gain insights into HL biology. Cytogenetic studies have revealed a high number of chromosomal breakpoints in advanced clinical stages of HL. The most frequent breakpoints mapped at 7q22, 7q32, 11q32, 13p11 and 14q32. CGH on microdissected HRS cells revealed recurrent gains of 2p, 9p, 12q and 17q and losses on 13q, 6q, 11q and 4q in cHL. The most commonly amplified regions were 2p and 9p including, respectively, the NF-κB family member c-REL and the tyrosine kinase JAK2. In NLP HL, gain was observed for chromosome arms 1q, 3p, 4q, 5q, 6q, 8q, 12q, and Xq and loss for chromosome 17. All the studies described above show that HL is characterized by many and often complex chromosomal aberrations. However, no consistent aberration that underlies the process of malignant transformation has been identified yet.

Only limited data is available on gene expression profiles of HRS and L&H cells. The main reason for this is that such studies are hampered by the rarity of the HRS and L&H cells in the tissue. To overcome this technical problem, HL cell lines or whole HL tissues were used for gene expression profiling. The only study using primary material was from Cossman and colleagues who performed cDNA sequencing on micromanipulated HRS cells. This approach precluded the generation of a global/large scale overview of the HRS transcriptome. A more comprehensive study, using serial analysis of gene expression (SAGE) and micro-array approaches to compare cHL cell lines with several normal B cell populations, revealed loss of B-lineage specific gene expression in HRS cells of cHL. So far no large scale gene expression study has been performed in NLP HL or the only available NLP HL derived cell line DEV. Overall, our knowledge on global gene expression in NLP HL is still very limited.
Due to these limited gene expression studies and lack of detection of consistent aberrations in genomic studies, HL is still poorly understood at the molecular level. In an effort to gain more insight into the pathogenesis of HL we combined data on global gene expression with results from chromosome copy number (CN) analysis. Besides a global correlation between CN changes and differentially expressed genes, two specific genes with a common increase in CN and expression level in cHL were identified.

Material & Methods

Cell lines
The HL cell lines L428, L1236, DEV, L591 and KM-H2 were cultured in RPMI-1640 medium (Cambrex Biosciences, Walkersville, MD) supplemented with ultraglutamine 1 (Cambrex Biosciences), 100 U/ml penicillin/streptomycin, 10% fetal calf serum (FCS) (Cambrex Biosciences) at 37°C in an atmosphere containing 5% CO₂. The final FCS concentration for DEV was 20% and for L428 5%.

Microarray-based Comparative Genomic Hybridization (aCGH)
The design and construction of the BAC-microarray used in this study is described in detail elsewhere. For the positioning of the 6465 BACs relative to the human sequence we have used the May 2004 human reference sequence (UCSC version hg16) that is based on NCBI Build 35. Genomic DNA (250 ng), isolated with a standard salt-chloroform extraction protocol, was labeled with either Cy3- or Cy5-dUTP (Perkin Elmer, Langen, Germany) using the BioPrime DNA Labeling System (Invitrogen Inc., Carlsbad, CA) as described. Samples were inversely sexmatched with the reference DNA to have an internal control for gain or loss at the sex chromosomes. Slides were processed according to the protocol of the manufacturer and as described. Briefly, the hybridizations were carried out under a coverslip, in a humidified chamber at 65°C for 40 hours. Post hybridization washes were done as recommended by the manufacturer of the slides (Schott Nexterion). Arrays were scanned using the Affymetrix 428 scanner (Affymetrix Inc., Carlsbad, CA). The resulting images were analyzed with ImaGene software package 5.0 (BioDiscovery Inc., Marina Del Rey, CA). Data were further processed with in-home designed data-analyses software. Briefly, spots are eliminated if the absolute reference signal is less than two times the average signal of a set of control spots consisting of Drosophila DNA. Raw sample/reference ratios are calculated for all spots without any background correction. Normalization is carried out for each subarray separately, assuming that the median ratio of all spots will be “1”. As a second threshold a spot is eliminated if it differs more than 20% from the median ratio.
of all replicate spots that contain the same BAC. As a third threshold, all BACs are eliminated for which there is only one data point (spot) left. Finally, the average ratio is calculated for the remaining replicates of each BAC. All BACs whose mean ratio deviates more than 20% from the normal ratio were selected as possibly aberrant. However, high or low copy signals were only interpreted as possible gain or loss of DNA if at least two consecutive BACs on the array showed the same deviation from the normal ratio.

**Centroblast isolation for qRT-PCR**

Cell suspensions were prepared from tonsils taken from patients during routine tonsillectomy. Centroblasts (CB) were isolated using a double staining with CD20-PE (clone B-Ly1, DAKO) and CD77-FITC (clone 5B5, BD Pharmingen) by fluorescence activated cell sorting (MoFlo Cytomation, Fort Collins, Colorado, USA). CB1 consisted of a pool of centroblasts isolated from frozen tonsil cell suspensions of three patients and CB2 was a pool of centroblasts isolated from fresh tonsil cell suspensions of two patients. By reanalysis, each sample showed a purity of more than 90%. All protocols for obtaining and studying human tissues and cells were approved by the institution’s review board for human subject research.

**RNA isolation**

Total RNA from CB and HL cell lines was extracted using Absolutely RNA Miniprep Kit (Stratagene, La Jolla, CA) or Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. All RNA samples were DNase treated followed by a multiplex PCR with primer sets specific for genomic DNA to monitor the efficiency of the DNase procedure. The integrity of the isolated RNA was routinely checked on a 1% agarose gel and only good quality RNA samples were used for subsequent analysis.

**Generation of SAGE libraries**

SAGE libraries for L428 and DEV were generated using the I-SAGE kit (Invitrogen, Carlsbad, CA) according to the manufacturers protocol. The L1236 and CB SAGE libraries were generated previously. The computer program (SAGE2000 version 4.12) used for the analysis of gene specific tags was kindly provided by Dr KW Kinzler (John Hopkins Oncology Center, Baltimore, Maryland, USA) (see also http://www.sagenet.org). Linker tags and duplicate dimers were excluded and the tag numbers were normalized to 20000. The SAGE tags were linked to the CGAP best gene for a tag map (http://cgap.nci.nih.gov/SAGE) to identify the corresponding genes. Further analysis and comparisons were performed in Microsoft access and excel. Comparisons were focused on identification of (1)
HL specific up- and downregulated genes, (2) cHL specific up- and downregulated genes and (3) genes differentially expressed between cHL and NLPHL. Tags with >4 fold increase or decrease and a tag count of more than 4 in at least one of the libraries were considered to be differentially expressed. The fold change in expression was determined by dividing the (average) number of tags of one or more profiles through the (average) number of tags of other profiles. The Reference Sequence (RefSeq) code of every gene was used to define its genomic position in the aCGH profiles. SAGE tags that could not be assigned to a gene or Unigene cluster to map it on the genome were not included in this analysis. For L428, L1236 and DEV, 25, 13 and 34 genes, respectively, could not be accurately mapped to the genome.

**Quantitative RT-PCR**

TaqMan® Low-density arrays (Applied Biosystems, Foster City, CA) loaded with TaqMan® Gene Expression Assays (Applied Biosystems) were used for quantitative RT-PCR analysis of selected genes. Complementary DNA (cDNA) was synthesized from 500 ng of total cellular RNA by First Strand cDNA Synthesis System using Superscript II RT (Invitrogen, Carlsbad, CA) using random hexamers in a volume of 20 µl. 50 µL cDNA was mixed with sample-specific PCR mix (Applied Biosystems) and loaded into the Micro Fluidic Cards according to the manufacturer’s protocol (final concentration of 2 ng cDNA/well) and the PCR reaction was performed in the ABI Prism 7900 Sequence Detection System (Applied Biosystems). Assays were performed in duplicate on different arrays. RNA polymerase II was used as a reference gene. In each sample, average Ct values for the target genes were subtracted from the average Ct value of the reference gene to yield the ΔCt value. 2^ΔCt values were calculated to indicate the relative amount of transcripts in each sample.

**Results**

**Array CGH analysis**

Array CGH (aCGH) analysis of cHL cell lines revealed many aberrations. L428, L1236 and KM-H2 all showed aCGH profiles with more than 20 CN changes. The L591 profile showed 8 gains and 7 losses. In contrast, analysis of the NLPHL cell line DEV revealed only 5 gains and 3 losses including a ~3-Mb homozygous deletion at 17q24.1-24.2 (Fig. 1)\(^5\). Comparison of aCGH profiles revealed two frequently overrepresented regions previously reported in cHL. Gain of 2p was observed in all 4 cHL cell lines with the smallest region of overlap (SRO) spanning from ~59-71 Mb relative to 2pter, and gain of the telomeric region of 9p (~0-5 Mb) in 3/4 cHL cell lines. Other regions with gain identified in 3/4 cell
Figure 1. Whole genome aCGH profiles of cHL cell lines L428, L1236, KM-H2, L591 and NLPHL cell line DEV. The cHL cell lines have many more chromosomal aberrations than the NLPHL cell line DEV. Plotted on the y axis are the log₂ ratios for the individual BAC clones on the array and on the x axis the genomic order of the BAC clones from 1p-Yq. Shaded vertical boxes indicate the smallest region of overlap (SRO) with increased CN in at least 3 cHL cell lines. Open vertical boxes indicate SROs with loss in at least two cHL cell lines. The positions and sizes of the SROs are shown in megabase (Mb) below the vertical boxes.
lines were detected on 7p (2-6 Mb), 9p (26-36 Mb), 11q (128-134 Mb) and Xq (151-155 Mb). No consistent loss was identified in the cHL cell lines. Two regions showed loss in two cell lines, a ~7 Mb deletion on chromosome arm 4q in L591 and KM-H2 and a ~6 Mb deletion on chromosome arm 11q present in L591 and L428 (Fig. 1). None of the SROs identified in the cHL cell lines was present in DEV.

**SAGE analysis**

SAGE profiles of the cHL cell line L428 and the NLP HL cell line DEV consisted in total of 20442 tags representing 1224 unique tags and 19852 tags representing 1132 unique tags, respectively (Table 1). The previously reported SAGE profiles of cHL cell line L1236 and centroblasts (CB) were included for further analysis. In total almost 100,000 tags were compared.

**Identification of differentially expressed genes**

To identify differentially expressed genes we used an arbitrary >4 fold increase or decrease in tag count. Comparison of all HL libraries with the CB library revealed only 7 consistently upregulated genes (Table 2, 1A). In contrast, 125 genes were consistently downregulated (Table 2, 1B). Among these were many known B cell and/or hematopoietic genes like CD22, CD79A & B, BOB.1, SWAP70, CD45-AP, CD37, CD72 and several HLA and Ig heavy and light chain genes (Table 2).

Comparison of classical HL cell lines L428 and L1236 with CB revealed 14 genes with overexpression in L428 and L1236. This included FSCN1 (Fascin), CCL17 (TARC) and IRAK1, that are known to be overexpressed in cHL (Table 2, 2A). 141 genes were consistently downregulated in both cHL cell lines compared to CB (Table 2, 2B). Several genes were also downregulated in the DEV cell line, but others like LRMP1, CD45, PLC-γ2, CD20 and CD19 were only downregulated in the cHL cell lines and demonstrated similar expression levels in CB and DEV.
Between the cHL cell lines and the NLPHL cell line DEV 103 genes were differentially expressed. 13 were expressed at higher level in L428 and L1236 (Table 2, 3A), including CCL17 and IRAK1. 90 were expressed at higher level in DEV, including potential oncogenes like PIM2, BCL2A1 and MAGE-A9 and several hematopoietic or B cell markers like LRMP1, CD48, CD45 and PLC-γ2 (Table 2, 3B).

**qRT-PCR validation of selected genes in HL cell line panel and CB**

Based on the different comparisons (1A/B, 2A/B and 3A/B), we selected 45 genes for verification by qRT-PCR on 5 HL cell lines and two pools of CB (Table 2 and Table 3). cHL cell lines KM-H2 and L591 were included in this analysis to determine whether the deregulated expression was a more general feature in HL.

For the CB > HL (comparison 1B) comparison, 20/21 genes could be validated (Fig. 2A). Consistent results were observed for all genes in KM-H2 and for 14 genes in L591. Several B cell genes present in 1B, e.g. CD79B, CD45AP, CD79A, BOB.1 and SWAP70, demonstrated a reduced expression in DEV in comparison to CB, but the levels are higher as those observed in most cHL cell lines. For 11 genes, including most of the HLA genes, DEV displays expression levels similar to cHL which were lower than the levels in CB.

SAGE results could be validated for FSCN1, CCL17, IRAK1, GNG5 and LGALS1 identified as upregulated in cHL compared to CB (comparison 2A, Fig. 2B). Results were consistent for 4/5 genes in KM-H2 and all in L591. Though CCL17 levels appear to be much lower in KM-H2 and L591, they are still, respectively, a ~100-fold and a ~10-fold higher expressed than in CB. Seven out of 8 genes downregulated in cHL and not in DEV as compared to CB, indeed showed reduced expression in L428, L1236 and KM-H2 (comparison 2B, Fig. 2C). For L591 results were consistent for 5 genes. DEV demonstrated for most genes expression levels that were higher than the levels in cHL and lower than the levels in CB.
Table 3. List of the genes selected from different comparisons for qRT-PCR analysis.

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<th>Comparison</th>
<th>Gene</th>
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<th>L1236</th>
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<th>CB</th>
<th>UniGene</th>
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The SAGE data for CCL17, IRAK1, GNG5 and LGALS1, selected as elevated in cHL in comparison with DEV, could all be validated (comparison 3A, Fig. 2B). KM-H2 showed elevated expression compared to DEV for 3/4 genes and L591 for all. Finally, only 5 of the 17 genes selected as upregulated in DEV compared to L428 and L1236 could be validated (comparison 3B, Fig. 2D). Consistent results were obtained for all genes in KM-H2 and for only 1 gene in L591.

**Global correlation between gene expression and DNA Copy Number**

A genome wide comparison of DNA CN changes with differentially expressed genes revealed that in total, 18% of the differentially expressed genes of L428 and L1236 map to regions with gain or loss of CN. In DEV, only 7% of the differentially expressed genes map to regions with abnormal CN. A high percentage of the genes that mapped to genomic regions with loss showed decreased mRNA levels. This percentage declines in regions with gain (Fig. 3). On the other hand, the percentage of genes with increased mRNA levels that mapped to regions with loss is lower than the percentage in regions with gain. This trend is most pronounced in the L428 cell line, where the percentage of overexpressed genes mapping in regions with loss increases from 27% to 73% in regions with gain.

**Chromosomal aberrations and gene expression**

Of the 14 commonly overexpressed genes in L428 and L1236, FSCN1, and IRAK1 map within amplified regions identified in 3/4 cHL cell lines (Fig. 1). FSCN1 and IRAK1 are located on the telomeric ends of 7p and Xq, respectively, and show an increased expression
A) comparison 1B (CB>HL)

B) comparison 2A and 3A (cHL>CB and cHL>DEV)
in cHL cell lines compared to CB and DEV (Fig. 2B).

Chromosomal region 2p is frequently amplified in cHL and includes the c-REL gene at 2p13 (position 61 Mb) as the proposed target gene. All 4 cHL cell lines have complete or

Figure 2. qRT-PCR validation of SAGE results. A) Analysis of downregulated genes in all HL cell lines compared to CB (comparison 1B). B) Analysis of genes upregulated in cHL compared to CB (comparison 2A) or DEV (comparison 3A). C) Analysis of genes downregulated in cHL compared to CB (comparison 2B). All genes from comparison 1B are also valid for this comparison. D) Analysis of downregulated genes in cHL compared to DEV (comparison 3B). LRMP1, CD45, PLCG2, IFI30, EVL and GGA2 from comparison 2B are also valid for this comparison.
partial gains of chromosome arm 2p with an SRO from ~59-71 Mb (Fig. 4A). However, in none of the SAGE libraries, tags (or alternative tags) corresponding to c-REL could be identified. Analysis of c-REL mRNA levels by qRT-PCR revealed an on average 2-fold reduced transcript level in cHL cell lines compared to CB (Fig 4A). None of the genes overexpressed in L428 and L1236 as compared to CB map within the 2p-SRO.

The SRO at 9p includes the JAK2 gene at 9p24 (Fig. 4B). Again, no tag(s) representing JAK2 were identified in the cHL SAGE libraries. qRT-PCR analysis revealed that JAK2 mRNA levels in 3/4 cHL cell lines were comparable to CB. L1236 showed a ~10-fold higher JAK2 expression (Fig.4B). No other commonly overexpressed gene mapped within the SRO on 9p.

Many of the downregulated genes shared between L428, L1236 and DEV mapped to the HLA region on chromosome 6 (Fig. 4C). The decreased expression of 7 of those genes could be confirmed by qRT-PCR (HLA-genes in Fig. 2A, comparison 1B). All 7 genes were also downregulated in KM-H2 and 5/7 were downregulated in L591. None of the HL cell lines showed loss of the HLA region on 6p.

The homozygous deletion in the DEV cell line at 17q24.1-24.2 contains 12 Reference Sequence (RefSeq) genes. As expected, no tags corresponding to any of the 12 genes were
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detected in the DEV library. Tags for 2/12 RefSeq genes, i.e. GNA13 and PRKCA, were detected in one or more of the other SAGE libraries. qRT-PCR analysis confirmed the absence of GNA13 and PRKCA transcripts in DEV and their presence in cHL cell lines and CB (Fig. 4D).

Discussion

Chromosomal aberrations in HL

In the present study we used aCGH to identify and map segmental CN changes in HL cell lines. Besides the already known SROs with gain on 2p, 9p and 11q\(^{59,63}\), we detected two new segments of ~4 Mb with an increased CN mapping to 7p and Xq (Fig. 1). Previous studies have not recognized these small SROs, perhaps due to the limited sensitivity of conventional CGH\(^{128,129}\).

Gene expression in HL

To validate our SAGE libraries, 45 different genes were selected for qRT-PCR validation. More than 90% of the selected genes could be validated for 4/5 comparisons. For unclear reasons, the validation frequency of the comparison between chHL and DEV was much lower. KM-H2 and L591 showed consistent gene expression changes for all but one (GNG5) and ~73% of the genes, respectively. The fact that L591 is more often an “outlier”
among the cHL cell lines may be due to the fact that this cell line shows a, for cHL, unusual EBV latency III gene expression program, i.e. expression of the transcriptional activator EBNA2 \(^{130,131}\).

Comparison of the HL SAGE libraries with the CB library revealed only 7 commonly upregulated genes in HL. This may not be very surprising since it is known that cHL and NLPHL are distinct types of HL based on several clinical, immunohistochemical and genetical differences. More surprising was the fact that also in L428 and L1236 very few genes (14) were commonly overexpressed compared to CB. Lowering the threshold for differences in tag count does not increase this number much: placing the threshold to >3 fold difference in tag count in cHL compared to CB resulted in 29 commonly overexpressed genes.

In contrast to overexpression, many genes are commonly downregulated in HL (125) and even more in cHL cell lines (141) compared to CB. Especially many B lineage or hematopoietic lineage genes were downregulated in cHL, in line with a previous report\(^ {100}\). Several of those genes, e.g. CD79B, CD79A, BOB.1 and CD22 were also downregulated in NLPHL cell line DEV. However, several other B cell expressed genes, e.g. LRMP1, CD45, CD20, CD19 and PCLG2 are also expressed in DEV. This suggests that the NLPHL cell line DEV may have an intermediate “loss of B cell phenotype” at the mRNA level. Whether primary L&H cells also display this phenotype, remains to be investigated. At the protein level at least, expression of several of these B cell genes was comparable in DEV and primary L&H cells to normal B cells\(^{8,55}\).

The common loss of B cell characteristic gene expression suggests that it may have an important function in the pathogenesis of cHL. This may be achieved via downregulation of one or more B cell associated transcription factors like E2A, EBF or Pax5. Recently, it was shown that in HRS cells inhibition of E2A by the known antagonists ID2 and/or ABF-1 results in reduced expression of several B cell specific genes\(^ {117}\). Indeed, ID2 and ABF-1 were shown to be highly expressed in cHL\(^ {99,117}\). Besides deregulation of transcription factors, epigenetic mechanisms were also reported to be involved in the B lineage specific gene silencing in cHL. It was shown that the expression of many B lineage genes, including CD19, CD20, CD79B, SYK, PU.1, BOB.1, BCMA and LCK, could be reactivated by demethylation of the DNA\(^ {115,132}\).

The common downregulation may also be caused by the overexpression of microRNA (miRNA). This recently discovered new class of small regulatory RNA molecules can downregulate the expression of several target genes by mRNA degradation and/or translational repression\(^ {133}\). We and others have shown that miR-155 is strongly upregulated in HL, regardless of subtype\(^ {134,135}\). Studies defining the targets of miR-155 and possible other highly expressed miRNA in HL will help determining their role in the global downregulation of B cell genes.
Association between copy number changes and gene expression

Mapping of differentially expressed genes to aCGH profiles of the HL cell lines revealed that most genes map to regions without gain or loss. This implies that the differential expression of most genes is not directly caused by CN changes. However, a clear trend was observed for the percentage of up- or downregulated genes in relation to the CN. The percentage of upregulated genes is much higher in regions with gain than in regions with loss and vice versa (Fig. 3).

Two genes upregulated in L428 and L1236 mapped within a SRO, FSCN1 on chromosome arm 7p and IRAK1 (interleukin-1 receptor-associated kinase 1) on chromosome arm Xq. High expression of FSCN1 protein in cHL and not NLPHL was reported previously. FSCN1 is known to be involved in actin cytoskeletal organization and cell migration. More recently it was shown that FSCN1 downregulation in carcinoma suppresses cell proliferation and invasion. The increased CN in the majority of cHL cell lines, the elevated expression at the mRNA and protein level and the potential functional role of FSCN1 together suggest an important role for FSCN1 in the pathogenesis of cHL.

The high IRAK1 mRNA levels in cHL in comparison to CB correlated with a common CN increase in cHL. A high IRAK1 mRNA expression level was demonstrated previously in cHL, but its expression was not studied in NLPHL. Unfortunately, detection of IRAK1 protein by immunohistochemistry was not possible since none of the available anti-IRAK1 antibodies showed (reliable) staining. IL1 bound to type 1 IL1 receptor (IL1R) induces recruitment of IRAK1 to the receptor complex, leading to IRAK1 phosphorylation. Upon phosphorylation, IRAK1 dissociates from the receptor complex and interacts with downstream molecules ultimately leading to NF-κB activation. Expression of IL1 has been shown in HRS cells and elevated expression of IL1R was also shown in HRS cells compared to germinal center B cells. Constitutively activated NF-κB is a hallmark of HRS cells that can be induced by diverse mechanisms. Autocrine or paracrine signalling via IL1 - IRAK1 may provide an additional mechanism leading to constitutive activity of NF-κB in HRS cells.

The previously reported c-REL and JAK2 genes as targets of the SROs on 2p and 9p were also studied by qRT-PCR (Fig. 4A and B). Surprisingly, c-REL and JAK2 mRNA levels were not elevated in the majority of cHL cell lines compared to CB. These results raise the question whether c-REL and JAK2 are the real targets of the SROs on 2p and 9p.

A striking downregulation of genes mapping within the HLA region was detected in all HL cell lines. This downregulation did not correlate with a loss of genomic DNA (Fig. 4C). It is known that HLA class I protein is usually not expressed by HRS cells. Although this was previously not appreciated, a recent study by Diepstra et al. showed absence of membranous HLA class II expression in almost 40% of the cHL cases.
A small homozygous deletion of ~3-Mb at 17q24.1-24.2 was detected in the DEV cell line\(^5\) (Fig. 4D). In a series of 19 NLPHL cases 6 showed loss of whole chromosome 17 and 1 showed loss of 17p\(^4\). In contrast to NLPHL, cHL is recognized by frequent gain of 17q instead of loss\(^6\). The homozygous deletion in DEV contains 12 RefSeq genes. Several of those genes have potential interesting functions like protein kinase C alpha (PRKCA), a gene with well known signal transduction capacity, and the tumor suppressor genes AXIN2 and HELZ\(^146,147\). So far, the relevance of the loss of expression of any of the genes within the homozygous deletion in relation to the pathogenesis of NLPHL is unknown.

In summary, we have confirmed the “loss of B cell phenotype” in cHL cell lines and show a more intermediate phenotype in the NLPHL cell line DEV. Comparison of gene expression profiling results with CN alterations revealed a good correlation for L428 and L1236. Two consistently overexpressed genes mapped to two common SROs in cHL, \(FSCN1\) on 7p and \(IRAK1\) on Xq. The fact that \(IRAK1\) and \(FSCN1\) are commonly amplified and overexpressed in cHL suggests an important role for these two genes in the pathogenesis of cHL.

**Acknowledgements**

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