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Recurrent Somatic Loss of *TNFRSF14* in Classical Hodgkin Lymphoma

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Abstract

Investigation of the genetic lesions underlying classical Hodgkin lymphoma (CHL) has been challenging due to the rarity of Hodgkin and Reed-Sternberg (HRS) cells, the pathognomonic neoplastic cells of CHL. In an effort to catalog more comprehensively recurrent copy number alterations occurring during oncogenesis, we investigated somatic alterations involved in CHL using whole-genome sequencing-mediated copy number analysis of purified HRS cells. We performed low-coverage sequencing of small numbers of intact HRS cells and paired non-neoplastic B lymphocytes isolated by flow cytometric cell sorting from 19 primary cases, as well as two commonly used HRS-derived cell lines (KM-H2 and L1236). We found that HRS cells contain strikingly fewer copy number abnormalities than CHL cell lines. A subset of cases displayed non-integral chromosomal copy number states, suggesting internal heterogeneity within the HRS cell population. Recurrent somatic copy number alterations involving known factors in CHL pathogenesis were identified (*REL*, the PD-1 pathway, and *TNFAIP3*). In eight cases (42%) we observed recurrent copy number loss of chr1:2,352,236–4,574,271, a region containing the candidate tumor suppressor *TNFRSF14*. Using flow cytometry, we demonstrated reduced *TNFRSF14* expression in HRS cells from five of 22 additional cases (23%) and in two of three CHL cell lines. These studies suggest that *TNFRSF14* dysregulation may contribute to the pathobiology of CHL in a subset of cases.

Introduction

Classical Hodgkin lymphoma (CHL) ranks among the most frequently occurring lymphomas in the western world and is marked by characteristic Hodgkin and Reed-Sternberg (HRS) cells, frequently multi-nucleated neoplastic cells derived from germinal

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center B lymphocytes (Kuppers, 2009; Kuppers et al., 2012). While most patients with CHL generally respond well to combination chemo- or radiation therapy, approximately 15 to 20% fail initial treatment or undergo relapse (Cuccaro et al., 2014). Further, even in patients in whom initial treatment is successful, secondary complications, including gonadal dysfunction, growth retardation, and secondary malignancies may occur (Evens et al., 2008).

The genetic pathways involved in CHL have been difficult to illuminate because HRS cells are rare and surrounded by robust, non-neoplastic infiltrates such that they may represent less than 0.1% of cells within involved lymph nodes (Kuppers, 2009), limiting material for research. Historically, discovery of factors contributing to CHL pathogenesis primarily occurred through studies utilizing more facile HRS-derived cell lines (Joos et al., 2003), genome-wide association studies (Enciso-Mora et al., 2010), and candidate gene identification in CHL predisposed pedigrees (Salipante et al., 2009). Other work has examined HRS cells directly by methods including karyotype (Atkin, 1998), fluorescence in situ hybridization (FISH) (Van Roosbroeck et al., 2011), and gene expression profiling (Steidl et al., 2012; Tiacci et al., 2012) or array comparative genomic hybridization (aCGH) of microdissected tumor cells (Hartmann et al., 2008; Steidl et al., 2010). More recently, targeted exome sequencing has been applied to enriched HRS cells (Reichel et al., 2015).

Several oncogenes and pathways have been successfully implicated in the disease (Hinz et al., 2002; Mathas et al., 2002; Weniger et al., 2006; Kuppers, 2009; Green et al., 2010; Kuppers et al., 2012; Steidl et al., 2012; Gunawardana et al., 2014; Reichel et al., 2015); regardless, technical challenges associated with HRS cell rarity have proven to be a significant barrier which limits genomic resolution (Atkin, 1998; Hartmann et al., 2008; Gunawardana et al., 2014). Consequently, pathogenic mutations have likely not been fully delineated, and work to date has failed to reveal any universal genetic lesion occurring in CHL.

Here, to better characterize genomic aberrations underlying CHL, we performed high-resolution genomic copy number analysis of HRS cells based on low-coverage whole genome sequencing data. Small pools of flow cytometric sorted neoplastic cells and patient-matched non-neoplastic B cells were examined from 19 sporadic, pre-treatment patient cases.

Materials and Methods

Patients and HRS Cell Isolation

High purity HRS and non-neoplastic B cells were isolated from lymph node suspensions via flow cytometric cell sorting as described in full elsewhere (Fromm et al., 2006). We selected cases only on the basis of having adequate residual excisional biopsy material after clinical immunophenotyping, with priority given to specimens where the fraction of HRS cells was relatively high (typically, 0.1-1%). Briefly, cell suspensions from morphology confirmed CHL cases (Supplementary Table 1) were blocked with unlabeled antibodies on ice for one h to reduce the number of bound T cells [anti-CD58 (Endogen, Woburn, MA; clone TS2/9), anti-CD54 (Serotec, Oxford, United Kingdom; clone 84H10), anti-CD2 (Becton Dickinson, San Jose, CA; clone RPA-2.10), and anti-LFA-1 (Dako, Carpinteria, CA; clone MHM23)]

and then stained for 15 min at room temperature with labeled antibodies purchased from Becton Dickinson (BD, East Rutherford, NJ), Beckman Coulter (Pasadena, CA), or Invitrogen (Carlsbad, CA): CD95-PB (Pacific Blue label; clone DX2), CD64-FITC (fluorescein isothiocyanate label; clone 22), CD30-PE (phycoerythrin label; clone Ber-H83), CD5-PETx (phycoerythrin-Texas red label; clone BL1a), CD40-PECy5.5 (phycoerythrin-Cyanine5.5 label; clone mAb89), CD20-PECy7 (phycoerythrin-Cyanine7 label; clone B9E9), CD15-APC (allophycocyanin label; clone H198), CD71-APCA700 (allophycocyanin-AlexaFluor700 label; clone YDJ.1.2.2), and CD45-APCCy7 (allophycocyanin-Cyanine7 label; clone 2D1). Cells were then washed once (3 ml PBS-BSA), reconstituted (1 ml RPMI), and sorted using an Aria II cell sorter (BD). HRS cells with attached T cells (including T cell rosettes) were excluded during cell sorting by gating on HRS cells without expression of CD5. When possible, HRS cell purity was experimentally determined by re-analyzing an aliquot of the purified cells on the cell sorter (Supplementary Table 2). All material was from primary diagnosis, before initiation of therapy. HRS cell lines KM-H2, L1236, and L428 were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). This project was approved by the University of Washington Human Subjects Division, and was conducted in accordance with the Declaration of Helsinki.

Library Preparation and Sequencing

DNA was extracted from samples using Gentra Puregene kit (Qiagen, Germantown, MD) using the hair follicle protocol, with matched numbers of HRS cells and B cells extracted per patient. Patient sequencing libraries were prepared with Nextera enzyme (Epicentre, Madison, WI) using DNA from 500-cell equivalents as described (Adey, et al. 2010), except that PCR utilized KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Woburn, MA). If greater than 1,000 cells were collected from a specimen, individual libraries were prepared from aliquots of 500-cell equivalents using separate sequencing indices. Cell lines were prepared as shotgun libraries using standard ligation methods and non-limiting amounts of template genomic DNA. Sequencing was performed on an Illumina HiSeq2000 (San Diego, CA). Sequencing statistics are summarized in Supplementary Table 3.

Copy Number Calling and Recalibration

Sequencing reads aligned to GRCh37 for both HRS- and B-cell libraries were tallied over windows of unique mappability followed by normalization to a constant. The ratio of HRS-cell read depth over B-cell read depth (Supplementary Fig. 1) was then used in a segmentation Hidden Markov Model (HMM) described elsewhere (Adey et al., 2013), which was designed to call broad copy number states (short copy number variations are detected later via outlier calling). Copy number windows of two sizes were used based on uniquely alignable sequence with a mean size of 1,497.7 and 76,937.4 bp for high and low resolution calls, respectively. For each case, a number of initial state mean ratio scores were analyzed to find the model that gave the best fit to the data. Upon the best fitting segmentation, recalibration and absolute copy number identification was performed (Supplementary Figs. 2 and 3). For each sample, all possible copy number state assignment hypotheses were tested allowing for a maximum copy number of 12. This process also took into account sample purity and resulted in a genetic material ratio (GMR) for the sample

which represents the ratio of genetic material in the sample compared to its B-cell diploid counterpart. Empirical estimations of HRS cell purity were used when available, and were assumed to be 70% in cases where these data were not available. Each ratio score was then recalibrated by multiplying the ratio by the GMR. After recalibration, outliers were identified using a sliding window approach with thresholds based on the standard deviation of the state such that segments for which three of five windows were all above or below two standard deviations were called as outliers and assigned to a copy number.

A number of cases evidenced chromosomes which did not fit well with the rest of the data and for which the called state would switch back and forth between two states, suggesting potential “half”, or non-integer, states. To systematically identify these instances, the standard deviation from the called states was calculated for each chromosome, followed by calculation of the standard deviation if the entire chromosome was instead called at a half copy number state. The improvement score was determined as the standard deviation improvement over the original standard deviation. Chromosomes with marked improvement scores were classified as putative “half-state” chromosomes.

Recurrent Copy Number Enrichment and Depletion Events

Recurrence of enriched or depleted genomic regions was carried out on copy number segmented and outlier-called low-resolution (mean window size 76,937.4 bp) maps for the entire set of 19 primary cases. First, an enriched copy number threshold was determined for each sample by determining the lowest copy number for which the top 1% of windows fell above or, for the depleted threshold, the highest copy number for which the bottom 1% of windows fell below. This resulted in ranges between 29% and 1.2% of all windows across a sample being considered enriched or depleted. Each window was then examined for the number of samples with that window in excess above or below the respective thresholds. Permutation iterations were next performed by shuffling the threshold status assignments while maintaining the same number of enriched or depleted windows for each sample, followed by examining the number of enriched or depleted samples at each individual genomic window. A total of 10 million iterations were performed for enrichment detection and 1 million for depletion detection (as *P*-values were slightly lower for depletion and 1 million iterations were sufficient in establishing confident *P*-values). The *P*-values were then FDR-corrected into *q*-values using the R package “qvalue”. Significance thresholds were then set at 1% or 5% false discovery rates with all reported enriched or depleted segments passing the 1% FDR mark.

TNFRSF14 Expression

Patient lymph node suspensions or CHL cell lines mixed with normal peripheral blood were labeled and analyzed as above with respect to HRS and non-neoplastic B cells (Fromm et al., 2006), with the addition of PE-labeled anti-*TNFRSF14* (anti-CD270) (Biolegend) and the following combination of lineage-identifying antibodies: CD95-PB/CD64-FITC/CD270-PE/CD5-ECD/CD40-PECy5.5/CD20-PECy7/CD15-APC/CD30-APCA700 (Beckman Coulter, clone HRS4)/CD45-APCCy7. Anti-*TNFRSF14* signal and fluorescence minus one (FMO) controls were evaluated for HRS cells or HRS-derived cell lines (the latter evaluated in triplicate) relative to internal, normal B-cell controls.

Results

Sequencing and Copy Number Calls

Purifying intact HRS cells using flow cytometric cell sorting (Fromm et al., 2006) allowed for isolation of high-purity neoplastic cells from CHL patient lymph nodes, bearing high-quality DNA. However, due to the small size of many clinical biopsies, the yield of purified cells from residual material remaining after clinical diagnosis was limited, with only a few hundred cells available for most cases. We therefore adapted transposase-mediated library preparation for use with small quantities of DNA (Adey et al., 2010) in order to generate sequencing libraries from as few as 500 cells. In parallel, we performed deep sequencing of two spontaneously immortalized CHL cell lines commonly used in research, KMH2 (Kamesaki et al., 1986) and L1236 (Wolf et al., 1996).

Limited library complexities, and subsequently, limited effective read depths, were achievable from patient material (Supplementary Table 3), preventing us from performing reliable calling of somatic sequence variants (single nucleotide variants and insertions and deletions). Nevertheless, it was possible to robustly catalog somatic copy number alterations (SCNAs) (Beroukhi et al., 2010; Zack et al., 2013) using these genomic data (Adey et al., 2013). Previous studies have documented inconsistently recurrent SCNA of several loci in CHL, while limited to a resolution of 50-160 kb by existing aCGH methods (Hartmann et al. 2008; Steidl et al. 2010). Here, we inferred SCNAs from sequencing read depth, reducing DNA requirements by orders of magnitude, while providing enhanced resolution of up to ~1.5 kb (Adey et al., 2013) for nine of our cases with sufficient sequencing library complexity. We compared HRS cells to patient-matched lymphocytes, and cell lines against a copy-number neutral reference (Adey et al., 2013) in order to normalize for patient-specific constitutional variation (Fig. 1 and Supplementary Fig. 3).

Inferred SCNAs were overwhelmingly assigned to integers (Supplementary Figs. 3 and 4), suggesting that HRS populations derive from a clonal origin (Stein and Hummel 1999) and therefore share identical SCNAs. However, 6 cases included a subset of chromosomes that did not conform to these states and were instead best fit by intermediate, non-integer states (Supplementary Fig. 4), indicating a substantial degree of heterogeneity within those specimens. We also noted that cell lines contained strikingly more SCNA events than were demonstrated by HRS cells (Fig. 1).

Cataloging Recurrent SCNA Events

We identified genomic regions with statistically significant recurrent copy number gains or depletion through the use of a permutation analysis. Several genomic regions previously reported as sites of recurrent copy number alteration in other work (Joos et al., 2002; Chui et al., 2003; Hartmann et al., 2008; Steidl et al., 2010) were variably impacted in our study, yet bore insignificant q -values within our cohort. However, we note poor correlation among results of these previous studies, suggesting a lack of cross-study validation.

We identified two recurrent regions of copy number gain which crossed significance ($q < 0.01$, Fig. 2): chr2:59,373,061-64,564,824 (31 genes, eight cases) and chr9:4,055,292-8,654,471 (29 genes, eight cases). Encouragingly, previous aCGH studies

have implicated both of these loci in CHL pathogenesis (Joos et al., 2002; Chui et al., 2003; Green et al., 2010;), offering independent validation of the approach. The identified region of chromosome 2 contains the *REL* proto-oncogene (Joos et al., 2002), which participates in the mitogenic and tumorigenic NF-kappaB signaling pathway (Rayet and Gelinas, 1999) and is frequently overexpressed in CHL (Kuppers et al., 2012). Similarly, the chromosome 9 locus corresponds to the immunomodulating PD-1 pathway (*CD274*, *PDCD1LG2*, and *JAK2*), whose upregulation has similarly been observed in CHL and may play a role in immune evasion (Green et al., 2010).

Two regions of significantly recurrent copy number depletion were also identified (Fig. 2): chr6:135,344,040-147,103,224 (63 genes, 7 cases) and chr1:2,352,236-4,574,271 (29 genes, eight cases). Recurrent loss of 6q23-24 in CHL has been observed using aCGH (Hartmann et al., 2008), and focused deletions of tumor suppressor *TNFAIP3*, located within this region, have been shown in HRS cells (Kato et al., 2009; Schmitz et al., 2009; Nomoto et al., 2012). In contrast, although loss of a larger (>4 Mb) genomic region encompassing the chromosome 1 locus has been previously cataloged in one study (Steidl et al., 2010), this locus has not yet been externally validated or experimentally explored as a contributor to CHL pathogenesis. Intersecting our higher resolution map of this chromosome 1 locus (Supplementary Table 4) with a genomic catalog of genes frequently mutated in cancer (Lawrence et al., 2014) revealed overlap of only one factor, *TNFRSF14*, identifying it as a strong candidate gene in CHL, also formerly speculated as a contributory gene (Steidl et al., 2010).

***TNFRSF14* Expression in HRS Cells**

To further investigate a possible role of *TNFRSF14* in HRS cells, we quantified its expression in 22 additional and independent primary cases using flow cytometry (Fig. 3) (direct evaluation of *TNFRSF14* in sequenced cases was not possible, as no residual material suitable for flow cytometry was available). HRS cells from five cases (23%) demonstrated decreased *TNFRSF14* expression compared to patient-matched B cells. Further, two of the three CHL cell lines examined exhibited decreased *TNFRSF14* by flow cytometry relative to normal B cells. These studies suggest that reduced *TNFRSF14* protein expression occurs frequently in CHL.

Discussion

Unlike solid tumors, in which tumors cells are abundant and readily outnumber admixed stromal or inflammatory cells, genomic studies of HRS cells pose technical challenges for genetic and genomic studies. Here, we performed low-coverage whole genomic sequencing of small numbers of isolated HRS cells (Fromm et al., 2006) (Reichel, et al. 2015) using transposase-mediate library construction techniques (Adey et al., 2010) to define the somatic copy number profiles of primary, untreated CHL cases. In contrast to single-cell protocols that require whole-genome amplification which may introduce copy number artifacts (Pugh et al., 2008), transposase-mediated library construction is minimally biased and efficient enough to perform directly on trace material (Adey et al., 2010). Similarly, using HRS cells purified by flow cytometric cell sorting (Fromm et al., 2006; Reichel et al., 2015) avoids

fixation and sectioning artifacts (“truncation effect”) associated with laser microdissection techniques (Steidl et al., 2010), and offers advantages in overall throughput. Whole-genome SCNA analysis from limited, intact HRS cells thereby overcomes several challenges presented by CHL pathobiology, offering advantages in genomic resolution and permitting studies directly from patient material.

We observed substantial differences in the overall burden of SCNA observed in primary HRS cells compared to that seen in HRS-derived cell lines (Fig. 1). For copy number calls performed across both primary cases and cell lines, the standard deviation of windows within copy number states was low compared to the magnitude of differences observed between states, thus yielding a strong signal for copy number state segmentation and providing high confidence in the copy number alterations identified. The robustness of these copy number calls supports our conclusion that differences in the abundance of copy number alterations between primary cases and cell lines reflects a real, biological observation rather than a technical artifact, which would instead manifest as an increase in overall noise. Given the absence of patient-matched material for the cell lines, a limited number of identified sites of copy number variation are potentially due to neutral, constitutional differences exhibited between the cell line donor and the control DNA used to normalize copy number calls; nevertheless, such regions are expected to be limited in both size and pervasiveness (Conrad et al., 2010) and would consequently not explain the abundance of copy number alterations spanning multi-megabase lengths. HRS-derived cell lines were obtained from late stage or recurrent disease and represent unusual instances of CHL where HRS cells have become spontaneously immortalized and have gained the capability to replicate in vitro (Joos and Siebert, 2007), distinguishing them biologically from the vast majority of primary cases. Our findings suggest that the genomic SCNA landscape of these cell lines, too, is distinct from primary HRS cells, a conclusion also supported by comparative gene expression profiling studies (Steidl et al., 2012). These findings imply not only that CHL cell lines do not accurately model the genomics of primary disease but also that transforming lesions of CHL may be less complex than suggested by cell line research.

Interestingly, our analysis of primary HRS cells identified a few isolated cases evidencing chromosomes or chromosomal segments assigned to non-integral copy number values, indicating a mixture of cells bearing higher and lower genomic copy number complements for those regions (Supplementary Fig. 4). Given that HRS cells are known to be of a clonal origin (Inghirami et al., 1994; Kuppers et al., 1994; Hummel et al., 1999), this finding would be consistent either with rare CHL cases being marked by the presence of genetically-distinct HRS subclones, or alternatively, a single, uniform HRS cell population demonstrating internal chromosomal heterogeneity (Weber-Matthiesen et al., 1995), possibly among individual nuclei of multinucleated cells.

Several studies have sought to catalog the sites of recurrent copy number alterations in CHL (Joos et al., 2002; Chui et al., 2003; Hartmann et al., 2008; Steidl et al., 2010). For nearly half of the cases studied here, copy number calls were produced at a substantially higher resolution than previously achievable with existing methodologies. Our survey implies that the frequently occurring recurrent SCNA relevant to CHL pathogenesis have now been identified. At least one of the four statistically significant copy alterations identified by our,

and other studies, was identified in 18 of 19 cases (Fig. 2B), suggesting both that these events are quite frequent in CHL and that one or more SCNAs from this limited pool could potentially account for oncogenic changes underlying the majority of cases.

Genomic data identified somatic copy number loss of *TNFRSF14* as a candidate event in CHL, and we present observational data implicating the factor as a novel tumor suppressor pertinent to CHL pathogenesis. There is a significant correlation between genomic copy number and gene expression levels in CHL (Steidl et al., 2012), and significantly, we observed reduced expression of the *TNFRSF14* in HRS cells from nearly 23% of unselected primary CHL cases (Fig. 3). Although loss of a larger (>4.4 Mb) genomic region encompassing chr1:2,352,236-4,574,271 was previously reported as recurrent in CHL through aCGH studies of laser microdissected HRS cells (Steidl et al., 2010), outside of speculation, the *TNFRSF14* gene has not previously been demonstrated to be of importance in this malignancy. Additionally, the higher resolution of our study permitted us to further define a minimal region of recurrent copy number loss reported in our study which eliminates the *PRKCZ* gene (Steidl et al., 2010), previously speculated to contribute to CHL pathogenesis, from consideration. *TNFRSF14* is a tumor suppressor and immunogenicity regulation factor whose mutation has been demonstrated in the pathogenesis of both diffuse large B-cell lymphoma (Lohr et al., 2012) and follicular lymphoma (Cheung et al., 2010; Li et al., 2014; Mamessier et al., 2014), offering plausible support for a biological role in CHL. The mode by which *TNFRSF14* exerts oncogenic effects remains unclear, both because it is capable of sending both stimulatory and inhibitory signals to T cells depending on the particular ligand it binds (Kridel et al., 2012), and because it displays numerous splice variants (Cheung et al., 2010). Some have speculated that the gene plays a role in the oncogenic NF-kappaB signaling pathway (Steidl et al., 2010), which is frequently perturbed in CHL (Kuppers et al., 2012; Lake et al., 2009). Further exploring the role of *TNFRSF14* in CHL pathogenesis may help to elucidate the gene's mode of action in all three malignancies. Nevertheless, it is a possibility that additional genes in this region may contribute to oncogenesis, and targeted functional analyses as well as the study of supplementary cases may provide increased power to delineate additional relevant factors.

The limitations of our current work necessarily reflect the limited availability of patient cases with suitable quality and viability for flow cytometry enrichment, the paucity of clinical material available per patient, and also the inherent difficulties posed by this challenging and material-limited lymphoma. Due to a lack of sufficient material, we were unable to perform sequencing and expression analysis on the same cohort of patient samples, and are consequently unable to demonstrate a direct link between copy number loss and reduced *TNFRSF14* expression in CHL. It should also be noted, as in other disorders in which *TNFRSF14* mutation has been recurrently observed (Cheung et al., 2010; Lohr et al., 2012), that protein downregulation may result from inactivating sequence mutations or copy-neutral loss of heterozygosity, in addition to copy number loss. Thus, observed disruption of protein expression by flow cytometry in a fraction of our patient cases may reflect these alternative mechanisms and not necessarily copy number loss alone: indeed, cell lines KH-M2 and L1236 do not evidence overt copy number loss at this locus but display reduced *TNFRSF14* by flow cytometry. Another limitation of our study is that

insufficient numbers of flow sorted cells were available from patient cases to cross-validate the results of genomic sequencing using conventional methods, such as fluorescence in situ hybridization. Regardless, use of sequencing data to infer copy number states has been independently validated against conventional methods by several groups in a variety of contexts (Adey et al., 2013; Dumas et al., 2014; Liang et al., 2014; Wang et al., 2014). The various loci identified in the our study are supported by aCGH studies (Joos et al., 2002; Chui et al., 2003; Hartmann et al., 2008; Green et al., 2010; Steidl et al., 2010), including a deletion encompassing *TNFRSF14* (Steidl et al., 2010), providing external support for the validity of our approach and findings. We note that although the rate of *TNFRSF14*-region copy number loss identified by Steidl et al. (2010) (13%) was markedly lower than estimated in our study, this difference could be an artifact of the relatively small number of specimens examined. Nevertheless, and regardless of the underlying genetic mechanism or mechanisms that may be responsible for our observations, the data indicate that functionally reduced *TNFRSF14* protein expression occurs recurrently and frequently in CHL. The observation of genomic and protein-level *TNFRSF14* dysregulation in independent patient cohorts provides encouraging and orthologous support for a role of the gene in this malignancy.

It is unlikely that the present study encompasses a comprehensive catalog of all CHL-relevant genes. Evaluating increased numbers of cases may reveal less frequent, but biologically significant, recurrent SCNA. Moreover, reliably calling genomic point mutations from limited numbers of input cells remains challenging: library complexities in our study were insufficient to permit such calls. Indeed, whole genome characterization of HRS cells at this level of resolution has not yet been achieved (Gunawardana et al., 2014; Reichel et al., 2015). Future improvements to sequencing library preparation techniques may enable more comprehensive genomic characterization of CHL, permitting interrogation of both copy number and smaller sequence variants contributing to pathogenesis. As the cost of genomic sequencing continues to decrease, and single-cell sequencing protocols are further developed (Macaulay and Voet, 2014), we anticipate that direct sequencing of primary HRS cells will become increasingly feasible in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Representative genomic copy number alterations in primary HRS cells and HRS-derived cell lines. Copy number states are expressed as read depth ratio (left) compared to matched normal B cells for patient cases or a population-level control for cell lines, and as absolute copy number (right) for each sample. Copy number states for CHL cell lines KM-H2 (A) and L1236 (B) are shown relative to representative patient cases (C-D).

Figure 2. Statistically significant recurrent copy number alterations. (A) FDR-corrected q -values for recurrently enriched (blue) or depleted (red) genomic segments across all patients. Annotated genes within four genomic regions with an associated FDR rate of $<1\%$ are diagrammed, and known or speculated CHL-associated factors are indicated. (B) Copy number calls for the four statistically significant genomic regions in primary patient cases. Copy number calls are shaded according to depletion (copy number = 1, red), modest enrichment (copy number = 4, light blue), or strong enrichment (copy number > 4 , blue).

Figure 3.

Representative expression levels of *TNFRSF14* in primary HRS cells and CHL cell lines. (A-B) Primary HRS cells identified from patient lymph nodes using the antibody combination described in the Methods sections. For each panel, leftmost plot demonstrates distinct expression of CD40 and CD15 by the neoplastic HRS population (in red; all other lymph node cells in blue). Histograms, at right, display fluorescence of *TNFRSF14* (CD270) antigen on gated HRS cells in red, fluorescence minus one control for HRS cells in teal, *TNFRSF14* antigen on CD20-positive B cells in purple, and fluorescence minus one controls for CD20-positive B cells in green. Panel A demonstrates decreased *TNFRSF14* antigen on HRS cells compared to non-neoplastic B cells, panel B shows equivalent expression. Three representative cases are shown for each outcome. (C) CHL cell lines mixed with normal peripheral blood. Histograms display fluorescence of *TNFRSF14* antigen on CHL cell lines in red, fluorescence minus one controls for CHL cell lines in teal, *TNFRSF14* antigen on CD20-positive B cells in purple, and fluorescence minus one controls for CD20-positive B cells in green. Cell lines KM-H2 and L1236 exhibit reduced expression of *TNFRSF14* antigen compared to non-neoplastic B cells, cell line L428 demonstrates equivalent expression. Note log scale on all axes.