Genetic instability in Hodgkin’s lymphoma

D. Re, T. Zander, V. Diehl & J. Wolf*

Department of Internal Medicine I, University Hospital Cologne, Cologne, Germany

Genetic instability is a characteristic feature of the malignant Hodgkin and Reed–Sternberg (HRS) cells in classical Hodgkin’s lymphoma and the lymphocytic and histiocytic (L&H) cells in lymphocyte predominant Hodgkin’s lymphoma. Genetic instability can be classified into four major categories: distinct DNA mutations (microsatellite instability); numerical aberrations (chromosomal instability); structural aberrations (translocation instability); and gains and losses of chromosomal regions. In Hodgkin’s lymphoma (HL), HRS cells and L&H cells show somatically mutated clonally rearranged immunoglobulin genes, thus characterizing these cells genetically as germinal center B cells. These cells furthermore show mutations of oncogenes and tumor suppressor genes in some cases (p53, IκBα, CD95/Fas). They do not, however, display microsatellite instability, as they have a proficient mismatch repair machinery.

In contrast, HRS and L&H cells frequently harbor recurrent but not specific numerical and structural aberrations as detected by classical cytogenetics and fluorescence in situ hybridization analysis. Results from molecular genetic studies using comparative genomic hybridization and allelotyping (LOH) indicate typical genetic patterns in HL with gains and losses of distinct chromosomal regions. In some instances, candidate genes possibly involved in the malignant transformation of HRS cells and L&H cells have been characterized (JAK2, c-REL, MDM2). In summary, using molecular genetics it might be possible in the near future to elucidate some of the complex genetic instabilities observed in HL.

Introduction

Hodgkin and Reed–Sternberg (HRS) cells and lymphocytic and histiocytic (L&H) cells represent the malignant cells in classical Hodgkin’s lymphoma (cHL) and lymphocyte predominant HL (LPHL), respectively. Interest in the genetics of HL has risen since the discovery that HRS cells and L&H cells derive from germinal center (GC) B cells. It was the application of a PCR-based single-cell approach that allowed the detection of monoclonal immunoglobulin (Ig) gene rearrangements harboring somatic mutations within the heavy chain variable region [1]. Fundamental differences between L&H and HRS cells have been observed. In LPHL, L&H cells are characterized by the presence of ongoing mutations (intrachromosomal diversity) within the rearranged Ig gene. The pattern of somatic mutations in HRS cells, in contrast, indicates absence of an active hypermutation process, but destructive mutations have been described in ~25% of cHL cases (reviewed by Küppers [2]).

Although these genetic findings had a strong impact on the understanding of the biology of HL, many other features of HRS and L&H cells are not yet understood. Among these, genetic aberrations involved in the malignant transformation of HRS and L&H cells are still enigmatic. According to Lengauer et al. [3], in human tumors, genetic alterations can be divided into at least four major categories: subtle DNA sequence changes including microsatellite instability; chromosomal instability; chromosome translocations; and gene amplifications. This review focuses on these forms of genetic instabilities in HL.

Subtle DNA sequence changes

Subtle DNA sequence changes, i.e. mutations affecting one or several base pairs, seem to be rare events in HL. For example, analysis of the oncogenes bcl-2 and n-ras in HL showed absence of mutations in most instances [4, 5]. Results from the analysis of tumor suppressor genes are somewhat more promising. Somatic mutations were found in genes encoding for IκBα or IκBε in several cases [6–9]. This may be of interest, as these proteins negatively regulate the constitutively activated nuclear factor (NF)-κB expression in HL. Rarely, somatic mutations were also found in the apoptosis controlling genes p53 [10–12] and Fas/CD95 [13, 14]. In B cell-derived HRS cell lines, sequence analysis of bcl-10 [15] did not reveal mutations, while mutations of unknown significance were detected in the 5′ region of the bcl-6 gene in cell lines [16] as well as in primary cases of HL [17].

The described subtle DNA sequence changes within tumor suppressor genes might be the result of a defect mismatch repair machinery or of a (deregulated) hypermutation machinery. It has been speculated that mutations observed in HL...
could be the result of a defective mismatch repair (MMR) machinery, which itself might be linked to the hypermutation machinery [18]. The human DNA MMR system is responsible for the post-replication MMR involving homologs of yeast *mutS*- and *mutL*-related proteins [19]. In humans, defects in MMR genes have been linked to several solid tumors and hematological malignancies displaying microsatellite instability (MSI) [20]. Recently, MSI has been analyzed in cHL using a single-cell approach, proving the absence of this form of genetic instability in HRS cells [21, 22]. In accordance with this finding and their GC B cell derivation, it was found that HRS cells express the MMR proteins hMSH2 and hMLH1 [23]. It therefore is concluded that HRS cells are MMR-proficient and do not display a mutator phenotype explaining subtle sequence changes in tumor suppressor genes in HL.

Alternatively, it has been suggested that there might be a link between the hypermutation machinery and somatic mutations outside the Ig gene region. The hypermutation machinery is usually site specific (Ig genes), differentiation specific (GC) and lineage specific (B cell). As malignant cells in HL are GC-derived B cells harboring Ig gene mutations, these cells must have been under the influence of the hypermutation machinery at some point in their differentiation. As shown recently, it is likely that genes other than Ig genes might also be affected by this process [24–26]. The occurrence of the observed mutations in HL thus might be a by-product of the hypermutation machinery in some instances.

**Chromosomal instability**

Chromosomal instability is defined as the occurrence of numerical chromosomal aberrations. In hereditary non-polyposis colorectal cancer, this form of genetic instability correlates inversely with MSI. It is therefore not surprising that the MMR-proficient multinuclear Reed–Sternberg cells typically show a grossly abnormal karyotype, including gains and losses of whole chromosomes. Although classical cytogenetics has been used in the analysis of HRS cells for decades, there are at best several hundred metaphases that can be evaluated [5, 27, 28]. Moreover, results from these studies show a large variability regarding the percentage of abnormal metaphases (ranging from 13 to 92%), suggesting that the majority of HRS cells are diploid. If abnormal karyotypes were observed in HRS cells, gains would be observed more frequently than losses, with the exception of chromosomes 13, 15, 22 and Y. In these cases, gains were found for all chromosomes with gains of chromosomes 12 and X being detected in almost every other HL case [5].

In contrast to the classical cytogenetic approach, a study performing fluorescence in situ hybridization (FISH) in combination with immunohistochemistry (FICTION) revealed a more uniform picture: all HRS cell karyotypes analyzed showed numerical chromosome aberrations in the hyperdiploid range [29]. It has therefore been concluded that chromosomal instability is a characteristic feature of malignant cells in HL.

The mechanisms leading to chromosomal instability in HRS cells are not yet understood. Recently, there have been two studies showing that cell fusion is unlikely to explain polyploidy in HRS cells [30, 31]. It might be speculated that polyploidy is the result of a deregulated differentiation process of the HRS cells or of an altered expression of mitotic spindle checkpoint genes (‘endomitosis’).

**Chromosomal translocations**

The simple type of translocation is observed frequently in leukemias and non-Hodgkin’s lymphoma. This type includes distinct translocations that are typically and reproducibly found in the neoplastic cells. One example of such a simple translocation is t(14;18)(q32;q21), involving the B cell lymphoma/leukemia (bcl-2) locus in follicular center B cell lymphomas. Another example is t(2;5)(p23;35), involving the anaplastic lymphoma kinase (alk) and nucleophosmin genes in anaplastic large-cell lymphoma. Neither the t(14;18), nor the t(2;5) nor other known simple translocations are characteristic for HL [32, 33]. It is therefore concluded that in HL there are no known specific simple genetic aberrations that would appear to be necessary for the malignant transformation. As in HL, the occurrence of complex marker chromosomes involving several chromosomes is observed frequently. It is therefore conceivable that these complex alterations mask important simple translocations.

In HRS and L&H cells, complex structural chromosome aberrations have been described in numerous classical cytogenetic studies as well as in FISH analyses, and some of these aberrations are detected recurrently. Among these recurrent changes, alterations of chromosomes 2p, 3q, 6q, 7q, 9p, 13p, 14p and 17q are found more frequently than expected. Several breakpoints are detected non-randomly in HL, including 3q27, 6q15, 7q22, 11q23, 14q32 [5, 28], but translocation partners have not yet been identified. Since several of these alterations have been described in HRS cell lines as well, it might be that a more precise genetic analysis of these cell lines using modern molecular genetic tools will help identification of pathogenetically relevant genes [34].

**Gene amplification and deletion**

Recently, the application of sophisticated molecular techniques, including the micromanipulation of single HRS and L&H cells, allowed the genetic analysis of neoplastic cells in HL. Tumor DNA was isolated from single or pooled cells, pre-amplified in some instances and analyzed for genetic imbalances using genomic comparative hybridization (CGH) or loss of heterozygosity (LOH). All but one study included only cases of cHL. The one study analyzing LPHL with CGH found complex chromosome aberrations with gains of chromo-
somes 2q, 4q, 5q, 6 and 11q, which might be a characteristic feature of LPHL since these aberrations are rarely observed in other lymphomas.

As mentioned, most investigators have focused on the genetic analysis of HRS cells in cHL. So far, two groups have presented their CGH results, primarily looking for amplifications of oncogenes. Joos et al. [35] detected recurrent gains on chromosomes 2p, 9p and 12q with high-level amplifications on 4q16, 4q23–q24 and 9p23–p24. FISH studies performed with these samples led to the identification of gene amplifications, i.e. amplifications of the JAK2 gene on 9p23–p24 and the MDM2 gene on 12q14 [36]. As reported at the Fifth International Symposium on Hodkgin’s Lymphoma in Cologne (Germany) in September 2001, Barth et al. [37] found an amplification of the c-Rel locus on chromosome 2p, which might be one explanation for the constitutive activity of NF-κB in HRS cells. This pathway seems to be specific for cHL of the nodular sclerosis subtype [38]. Independently, a second group described gains on 1p13 and 7q35 –q25, which is also a characteristic feature of other B cell lymphomas [41]. Candidate tumor suppressor genes located within the mentioned chromosomal region are currently being analyzed.

Conclusions

Neoplastic cells in HL are derived from GC B cells, as they harbor somatically mutated clonally rearranged Ig genes. HRS and L&H cells are genetically instable. (i) They show somatic mutations of p53, IκBα and CD95, which are observed at varying frequencies. They do not, however, display a mutator phenotype. (ii) They typically show chromosomal instability within the hyperdiploid range. (iii) They harbor recurrent unspecific complex but not simple structural chromosomal aberrations, which are distributed non-randomly. (iv) They are characterized by genetic imbalances in distinct chromosomal regions showing gains (suggesting amplification of oncogenes) and losses (suggesting deletion of tumor suppressor genes). Based on the latter results, further studies using modern molecular techniques will focus on these chromosomal regions in order to identify distinct pathways involved in the malignant transformation of GC B cells.

In summary, as yet neither the application of classical cytogenetics nor the use of more sophisticated techniques such as FISH, CGH and LOH analyses has resulted in the identification of one or more consistent aberrations that might underly the process of malignant transformation. It therefore might be speculated that the complex aberrations observed in HL are an epiphenomenon, and develop secondary to the initial transformation event(s) in the course of tumor progression. Alternatively, and this seems more probable to us, modern molecular genetic methods (like for instance, the microarray technology) will enable us to describe—and hopefully, to understand—the complex total gene expression pattern of a given cell and thus also the process of malignant transformation in HL in the near future.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft, grant no. SFB502 TP1.

References