Cytogenetic Features of Hodgkin's Disease Suggest Possible Origin From a Lymphocyte

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Surface marker and gene rearrangement data have supported various hypotheses about the origin of the malignant cell in Hodgkin's disease. Cytogenetic data about this disorder, however, are very scanty. To determine if any chromosomal abnormalities that could add further information to this controversial point are present, we studied tumor samples from 49 patients. Abnormal metaphases were obtained in 18 cases. The most common breakpoints were in 11q23, 14q32, 6q11-21, and 8q22-24. These are common breakpoints in lymphoma and raise the possibility that the malignant cell in Hodgkin's disease may be derived from a lymphocyte. The 11q23 breakpoint is also seen in t(4;11) and t(9;11), which is typical of a type of childhood B-cell acute lymphoblastic leukemia characterized by the presence of aberrant myeloid and monocytic markers. Myeloid and monocytic markers are common in Reed-Sternberg cells.

THE LINEAGE of the malignant cell in Hodgkin's disease is an issue surrounded by considerable controversy. That most of the cells in lymph nodes involved by this tumor are reactive lymphocytes, eosinophils, histiocytes, and plasma cells compounds the difficulties of identifying the lineage of the malignant cell. Currently available surface marker and gene rearrangement data have supported diverse origins including histiocytes, myeloid cells, B or T lymphocytes, activated lymphoid cells, and interdigitating dendritic reticulum cells.[1-7] Cytogenetic data could be useful in elucidating the cell lineage in this disorder. However, such data in Hodgkin's disease are scanty, in contrast to lymphoma.[8,9]

We studied the karyotype of 49 samples from 40 previously untreated and 9 treated patients with Hodgkin's disease to determine if this could add valuable information regarding cell lineage and to compare the findings with those we have observed in lymphoma.

MATERIALS AND METHODS

A cell suspension was prepared from solid tissues submitted at the time of biopsy. The cells were cultured in RPMI 1640 or Ham's F10 medium, each supplemented with 20% fetal calf serum (FCS). Colcemid (0.06 μg/mL) was added for the last 3 hours of culture to the cells in RPMI 1640, and colchicine (1.0 μg/mL) was added to the cells in Ham's F10 for the last 30 minutes of culture. The cells were then harvested using cell synchronization procedures.[10,11]

In 9 of the 49 cases, the material was obtained by means of a lymph node aspiration. In view of the small number of cells in lymph node aspirates, synchronization techniques using 5-fluorouracil (5-FU) or cold shock were used to increase the mitotic index and improve chromosome morphology of such samples. Several banding techniques were used.[12]

All histologic material was reviewed by at least one member of the Hematopathology Section, Department of Pathology, M.D. Anderson Hospital. Lymph node aspirates and body fluid samples were reviewed by a member of the cytopathology section; however, the definitive diagnosis of Hodgkin's disease was always made on tissue sections and was never based exclusively on cytology samples. Evaluable abnormal metaphases were obtained in 18 cases; 14 of these had pathologic confirmation of Hodgkin's disease in the same lymph node from which the cytogenetic study was obtained. In the remaining four cases, pathologic confirmation was done on a separate lymph node biopsy within 2 weeks.

RESULTS

Of the tissue samples taken from 49 patients with Hodgkin's disease, 59% yielded evaluable metaphases. Thirty-seven percent yielded abnormal metaphases, 22% showed normal diploid metaphases, and 41% had either insufficient or inevaluable metaphases. Of 15 cases of Hodgkin's disease mixed cellularity, 33% showed abnormal and 40% showed normal diploid metaphases. Of 32 cases of Hodgkin's disease nodular sclerosis, 34% showed abnormal metaphases and 16% showed normal metaphases. Of 18 cases with abnormal metaphases, 72% showed both numerical and structural abnormalities, 17% had only numerical abnormalities, and 11% had only structural abnormalities.

Of the nine previously treated patients, four yielded abnormal metaphases. The possibility that these four cases were instances of large cell lymphoma transformed from Hodgkin's disease was ruled out by means of a lymph node biopsy carried out on the same sample from which the cytogenetic study was obtained in three of the four cases.

The most frequently observed structural abnormalities are summarized in Table 1, in order of frequency. One case had a typical t(14;18)(q32;q21); another had a t(11;14)(q23;q32). With the exception of one case, all abnormalities listed in Table 1 were observed in at least two metaphases. Figure 1 summarizes the distribution of ploidy by cytogenetic technique in the 18 cases of Hodgkin's disease with abnormal metaphases and compares it with our previous experience with large cell lymphoma (LCL). The chromosomes most frequently involved in structural abnormalities are in descending order of frequency: 11q (10 breakpoints in 7 patients), 14q (9 breakpoints in 7 patients), 7p (7 breakpoints in 5 patients), and 1p (7 breakpoints in 4 patients).

DISCUSSION

It was possible in our experience to obtain metaphases in most cases of Hodgkin's disease. However, of the 29 cases in

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Table 1. Characteristic Structural Cytogenetic Abnormalities Seen in Hodgkin's Disease

Abnormality Location	No. With Abnormality	No. of Deletion/ No. of Translocations	Possible Gene	Nodular Sclerosis	Mixed Cell	Other Types
11q23*	6	2/4	c-*ets*	5	1	—
14q32†	5	0/5	IgH	4	1	—
6q11-21	5	3/2	c-*myb*	4	—	1
8q22-24	4	2/2	c-*myc*	3	1	—
11q13	1	0/1	*bcl*-1	—	—	1

*Includes 1 t(11;14)(q23;q32).
†Includes 1 t(14;18)(q32;q21).

which metaphases were obtained, 11 (39%) were normal diploid at 500-band level, suggesting that these probably originated from the normal reactive cells and not from the malignant Hodgkin's cells. This is not surprising because most cells in Hodgkin's disease tissues are reactive. This finding contrasts to LCL, in which most cells in the involved tissues are malignant; consequently, the karyotype is almost uniformly abnormal.[12-14]

The most frequent abnormalities in this series (Table 1) were strikingly similar to what has been described in the past for lymphoma. The 11q23, 14q32, 6q, 8q24, and 11q13 breakpoints have all been described frequently, although not exclusively in lymphoma.[11-13] The breakpoint in 14q32 has been associated very frequently with B-cell lymphoma, where it occurs characteristically in the follicular types as t(14;18)(q32;q21).[13] Immunoglobulin heavy-chain (IgH) genes reside in chromosome 14q32, whereas 18q21 harbors the *bcl*-2 gene. This most likely explains the common association of this breakpoint with B-cell lymphomas. Nevertheless, structural abnormalities of 14q32 have also been described in T-cell neoplasms, particularly T-cell CLL. Furthermore, a large number of genes reside in each chromosome band; thus, breakpoints in specific bands cannot be construed as final proof of the involvement of any one gene. For example, occasional nonhematopoietic malignancies such as renal cell carcinoma and retinoblastoma have been reported with chromosome abnormalities in 14q32. Whether the IgH chain genes are involved in these cases is not known. The occurrence of 14q32 abnormalities in these solid tumors is very unusual, however, when compared with the observed frequency of 28% in our series. Indeed, these instances are exceptions rather than the rule. In retinoblastoma, chromosome 13 is the most consistently involved, although three instances of 14q32 abnormalities have been reported. In renal cell cancer, in which the short arm of chromosome 3 is frequently involved, only two instances of involvement of the distal part of 14q have been reported. Finally, recent studies have shown that immunoglobulin gene rearrangements occur in Hodgkin's disease, which supports the possibility that some cases of Hodgkin's disease could be derived from a B lymphocyte.[3,4]

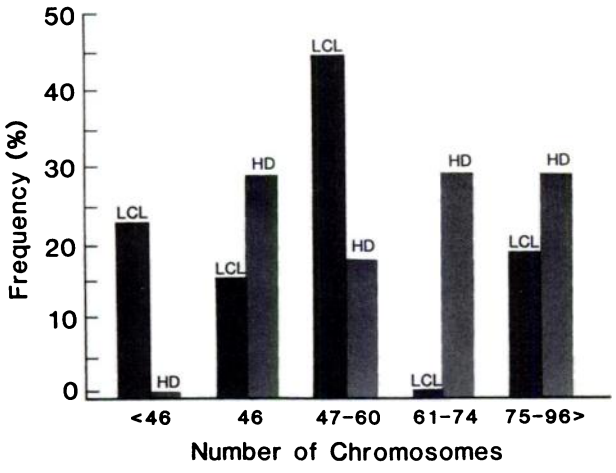

Fig 1. Distribution of ploidy in Hodgkin's disease (HD) *v* large cell lymphoma (LCL).

Of the five translocations involving 14q32 in our series, two involved characteristic translocations previously described in B-cell lymphomas: t(14;18) and t(11;14). The breakpoint in the latter translocation, however, was not in the *bcl*-1 site (11q13) but instead was in 11q23, which is the locus of c-*ets* oncogene that has been implicated in t(4;11) and t(9;11).[15-17] These two translocations, which involve 11q23, are associated with a unique type of childhood acute lymphoblastic leukemia characterized by the presence of aberrant myeloid or monocytic markers. This phenomenon has been referred to as lineage promiscuity or infidelity. In our series of Hodgkin's cases, the most commonly observed structural abnormality was precisely in the 11q23 region. These findings raise the intriguing possibility that in some cases the Hodgkin's cell might originate from a precursor B-lymphoid cell that might aberrantly express biologic markers associated with cells of myeloid and monocytic lineages. This could help explain why surface marker and gene rearrangement studies have supported the origin of Hodgkin's disease from divergent cell types, including histiocytes and lymphocytes. The presence of granulocyte-associated markers such as Leu M-1 (CD-15) could also be related to this cytogenetic abnormality.

Another commonly involved breakpoint in our series was 6q, which has been associated with LCL, usually a B-cell disorder.[13-15] The oncogene c-*myb* has been mapped to 6q. Structural abnormalities of chromosome 8q24, the site of c-*myc* oncogene, have been implicated in Burkitt's lymphoma, another B-cell disorder. We observed abnormalities of 8q24 in four instances. These data suggest that c-*ets*, c-*myb*, c-*myc*, and *bcl*-2 oncogenes should be studied for their potential involvement in the pathogenesis of Hodgkin's disease.

Despite the similarities between the breakpoints seen in Hodgkin's disease and those seen in other lymphoid disorders, certain differences emerge. The ploidy characteristics

of Hodgkin's disease are clearly different from those of LCL. In Hodgkin's disease, the number of chromosomes was commonly in the triploid to tetraploid range, in contrast to LCL (Fig 1). On the other hand, hypodiploidy was not observed in Hodgkin's disease but was common in LCL. Another difference between LCL and Hodgkin's disease was that both chromosomes 11q23 and 7p were involved more frequently in Hodgkin's disease.

The chromosomal breakpoints observed in our study, conducted exclusively on fresh tumor samples, are in agreement with those observed by Fonatsch et al in tissue derived from four Hodgkin's disease cell lines.[8] This lends credence to the possibility that the 11q23 and 14q23 breakpoints also observed in these cell lines are primary abnormalities and not secondary or acquired during in vitro growth.[8] Moreover, the 14q abnormality has been reported in 4 of 10 fresh samples studied independently by Hossfeld et al.[17-19] Rowley also quotes data from Fleischman, who identified this abnormality in 6 of 15 fresh samples studied cytogenetically.[9] The 11q abnormalities have also been observed in 3 of 6 cases studied by Hossfeld and 0 of 5 by Reeves.[17-19]

The cytogenetic data we have accumulated in Hodgkin's disease suggest, but do not conclusively prove, that the malignant cell in this disorder is of lymphoid lineage. Its cytogenetic features resemble more closely the LCLs. Some cases of Hodgkin's disease, particularly the lymphocyte depletion type, can be very difficult to differentiate from LCL. Furthermore, patients with Hodgkin's disease have been known to develop LCL later during their disease course. Finally, Reed-Sternberg-like cells are occasionally seen in lymph nodes of cases of LCL. This raises the interesting question of whether in some instances Hodgkin's disease and LCL share common biologic features.

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