6q Deletions Define Distinct Clinico-Pathologic Subsets of Non-Hodgkin’s Lymphoma


Commonly observed in lymphoid neoplasms, deletions of 6q have been correlated with histologic and clinical subsets of non-Hodgkin’s lymphoma (NHL). Our recent analysis of loss of heterozygosity of 6q loci in NHL showed two regions of minimal molecular deletion (RMD), an RMD1 at 6q25-27 and an RMD2 at 6q21-23. To establish correlations between these RMDs and regions of minimal cytogenetic deletions (RCDs) on 6q, and to define associations between RCDs and clinico-pathologic features, we have analyzed chromosome 6 abnormalities in 459 consecutively ascertained, karyotypically abnormal cases of NHL. Among these, 126 (27.5%) cases had structural abnormalities of chromosome 6, of which 94 were deletions. Analysis of these deletions identified three RCDs. An RCD1 encompassing 6q25-27 was seen in 45 intermediate-grade NHL. An RCD2 at 6q21 was observed in 11 high-grade NHL, 9 of which were of the immunoblastic subtype. An RCD3 at 6q23 was noted in 18 low-grade NHL lacking a t(14;18) translocation. Of these 18 cases, 12 were small lymphocytic NHL and, in 2 of these, del(6q) was the sole karyotypic abnormality. In 20 cases of low-grade NHL with t(14;18), the deletions spanned both RCD1 and RCD3. These data suggested the presence of at least 3 tumor suppressor genes on 6q within RCD1, RCD2, and RCD3; they also showed associations between RCDs in 6q and subsets of NHL, including a specific association between a group of well-differentiated lymphoid neoplasms and RCD3. The apparent heterogeneity of breakpoints when all NHLs are considered together explains the inability of previous studies to reliably establish correlations between recurring 6q deletions and histologic and clinical features of NHL.

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OBSERVED IN up to a third of the cases, structural abnormalities of chromosome 6 are among the most common recurring karyotypic abnormalities in non-Hodgkin’s lymphoma (NHL). The frequently encountered 6q deletions in these tumors have been inconsistently correlated with clinical features of the disease, as well as with tumor progression. Recurring 6q deletions have also been observed in other hematologic and solid tumors and have suggested the existence of one or more tumor suppressor genes (TSGs). Recently, based on an analysis of loss of heterozygosity (LOH) using a panel of probes mapped to 6q, we showed two regions of minimal molecular deletion (RMD) in NHL: an RMD1 at 6q25-27 and an RMD2 at 6q21-23. Because of the limited sample sizes of all previous cytogenetic and molecular series, it was not possible to establish associations between specific 6q deletions and subsets of NHL. In the current series of 126 consecutively ascertained NHL with structural abnormalities of chromosome 6, we identified three regions of minimal cytogenetic deletion (RCD) suggesting the involvement of at least three TSGs and noted new correlations between RCDs and clinical, histologic, and immunophenotypic features of tumors. Thus, a subset of low-grade NHL were associated with an RCD at 6q23 (RCD3) and subsets of high-grade and intermediate-grade NHL were associated with two additional RCDs, RCD1 and RCD2, respectively. These observations form the basis for further definition of RMDs, eventually leading to isolation and characterization of TSGs and correlation of their loss with histologic and clinical features of NHL.

MATERIALS AND METHODS

Between January 1984 and December 1991, 781 consecutively ascertained specimens of histologically confirmed NHL seen at Memorial Hospital were subjected to cytogenetic analysis. Biopsy material was divided for histopathologic, cytogenetic, and immunophenotypic/immunogenotypic analyses, which were performed as previously described. Karyotypes were defined and described according to the International System for Human Cytogenetic Nomenclature (ISCN, 1991). Of the 781 specimens, 480 specimens from 459 patients had clonal chromosomal abnormalities. Among the same 459 specimens, 126 (27.5%) showed structural abnormalities of chromosome 6, of which 94 were deletions. These 94 cases with del(6q) comprised the dataset for the analysis reported here. A summary of cytogenetic abnormalities in 46 of these 94 cases have been previously reported by us.13,15,17

For the purpose of this analysis, we defined deletions by the following criteria. Whenever 6q ended in a dark band without a recognizable pale end, the deletion was scored as terminal. In the case of deletions affecting the 6q24-27 region, when a pale band was recognized at the terminus, the deletion was scored as interstitial with the terminal pale band representing 6q27 or part of it. In such cases, loss of 6q24 and/or 6q26 could easily be recognized because of the positive G-staining of these two bands. Representative illustrations of interstitial deletions are shown in Fig 1. Recurring deletions were analyzed to identify the RCDs encountered in subsets of cases. As mentioned previously, our molecular analysis identified the limit of

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the distal deletion in this region (6q25-27) to be 6q27 (RMD1). Therefore, to define the molecular limit of deletion in cases for which the distal limit of the deletion was scored as 6q25 by cytogenetic analysis, LOH was assayed using restriction fragment length polymorphisms (RFLPs) mapped to 6q27. For this, Southern blot hybridization analysis was performed on 5 μg of tumor DNA, as previously described, using two highly polymorphic probes (>90% heterozygosity in normal DNA) mapped to 6q27: D6S132 and D6S133 (gift of Dr G. Vergnaud).

Cytogenetic, histologic, and clinical correlations were performed on subsets of low-, intermediate-, and high-grade NHL as defined by the International Working Formulation. The cases were further stratified according to presence or absence of t(14;18) (q32;q21) because this translocation has been shown to be a frequent aberration in low-grade (>70%) and intermediate-/high-grade (>25%) NHL. Comparisons of histologic and clinical subsets with 6q deletions were performed using the method of inferences from proportions. Survival analysis, using the method of Kaplan and Meier, was performed only on the subset of cases of diffuse large cell NHL (DLC), as described previously.

RESULTS

Interstitial (45 cases) or terminal (40 cases) deletions of 6q were noted in 85 cases; in 9 cases, poor banding morphology precluded precise identification of the deletion breakpoints. Whereas there was no single region deleted in every case, each of the 85 cases showed deletion of either band 6q21 or 6q25, or both. RCDs were defined by comparison of cases stratified according to histologic grade and presence or absence of t(14;18).

The largest histologic subset was comprised of 45 intermediate-grade NHL with and without the t(14;18) translocation (Fig 2). This subset included 31 diffuse large cell (DLC), 8 diffuse mixed cell (DMC), 5 follicular large cell (FLC), and 1 diffuse small cleaved cell (DSC) NHL. B-cell immunophenotype/genotype was shown in 42 of the tumors; 2 DLC were of T-cell lineage and 1 DMC was a null-cell NHL. The single chromosomal band most commonly deleted in this subset was 6q25, here designated as RCD1 (Fig 2). The same RCD was also noted when 28 of these intermediate-grade NHL cases, analyzed at the time of diagnosis, were compared with the remaining 17 cases analyzed at the time of relapse (posttreatment).

In this ascertainment, evidence for transformation from a lower grade was shown in 8 of 56 cases of intermediate-/high-grade NHL with (nos. 159, 316, and 501) and without (nos. 66, 158, 746, 766, and 824) the t(14;18) translocation. The common deleted segment in these cases extended from 6q23 to 6q25. In contrast, among 11 cases of high-grade NHL, of which 10 were immunoblastic (IMB) and 1 lymphoblastic (LYB), an RCD here designated as RCD2 was identified at 6q21 (Fig 3). The LYB NHL and one of the IMB tumors were of T-cell lineage; another IMB tumor was null cell by immunophenotypic/genotypic analysis. The remaining eight tumors in this subset were of B-cell lineage.

A del(6q) was observed in 18 cases of low-grade NHL without a t(14;18) translocation. In this subset, an RCD here designated as RCD3 was identified at 6q23 (Fig 4). This group included a subset of 12 small lymphocytic (sm lym) NHL, of which 7 were of chronic lymphocytic leukemia (CLL) subtype, and 5 were of plasmacytoid (pl) subtype. In 2 of these cases (nos. 1000 and 1046), the del(6q) was the only karyotypic abnormality; both cases were classified as sm lym NHL of CLL type. In the remaining cases, del(6q) was seen in association with additional chromosomal abnormalities.

A del(6q) was also observed in 20 low-grade NHL, with a t(14;18) translocation. The RCD in 19 of these cases spanned the region 6q23-27, which included the regions RCD1 and RCD3 (Fig 5). In case no. 732, the deletion did not include RCD3, but included RCD2; this case was one of a subset of t(3;22)(q27;q11) NHL which were included in a previous report.

The distal limits of RCD1 determined here by cytogenetic analysis (6q25) and RMD1 previously determined by RFLP analysis (6q27) unexpectedly were different. To establish if this discrepancy was attributable to real differences in the limits of the deletions or to a complex nature of the lesion that resulted in inconsistent cytogenetic resolution of the distal deletion break, we subjected cases with a distal cytogenetic break at 6q25 to RFLP analysis using the two highly polymorphic probes, D6S132 and D6S133, which have been mapped to 6q27. These comprised 8 cases of intermediate-grade NHL with or without t(14;18) (nos. 159, 430, 841, 843, 1041, 1163, 1172, and 1230) and 5 cases of low-grade NHL with t(14;18) (nos. 826, 846, 975, 1024, and 1125) (Figs 2 and 5). DNA for study was available from 5 of the intermediate-grade tumors and 4 of the low-grade lesions. Southern blot analysis showed a loss in intensity of one allele compared with the other, indicating deletion in 6q27 in 5 cases (nos. 826, 843, 1125, 1172, and 1230; Fig 6). Alleles of intensity comparable with that in normal DNA, indicating no loss, were seen in the remaining 4 cases (nos. 430, 946, 975, and 1163). However, histologic and immunohistochemical analysis of these cases showed infiltrates of large numbers (20% to 60%) of reactive T cells (nos. 430,
946, and 1163) in the setting of extensive necrosis, skeletal muscle invasion (no. 430), or focal sclerosis (no. 946). Therefore, the failure of the RFLP analysis to detect LOH in these 4 cases is considered most likely to be attributable to contaminating non-neoplastic cells in the tissue biopsy. Thus, where informative, the deletions in these cases extended to 6q27; therefore, we concluded that the distal limit of RCD1 was 6q27, rather than 6q25, equating RCD1 with RMD1.

The median survival of the 36 DLLC with cytogenetic analysis at the time of diagnosis was not reached. The median survival was only 21 months for the subset of 7 DLLC cases that showed only RCD1, compared with median not reached for 24 cases that showed both RCD1 and RCD2 ($P = .008$). These subsets did not differ in median age or LDH. The clinical behavior of low-grade NHL with 6q deletion was typical for this histology, with a median survival not yet reached at the time of last follow-up. There was no adverse prognostic impact of presence of the t(14;18) translocation in this subset.

**DISCUSSION**

Deletions of chromosome 6 have been observed in many types of tumors, notably acute lymphoblastic leukemia...
Fig 5. Deletion map in 20 cases of low-grade NHL with t(14;18). The RCD spanned 6q23-6q25 (upper bracket) by cytoge- netic analysis and 6q23-6q27 (lower bracket) by RFLP analysis (RCD1 and RCD3). Some areas of the biopsy specimen of case 1125 showed evidence of transformation to a diffuse large cell tu- mor. *Case 732 showed t(3;22)(q27;q21).

Fig 6. Analysis of LOH in the 6q27 region in selected cases of NHL with RCD1 and 6q25 distal break. Genomic DNAs were digested with (A) Haelll or (B) PvuII and subjected to Southern blot analysis using highly polymorphic probes (A) D6S132 and (B) D6S133, which mapped to 6q27. Cases 1230, 843, 1172, 1125, and 826 were scored as positive for deletion, based on the identification of only one allelic band or allelic bands of unequal intensities, the latter due to samples containing contaminating nor- mal cells. N, normal (nontumor) DNA.
Table 1. Correlation of RCD and RMD in 6q With t(14;18) Translocation and Histologic Subsets of NHL

<table>
<thead>
<tr>
<th>RCD</th>
<th>RMD</th>
<th>Chromosomal Location</th>
<th>Correlation With Histologic Subset t(14;18)</th>
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<tr>
<td>RCD1-2</td>
<td>RMD1-2</td>
<td>6q22-27</td>
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<tr>
<td>RCD2-2</td>
<td>RMD2-2</td>
<td>6q21</td>
<td>High grade</td>
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<tr>
<td>RCD1-3</td>
<td>RMD1-3</td>
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* Postulated.

The histologic correlations of del(6q) in high grade NHL presented here may also be considered in conjunction with patterns of deletion reported in acute and chronic lymphoid leukemias. All 45 cases of ALL with del(6q) in a large single-institution series showed a common deleted band at 6q21, suggesting a link between ALL and RCD2 observed by us in high-grade NHL. In contrast, in CLL, five cases with del(6q) as the only karyotypic abnormality have been reported, the deleted regions in these cases were consistent with the RCD3 observed here. Although the most frequent cytogenetic abnormality in CLL is trisomy 12, 4% of CLL in large series showed del(6q), albeit rarely as a solitary abnormality.

The large number of cases in this cytogenetic series enabled identification of clinico-pathologic subsets associated with RCDs. Two of these RCDs corresponded to RMDs deleted regions in these cases were consistent with the abled identification of clinico-pathologic subsets associated with acute lymphoid, and possibly other, cell types awaits their isolation and characterization.

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6q deletions define distinct clinico-pathologic subsets of non-Hodgkin's lymphoma

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