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Original contribution

Intrachromosomal rearrangement of chromosome 3q27: an under recognized mechanism of BCL6 translocation in B-cell non–Hodgkin lymphoma

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Summary Balanced reciprocal translocations involving chromosomal region 3q27, which led to identification of the BCL6 protooncogene, are one of the most common recurrent chromosomal abnormalities reported in B-cell non–Hodgkin lymphomas (B-NHL). Cloning of the breakpoints of these translocations has facilitated the identification of a number of BCL6 partners including immunoglobulin genes and more than 20 non-immunoglobulin genes on almost all human chromosomes. Fusion of BCL6 with these genes leads to deregulated BCL6 expression because of substitution of its promoter with that of the translocation partner. Despite the promiscuous nature of BCL6 translocations, intrachromosomal rearrangements of the BCL6 gene have not been well recognized. In the present study, we present evidence for intrachromosomal rearrangements, because of interstitial deletions and inversions, involving region 3q27 as an overlooked mechanism of BCL6 deregulation. These rearrangements accounted for 3/20 (15%) of all BCL6 translocations occurring in B-NHL, including follicular lymphomas, diffuse large B-cell lymphomas, and marginal zone B-cell lymphomas, diagnosed at our institute. In addition to confirming previously described partner loci on chromosome 3, we also identified a novel BCL6 partner locus (3p24) that to our knowledge has not been reported previously. Our data should help facilitate the identification of new BCL6 partner genes, which may enhance our understanding of the clinical and biological role of BCL6 in B-NHL pathogenesis.

1. Introduction

Nonrandom chromosomal translocations that lead to the deregulation of a variety of protooncogenes have been described in B-cell non–Hodgkin lymphomas (B-NHL), and several of these are considered defining features of B-NHL subsets. Balanced reciprocal translocations involving chromosomal band 3q27 and immunoglobulin (Ig) genes were first reported in B-NHL more than a decade ago [1], and cloning of these breakpoints led to identification of the BCL-6 (LAZ3) gene, which encodes a zinc finger protein that acts as a transcriptional repressor [2]. Subsequent

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studies by Fukuda et al [3], Dent et al [4], and Shafer et al [5] have established important functional roles for BCL6 in germinal center (GC) development and in the generation of T-helper type 2 immune responses.

Translocations involving BCL6 are considered one of the most frequent cytogenetic abnormalities in B-NHL [6], most commonly detected in GC-derived lymphomas, including 15% to 40% of diffuse large B-cell lymphomas (DLBCL) [6-11], 6% to 15% of follicular lymphomas (FL) [6,8,12,13], and 48% of nodular lymphocyte predominant Hodgkin lymphomas [14].

BCL6 translocation partners include Ig genes in 52% to 54% of cases and a variety of non-Ig genes in 38% to 40% of cases, many of which are recurrently targeted by interchromosomal rearrangements in B-NHL [15,16]. Only a few studies have described BCL6 partner genes or loci on chromosome 3 [15,17,18]. We decided to investigate whether structural alterations, such as deletions and inversions occurring within chromosome 3 and involving the 3q27 region, were indicative of intrachromosomal BCL6 rearrangements. We found intrachromosomal rearrangements of BCL6 in 3/20 (15%) of all B-NHL with BCL6 translocations, suggesting that such rearrangements represent a not infrequent mechanism of BCL6 deregulation in B-NHL.

2. Materials and methods

2.1. Selection of B-NHL with 3q27 abnormalities and morphologic analysis

G-banded karyotypes of 310 consecutive cases of B-NHL with clonal chromosomal abnormalities submitted for karyotypic analysis to our institute, over a 9-year period (1997-2005), were reviewed for chromosome band 3q27 abnormalities. Morphologic assessment was performed using formalin-fixed, paraffin-embedded tissue sections (3μ) stained with hematoxylin and eosin. All B-NHL were classified and graded according to the current WHO criteria [19].

2.2. G-banded karyotype analysis and fluorescent in situ hybridization for BCL6

Giemsa banding was performed using standard methods, and the karyotypes were described according to *International System for Human Cytogenetic Nomenclature* [20]. Fluorescent in situ hybridization (FISH) analysis was performed on fixed cells from all cases using dual-color break-apart probes for BCL6 that span the entire BCL6 locus (VYSIS, Downers Grove, Ill, USA) using standard protocols; 200 to 500 cells were analyzed, and fluorescence signals were captured after counterstaining with DAPI using the Cytovision Imaging system attached to a Nikon Eclipse 600 microscope (Applied Imaging, Santa Clara, Calif, USA).

3. Results

3.1. Patient characteristics, morphology, and grade of B-NHL with 3q27 abnormalities

Upon review of the G-banded karyotypes, a total of 21 B-NHL with clonal chromosomal abnormalities involving the 3q27 region were identified. These included 13/61 (21%) DLBCL (age 35-88 years, mean 62 years, male/female 4:9); 7/44 (16%) FL (age 33-79 years, mean 60 years, male/female 3:4), which consisted of 4 cases of FL grade 2 (FL2) and 3 cases of FL grade 3a (FL3a); and 1/14 (7%) marginal zone B-cell lymphomas (MZBCL), the latter occurring in a 79-year-old woman. All 13 cases of DLBCL had centroblastic morphology, and the MZBCL with the 3q27 abnormality had an increased number (10%) of large cells.

3.2. G-banded karyotype analysis and FISH for BCL6

All 21 cases with translocations involving band 3q27 had complex karyotypes (Table 1). Of these 21 cases, 17 (81%) showed interchromosomal translocations of 3q27 with a variety of partner chromosomes, whereas 4 (19%) cases had chromosomal abnormalities suggestive of intrachromosomal 3q27 rearrangements (cases 1, 2, 3, and 8; Table 1). FISH analysis using a BCL6 break-apart probe was performed on all cases because a prior study by Chaganti et al [21] had reported that BCL6 rearrangements could only be detected in approximately 50% of B-NHL with band 3q27 abnormalities. Moreover, we also wanted to explore the possibility whether, in addition to reciprocal chromosomal translocations, intrachromosomal rearrangements such as inversions and deletions involving band 3q27 reflected BCL6 rearrangements, similar to what has been described for the MLL gene in acute leukemias [22]. FISH analysis confirmed BCL6 rearrangement in 20/21 (95%) cases. Interchromosomal BCL6 rearrangements were noted in 17/20 (85%) cases, which included balanced reciprocal translocations in 16/17 (94%) cases (3/4 [75%] FL; 12/12 DLBCL; and 1/1 MZBCL) (cases 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 21; Table 1) and unbalanced translocations in 1/17 (6%) cases (1/4 [25%] FL2) (case 7; Table 1). The latter case demonstrated add(3)(q27). Of the 16 B-NHL with balanced reciprocal translocations involving BCL6, 6 (38%) cases (1 FL and 5 DLBCL) (cases 6, 9, 10, 14, 15, and 16; Table 1) had Ig, and 6 (38%) cases (2 FL, 3 DLBCL, and 1 MZBCL) (cases 4, 5, 17, 18, 19, and 21; Table 1) had non-Ig partner loci, the latter included 1q21, 2p11.2, 6p21.3, 10q11.2, 12q24.1, and 12q13 (n = 2). Partner loci in 4 cases (24%, 4 DLBCL) (cases 8, 11, 12, and 13; Table 1) could not be determined.

Four cases showed chromosomal abnormalities suggestive of intrachromosomal 3q27 rearrangements. These consisted of 2 inversions (pericentric and paracentric, 1 case each; cases 1 and 2; Table 1) and 2 interstitial deletions (cases 3 and 8; Table 1). The inversions involved