HHS Public Access

Author manuscript

Mod Pathol. Author manuscript; available in PMC 2010 March 01.

Published in final edited form as:

Mod Pathol. 2009 September ; 22(9): 1251–1257. doi:10.1038/modpathol.2009.81.

t(14;18)-negative Follicular Lymphomas Are Associated with a High Frequency of *BCL6* Rearrangement at the Alternative Breakpoint Region

Keni Gu[1], **Kai Fu**[1], **Smrati Jain**[2], **Zhongfen Liu**[1], **Javeed Iqbal**[1], **Min Li**[1], **Warren G Sanger**[2], **Dennis D Weisenburger**[1], **Timothy C Greiner**[1], **Patricia Aoun**[1], **Bhavana J Dave**[2], and **Wing C Chan**[1]

[1]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE

[2]Center for Human Genetics, University of Nebraska Medical Center, Omaha, NE

Abstract

A frequent chromosomal translocation in mature B-cell non-Hodgkin lymphoma affects band 3q27 and results in deregulation of the B-cell lymphoma 6 (*BCL6)* gene. Two breakpoint clusters have been described thus far, the major breakpoint region (MBR) and an alternative breakpoint region (ABR) that is located 245 - 285 kb 5' to *BCL6*. Translocation at the MBR predominates in diffuse large B-cell lymphoma, whereas translocation at the ABR is reported to be frequently associated with grade 3B follicular lymphoma. However, translocation at the ABR has not been studied in a large series of follicular lymphomas, particularly t(14;18)-negative follicular lymphomas. Therefore, we studied *BLC6* rearrangements at the MBR and ABR by using break-apart fluorescence in situ hybridization (FISH) probes in 142 cases of follicular lymphomas, including 63 t(14;18)-negative and 79 t(14;18)-positive cases. Conventional cytogenetic (karyotype) analysis was also performed in 58 of the 63 t(14;18)-negative cases. *BCL6* rearrangement was found in 26% of t(14;18)-negative and 19% of t(14;18)-positive follicular lymphoma. t(14;18)-negative cases showed a high frequency of rearrangement at the ABR (12%) with an ABR:MBR ratio of 0.86, compared to only 5% with an ABR:MBR ratio of 0.36 in the t(14;18)-positive cases. *BCL6* rearrangements were found in all grades of follicular lymphoma but were most frequent in grade 3 t(14;18)-negative follicular lymphoma (60%). FISH analysis had a higher sensitivity for detecting *BCL6* rearrangements than conventional cytogenetics. In conclusion, *BCL6* rearrangements occur at a similar frequency in t(14;18)-negative follicular lymphoma and diffuse large B-cell lymphoma. However, t(14;18)-negative follicular lymphoma appears to have a higher frequency of rearrangement at the ABR compared with t(14;18)-positive follicular lymphoma and diffuse large B-cell lymphoma. Therefore, it is important to perform

Correspondence to Wing C. Chan, M.D. Co-Director, Center for Lymphoma and Leukemia Research Department of Pathology and Microbiology 983135 Nebraska Medical Center Omaha, NE 68198-3135 Phone: (402) 559-7684 Fax: (402) 559-6018 e-mail: jchan@unmc.edu. Bhavana J. Dave, PhD., FACMG Associate Director, Human Genetics Laboratory Department of Pediatrics and Pathology Munroe Meyer Institute, University of Nebraska Medical Center Omaha, NE 68198-5440 Phone: 402-559-9914 Fax: 402-559-7248 bdave@unmc.edu.

Disclosure/Conflict of Interest All authors have no significant financial or other relationship (which may be perceived as influencing the content, or conclusions reached) with the commercial enterprise whose products and/or services will be discussed in this paper.

FISH analysis with ABR to determine possible involvement of *BCL6* rearrangement in follicular lymphoma, especially in t(14;18)-negative cases.

Keywords

Chromosomal translocation; t(14; 18); follicular lymphoma; fluorescence in situ hybridization (FISH); *BCL6* rearrangement; major breakpoint region (MBR); alternative breakpoint region (ABR)

Introduction

Follicular lymphoma is a neoplasm of germinal center B cells and comprises about 20% of all lymphomas with the highest incidence in the United States and Western Europe. It affects primarily adults, with a median age in the sixth decade and a male:female ratio of 1:1.7.1 Follicular lymphoma is generally considered to be an indolent, but incurable disease, with a median overall survival of 7 - 10 years. Transformation to an aggressive B-cell lymphoma occurs in about 25-35% of cases and is associated with a poor outcome.1 A chromosomal translocation, t(14;18)(q32;q21), is the genetic hallmark of follicular lymphoma. This translocation can be identified in 85-90% of nodal follicular lymphoma cases.2 Juxtaposition of the B-cell leukemia/lymphoma 2 gene (*BCL2*) at chromosome 18q21 and the immunoglobulin heavy chain gene (*IgH*) at 14q32 results in deregulation of *BCL2*, an anti-apoptotic protein. Additional genetic alterations result in progression to follicular lymphoma.3, 4 However, 10-15% of cases do not harbor the t(14;18)(q32;q21) and in these t(14;18)-negative cases, other mechanisms are thought to be involved in the pathogenesis.

A frequent chromosomal translocation in mature B-cell lymphoma is a rearrangement affecting chromosomal band 3q27, which results in deregulation of expression of *BCL6*, a human proto-oncogene.5, 6 *BCL6* encodes a 95 kd nuclear zinc finger phosphoprotein which functions as a sequence-specific transcriptional repressor.7 Chromosome 3q27 rearrangement/*BCL6* translocation is frequently found in diffuse large B-cell lymphoma, at a frequency of 25 - 37%, depending on the detection methods used and patient population studied.8-10 *BCL6* translocations have also been reported at a lower frequency in follicular lymphoma (13% and 14.3%).8, 10 The highest frequency of *BCL6* translocation has been reported in grade 3B follicular lymphoma (44%).11 Interestingly, a higher frequency of 3q27 abnormalities (22%) was also found in t(14;18)-negative follicular lymphoma,12, 13 including the t(3;14)(q27;q32), suggesting an important role for *BCL6* in t(14;18)-negative follicular lymphoma.12, 13

In addition to translocation at the major breakpoint region (MBR) that encompasses the noncoding first exon and part of the first intron of *BCL6*, an alternative breakpoint region (ABR) located 245 - 285 kb 5' to *BCL6* has been described.14, 15 Translocations of *BCL6* involving the ABR were reported to be associated with grade 3B follicular lymphoma.16 However, translocation at the ABR has not been examined in a large series of follicular lymphoma, particularly in t(14;18)-negative cases. The aim of this study was to determine the frequency of *BCL6* rearrangements at the MBR and ABR in a series of well-defined t(14;18)-negative and t(14;18)-positive follicular lymphoma cases. We have used break-

apart FISH probes for the MBR and ABR of *BCL6*. The splitting of the FISH signals could be caused by many different cytogenetic rearrangements occurring in that region, including translocations, inversions and insertions, we will therefore use the term `rearrangement' for all subsequent discussions. We also compared these results with our previously-published study of diffuse large B-cell lymphoma.17

Materials and Methods

Patient population

We selected 142 cases of follicular lymphoma (from year of 1982 to 2005), including 63 t(14;18)-negative and 79 t(14;18)-positive cases. Briefly, we retrieved all t(14;18)-negative follicular lymphoma cases from our cytogenetic database and the selected cases were further verified by FISH analysis to confirm the t(14;18) status. For t(14;18)-positive follicular lymphoma cases, the selective criteria were based on either conventional karyotype analysis or FISH analysis. The diagnosis of follicular lymphoma was confirmed by at least two hematopathologists in all cases. The Institutional Review Board of the University of Nebraska Medical Center approved this study.

FISH analysis

Interphase FISH analysis for *BCL6* rearrangements was performed on the 142 cases using tissue microarrays made from formalin-fixed, paraffin-embedded tissue blocks, as described previously.17 Briefly, hematoxylin and eosin-stained sections from each tissue block were used to define diagnostic areas, and 3 representative 0.6-mm cores were obtained from each case. Only follicular lymphoma components were selected for FISH studies in the composite lymphomas cases. A *BCL6* break-apart probe (Abbott-Vysis, Downers Grove, IL, USA) was used to detect *BCL6* rearrangement at the MBR, and a home-brew break-apart probe (Clones RP11-1144D2 and RP11-76L15, kindly provided by W. L. Lam and R. J. deLeeuw) was used for rearrangement at the ABR (Figure 1). Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI) in Antifade solution and the slides were visualized using an Olympus BX51 fluorescence microscope. Images were captured and archived using Cyto Vision software (Applied Imaging, Santa Clara, CA, USA). To analyze the hybridization, a total of 50-100 nuclei per case were scored for the presence of the *BCL6* rearrangement. A cutoff for positive FISH assays for both the MBR and ABR was established to be more than 20%. Interphase FISH analysis for *BCL2* rearrangement was also performed using a *BCL2* break-apart probe (Abbott-Vysis, Downers Grove, IL, USA) with a positive cutoff of 20%. Any ambiguous FISH results from the tissue microarrays were repeated using unstained whole tissue sections.

Conventional cytogenetic analysis

Karyotypes were available in 58 of the 63 t(14;18)-negative follicular lymphoma cases. Cytogenetic analysis was performed according to a previously described protocol.18 When available, at least 20 metaphases were analyzed. Karyotypes of Giemsa banded chromosomes were described according to the International System for Human Cytogenetic Nomenclature (ISCN 2005).

Statistical analysis

Fisher's exact test was used to calculate p value in the statistical analysis.

Results

Sixty-three t(14;18)-negative cases of follicular lymphoma had a median age of 62 years (range, 24 - 86 years; 59% female and 41% male). *BCL2* rearrangement status was verified by *BCL2* FISH analysis in all t(14;18)-negative follicular lymphoma cases. A total of 112 FISH assays for the *BCL6* rearrangements yielded interpretable results, including 63 MBR and 49 ABR studies. There were a total of 79 t(14;18)-positive cases of follicular lymphoma with a median age 55 years (range, 34 - 89 years; 48% female and 52% male). In these t(14;18)-positive cases, a total of 117 FISH assays for the *BCL6* rearrangements yielded interpretable results, including 79 MBR and 38 ABR studies. A higher technical failure rate was noted in *BCL6* ABR FISH studies.

The frequency of the *BCL6* rearrangement was 26% in t(14;18)-negative follicular lymphoma (*BCL6*-MBR: 14%, *BCL6*-ABR: 12%) and 19% in t(14;18)-positive follicular lymphoma (*BCL6*-MBR: 14%, *BCL6*-ABR: 5%) (Table 1). We found that t(14;18)-negative follicular lymphoma appeared to have a higher frequency of rearrangement at the ABR with an ABR:MBR ratio of 0.86, compared to t(14;18)-positive follicular lymphoma (ABR:MBR ratio of 0.36) and diffuse large B-cell lymphoma (ABR:MBR ratio of 0.32).17 There was no statistically significant difference detected in the distribution of *BCL6*-ABR rearrangement positive cases between the 2 groups (t(14;18)-negative and t(14;18)-positive cases) (p=0.46).

A comparison of clinical features and histological grade by sites of the rearrangement of *BCL6* in the t(14;18)-negative and t(14;18)-positive follicular lymphoma is listed in Table 2. The t(14;18)-negative follicular lymphoma group contains a higher frequency of grade 3 cases than t(14;18)-positive follicular lymphoma group (57% vs. 37%, p=0.02). *BCL6* rearrangements were found in all grades of follicular lymphoma, but were the highest in grade 3 t(14;18)-negative follicular lymphoma cases (60%) (Table 2).

The distribution of *BCL6* rearrangement was analyzed in grade 3 follicular lymphoma cases, particularly in grade 3 cases with a diffuse large B-cell lymphoma component. Due to higher technical failure rate in the *BCL6* ABR FISH analysis, only 49 t(14;18)-negative follicular lymphoma cases had interpretable FISH assays for the *BCL6*-MBR and *BCL6*-ABR rearrangements, and only two cases were grade 3B, both of which also contained a diffuse large B-cell component. One (case #15, Table 3) had a *BCL6*-MBR rearrangement. Twenty-eight of the 49 cases were grade 3A and half of the 28 cases were grade 3A with a diffuse large B-cell component. Among these 14 cases, one had *BCL6*-ABR rearrangement and four had *BCL6*-MBR rearrangement (Table-3).

Similarly, in the 38 t(14;18)-positive follicular lymphoma cases with interpretable FISH assays for both the *BCL6*-MBR and *BCL6*-ABR rearrangements, only two cases were grade 3B, both of which also contained a diffuse large B-cell lymphoma component, and one of these 2 cases had a *BCL6*-MBR rearrangement. Twelve of the 38 cases were grade 3A and 3 of the 12 cases were grade 3A with a diffuse large B-cell lymphoma component, and one of

these 3 cases had a *BCL6*-ABR rearrangement. Thus, none of the follicular lymphoma 3B cases studied had *BCL6*-ABR rearrangement. The numbers of positive cases in each group are too small for statistical analysis.

Conventional cytogenetic (karyotype) results were available in 58 of the 63 t(14;18)-negative follicular lymphoma cases. Eleven of these 58 cases showed either trisomy 3 or a chromosomal translocation involving band 3q27. Table 3 displays 21 cases that had either *BCL6* rearrangements detected by FISH (15 cases) or cytogenetic abnormalities by karyotype analysis. Three of these 15 cases also had a chromosomal translocation involving 3q27 identified by karyotype analysis, with 2 cases having t(3;22)(q27;q11.2) and one t(3;14)(q27;q32). Of the remaining 12 cases, one contained a chromosomal translocation involving 3q29 and three had trisomy 3. Karyotype analysis results were not available in the t(14;18)-positive follicular lymphoma cases, therefore, comparison between karyotype analysis and FISH studies was not performed.

Discussion

Chromosomal translocation involving band 3q27 (*BCL6*) is common in diffuse large B-cell lymphoma and is recognized to be pathogenetically significant.19 This translocation is also seen in other B-cell non-Hodgkin lymphoma, mainly in follicular lymphoma, but also in marginal zone lymphoma.20 However, most studies of *BCL6* translocation by FISH or Southern blot analysis in follicular lymphoma did not include probe(s) for the *BCL6*-ABR rearrangement.8-10, 13 Translocation at the ABR has been reported to occur more frequently in follicular lymphoma (2/20, 10%) than in diffuse large B-cell lymphoma (2/84, 2.4%).15 This finding questions the reported incidence of *BCL6* translocation especially in follicular lymphoma when the ABR was not examined. Therefore, we examined a large series of well-defined t(14;18)-negative and t(14;18)-positive follicular lymphoma for *BCL6* rearrangement at the MBR and ABR by FISH analysis. Our study shows an overall frequency of *BCL6* rearrangement of 26% in t(14;18)-negative and 19% in t(14;18)-positive follicular lymphoma. For the MBR alone, the frequency of *BCL6* rearrangement is very similar between t(14;18)-negative and t(14;18)-positive follicular lymphoma (Table 1). Using FISH analysis, Diaz-Alderete et al also reported similar frequencies of *BCL6* rearrangement at the MBR in t(14;18)-negative (14.6%) and t(14;18)-positive follicular lymphoma (13.1%).21 In addition, we found that the frequency of *BCL6* rearrangement at the ABR appeared to be higher in t(14;18)-negative (12%) than in t(14;18)-positive follicular lymphoma (5%) (Table 1). There is only a trend that t(14;18)-negative follicular lymphomas are associated with a higher frequency of *BCL6*-ABR rearrangement, though there was no statistically significant difference for this rearrangement between t(14;18)-negative cases and t(14;18)-positive cases due to the overall low incidence of *BCL6* rearrangements at the MBR and ABR.

The *BCL6*-ABR rearrangement occurred in all grades of follicular lymphoma but was most frequent in grade 3 t(14;18)-negative follicular lymphoma cases (67%) (Table 2). In a smaller series, Bosga-Bouwer et al selected eight cases of grade 3B follicular lymphoma with cytogenetic abnormalities at the 3q27 and reported that the ABR translocation was found in six of these cases.16 However, in our series of 87 follicular lymphoma cases with

interpretable FISH assays for both the *BCL6*-MBR and *BCL6*-ABR rearrangements, none of the four cases of grade 3B follicular lymphoma (2 t(14;18)-negative and 2 t(14;18)-positive) had a *BCL6*-ABR rearrangement. While *BCL6* rearrangement was common in grade 3 follicular lymphoma, especially in t(14;18)-negative cases, we did not observe a high frequency in grade 3B cases. However, the number of 3B follicular lymphoma is small and this issue needs further investigation.

We also found the overall frequency of *BCL6* rearrangement in t(14;18)-negative follicular lymphoma (26%) to be similar to that of our previously-published study for diffuse large B-cell lymphoma cases (25%),17 suggesting that *BCL6* may also be pathogenetically significant in t(14;18)-negative follicular lymphoma. However, t(14;18)-negative follicular lymphoma had a higher frequency of *BCL6* rearrangement at the ABR, whereas diffuse large B-cell lymphoma more frequently used the MBR. We found that the intergenic region around the ABR is actively transcribed, with transcripts found in germinal center B-cell lines but not in cell lines at other stages of B-cell differentiation (data not shown). The chromatin structure in germinal center B-cells may, therefore, permit rearrangements to occur at this location. However, why this region is preferentially used in t(14;18)-negative follicular lymphoma and the mechanism of rearrangement is not currently understood.

The mechanism of dysregulation of *BCL6* expression due to *BCL6* rearrangement at the MBR has been extensively investigated. Chromosomal translocations at the MBR place the intact coding region of *BCL6* under the control of heterologous promoters derived from partner chromosomes, resulting in deregulated expression of *BCL6*. 22-24 Moreover, most rearrangements at the MBR remove the first noncoding exon of *BCL6* gene that contains two *BCL6* binding sites, and thus disrupt a negative autoregulatory circuit. 25, 26 Some of the rearrangement may also disrupt an interferon regulatory factor 4 (IRF4) responsive region in the first intron of *BCL6* and block its downregulation by CD40 signaling.27 In contrast, chromosomal rearrangements at the ABR leave the regulatory regions intact in the first exon and first intron of *BCL6*. Thus, rearrangement at the ABR must introduce a strong enhancer signal to dysregulate *BCL6* expression. It has been reported that diffuse large B-cell lymphoma and follicular lymphoma cases with ABR rearrangement expressed BCL6 at levels comparable to phenotypically-similar cases with MBR rearrangement or with no 3q27 abnormality.15

It should be noted that dysregulation of *BCL6* expression may also result from mutations introduced by the somatic hypermutation affecting the 5' regulatory region of *BCL6*, as noted above.25 Thus, the frequency of cases with dysregulated *BCL6* expression may be significantly higher than the frequency of cases with *BCL6* rearrangement. Also, rearranged cases with a preserved 5' regulatory region may be further affected by these mutations.

Discrepancies between FISH analysis and conventional cytogenetics (karyotype study) were not infrequent in our study. The possible explanations include: 1) 3q27 rearrangements not involving the MBR and ABR regions, 2) compromised morphology of tumor chromosomes making it difficult to recognize rearrangements near the telomeric end of 3q, 3) the rearrangement involving 3q27 are hidden in partially characterized karyotypes containing `add' (additional uncharacterized chromosomal material) or `marker' chromosomes.28

In summary, our study demonstrates that the frequency of the *BCL6* rearrangement is similar in t(14;18)-negative follicular lymphoma and diffuse large B-cell lymphoma. However, t(14;18)-negative follicular lymphoma has a higher frequency of *BCL6* rearrangement at the ABR, whereas t(14;18)-positive follicular lymphoma and diffuse large B-cell lymphoma more frequently use the MBR than the ABR. Therefore, it is important to include the ABR for FISH analysis of *BCL6* rearrangements in follicular lymphoma, especially in t(14;18)-negative cases.

The high frequency of *BCL6* rearrangement in t(14;18)-negative follicular lymphoma suggests that *BCL6* could play an important role in the lymphomagenesis. The mechanism of *BCL6* rearrangement at the ABR is currently under investigation and may explain the differential use of this breakpoint in the different lymphomas. How rearrangements at the far 5'ABR affect BCL6 expression is intriguing and requires further study.

Acknowledgement

Statistical analysis was performed by Lynette Smith, MS, statistical coordinator, Department of Biostatistics, SLC 3040, 983135 Nebraska Medical Center, Omaha, NE 68198-4375, and Lixin Zhang, PhD. Department of Epidemiology and Center for Molecular and Clinical Epidemiology of Infectious Diseases, University of Michigan, 109 Observatory Street, Ann Arbor, MI 48109-2029

This work was supported in part by an NCI grant (5U01/CA 114778)

References

1. Nathwani, BN.; Harris, NL.; Weisenburger, DD., et al. Follicular lymphoma. In: Jaffe, ES.; Harris, NL.; Stein, H.; Vardiman, JW., editors. World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of Haematopoietic and Lymphoid Tissues. IARC Press; Lyon: 2001. p. 162-167.
2. Aster JC, Longtine JA. Detection of BCL2 rearrangements in follicular lymphoma. Am J Pathol. 2002; 160:759–63. [PubMed: 11891173]
3. Winter JN, Gascoyne RD, Van Besien K. Low-grade lymphoma. Hematology Am Soc Hematol Educ Program. 2004:203–20. [PubMed: 15561684]
4. d'Amore F, Chan E, Iqbal J, et al. Clonal Evolution in t(14;18)-Positive Follicular Lymphoma, Evidence for Multiple Common Pathways, and Frequent Parallel Clonal Evolution. Clin Cancer Res. 2008; 14:7180–7187. [PubMed: 19010834]
5. Baron BW, Nucifora G, McCabe N, Espinosa R 3rd, Le Beau MM, McKeithan TW. Identification of the gene associated with the recurring chromosomal translocations t(3;14)(q27;q32) and t(3;22)(q27;q11) in B-cell lymphomas. Proc Natl Acad Sci U S A. 1993; 90:5262–6. [PubMed: 8506375]
6. Ye BH, Lista F, Lo Coco F, et al. Alterations of a zinc finger-encoding gene, BCL-6, in diffuse large-cell lymphoma. Science. 1993; 262:747–50. [PubMed: 8235596]
7. Chang CC, Ye BH, Chaganti RS, Dalla-Favera R. BCL-6, a POZ/zinc-finger protein, is a sequence-specific transcriptional repressor. Proc Natl Acad Sci U S A. 1996; 93:6947–52. [PubMed: 8692924]
8. Bastard C, Deweindt C, Kerckaert JP, et al. LAZ3 rearrangements in non-Hodgkin's lymphoma: correlation with histology, immunophenotype, karyotype, and clinical outcome in 217 patients. Blood. 1994; 83:2423–7. [PubMed: 8167331]
9. Katzenberger T, Ott G, Klein T, Kalla J, Muller-Hermelink HK, Ott MM. Cytogenetic alterations affecting BCL6 are predominantly found in follicular lymphomas grade 3B with a diffuse large B-cell component. Am J Pathol. 2004; 165:481–90. [PubMed: 15277222]
10. Otsuki T, Yano T, Clark HM, et al. Analysis of LAZ3 (BCL-6) status in B-cell non-Hodgkin's lymphomas: results of rearrangement and gene expression studies and a mutational analysis of coding region sequences. Blood. 1995; 85:2877–84. [PubMed: 7742550]

11. Ott G, Katzenberger T, Lohr A, et al. Cytomorphologic, immunohistochemical, and cytogenetic profiles of follicular lymphoma: 2 types of follicular lymphoma grade 3. Blood. 2002; 99:3806–12. [PubMed: 11986240]
12. Horsman DE, Okamoto I, Ludkovski O, et al. Follicular lymphoma lacking the t(14;18)(q32;q21): identification of two disease subtypes. Br J Haematol. 2003; 120:424–33. [PubMed: 12580956]
13. Jardin F, Gaulard P, Buchonnet G, et al. Follicular lymphoma without t(14;18) and with BCL-6 rearrangement: a lymphoma subtype with distinct pathological, molecular and clinical characteristics. Leukemia. 2002; 16:2309–17. [PubMed: 12399977]
14. Chen W, Butler M, Rao PH, et al. The t(2;3)(q21;q27) translocation in non-Hodgkin's lymphoma displays BCL6 mutations in the 5' regulatory region and chromosomal breakpoints distant from the gene. Oncogene. 1998; 17:1717–22. [PubMed: 9796700]
15. Butler MP, Iida S, Capello D, et al. Alternative translocation breakpoint cluster region 5' to BCL-6 in B-cell non-Hodgkin's lymphoma. Cancer Res. 2002; 62:4089–94. [PubMed: 12124346]
16. Bosga-Bouwer AG, Haralambieva E, Booman M, et al. BCL6 alternative translocation breakpoint cluster region associated with follicular lymphoma grade 3B. Genes Chromosomes Cancer. 2005; 44:301–4. [PubMed: 16075463]
17. Iqbal J, Greiner TC, Patel K, et al. Distinctive patterns of BCL6 molecular alterations and their functional consequences in different subgroups of diffuse large B-cell lymphoma. Leukemia. 2007; 21:2332–43. [PubMed: 17625604]
18. Dave BJ, Hess MM, Pickering DL, et al. Rearrangements of chromosome band 1p36 in non-Hodgkin's lymphoma. Clin Cancer Res. 1999; 5:1401–9. [PubMed: 10389925]
19. Cattoretti G, Pasqualucci L, Ballon G, et al. Deregulated BCL6 expression recapitulates the pathogenesis of human diffuse large B cell lymphomas in mice. Cancer Cell. 2005; 7:445–55. [PubMed: 15894265]
20. Ye H, Remstein ED, Bacon CM, Nicholson AG, Dogan A, Du MQ. Chromosomal translocations involving BCL6 in MALT lymphoma. Haematologica. 2008; 93:145–6. [PubMed: 18166802]
21. Diaz-Alderete A, Doval A, Camacho F, et al. Frequency of BCL2 and BCL6 translocations in follicular lymphoma: relation with histological and clinical features. Leuk Lymphoma. 2008; 49:95–101. [PubMed: 18203018]
22. Chen W, Iida S, Louie DC, Dalla-Favera R, Chaganti RS. Heterologous promoters fused to BCL6 by chromosomal translocations affecting band 3q27 cause its deregulated expression during B-cell differentiation. Blood. 1998; 91:603–7. [PubMed: 9427715]
23. Ohno H. Pathogenetic and clinical implications of non-immunoglobulin; BCL6 translocations in B-cell non-Hodgkin's lymphoma. J Clin Exp Hematop. 2006; 46:43–53. [PubMed: 17142954]
24. Ye BH, Chaganti S, Chang CC, et al. Chromosomal translocations cause deregulated BCL6 expression by promoter substitution in B cell lymphoma. Embo J. 1995; 14:6209–17. [PubMed: 8557040]
25. Pasqualucci L, Migliazza A, Basso K, Houldsworth J, Chaganti RS, Dalla-Favera R. Mutations of the BCL6 proto-oncogene disrupt its negative autoregulation in diffuse large B-cell lymphoma. Blood. 2003; 101:2914–23. [PubMed: 12515714]
26. Wang X, Li Z, Naganuma A, Ye BH. Negative autoregulation of BCL-6 is bypassed by genetic alterations in diffuse large B cell lymphomas. Proc Natl Acad Sci U S A. 2002; 99:15018–23. [PubMed: 12407182]
27. Saito M, Gao J, Basso K, et al. A signaling pathway mediating downregulation of BCL6 in germinal center B cells is blocked by BCL6 gene alterations in B cell lymphoma. Cancer Cell. 2007; 12:280–92. [PubMed: 17785208]
28. Keller CE, Nandula S, Vakiani E, Alobeid B, Murty VV, Bhagat G. Intrachromosomal rearrangement of chromosome 3q27: an under recognized mechanism of BCL6 translocation in B-cell non-Hodgkin lymphoma. Hum Pathol. 2006; 37:1093–9. [PubMed: 16867873]

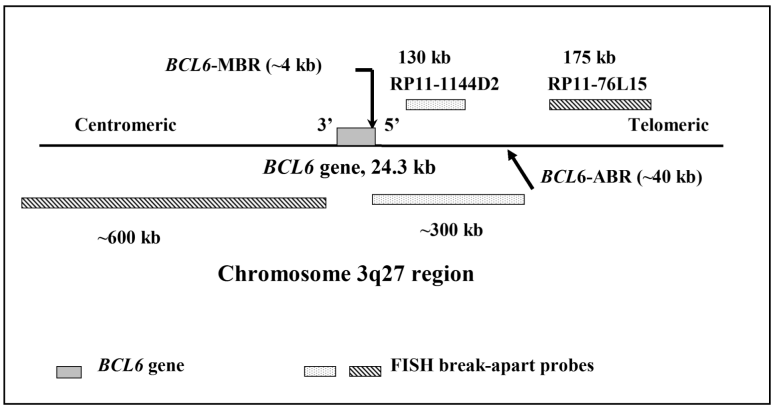

Figure 1.

Diagram of positions of FISH probes for *BCL6* rearrangements at the MBR and ABR of chromosome 3q27.

MBR: Major breakpoint region, ABR: Alternative breakpoint region

Table 1

FISH analysis for *BCL6* rearrangements at the MBR and ABR among t(14;18)-positive FL, t(14;18)-negative FL and DLBCL cases.

	t(14;18)-pos. FL		t(14;18)-neg. FL		DLBCL*	
FISH results	***BCL6* MBR**	***BCL6* ABR**	***BCL6* MBR**	***BCL6* ABR**	***BCL6* MBR**	***BCL6* ABR**
Positive cases	11	2	9	6	25	5
Negative cases	68	36	54	43	108	73
Total cases	79	38	63	49	133	78
Positive cases (%)	**14**	**5**	**14**	**12**	**19**	**6**

FL: Follicular lymphoma, DLBCL: Diffuse large B-cell lymphoma, pos.: Positive, neg.: Negative, MBR: Major breakpoint region, ABR: Alternative breakpoint region.

*From our previously-published data17

Table 2

Comparison of age, gender, sites of involvement and histological grade by *BCL6* rearrangement sites in t(14;18)-positive and t(14;18)-negative FL.

	t(14;18) -pos. total cases	t(14;18) -neg. total cases	t(14;18)-pos. with *BCL6*R	t(14;18)-neg. with *BCL6*R	t(14;18)-pos. with *BCL6*R at MBR	t(14;18)-neg. with *BCL6*R at MBR	t(14;18)-pos. with *BCL6*R at ABR	t(14;18)-neg. with *BCL6*R at ABR
Age range (median)	34-89 (55)	24-86 (62)	38-80 (56)	29-83 (63)	44-80 (56)	29-83 (58)	38-79 (59)	41-73 (68)
Female	48%	59%	31%	87%	36%	100%	0%	67%
Nodal	87%	78%	85%	87%	82%	78%	100%	100%
Grade 3	37%	57%	46%	60%	45%	56%	50%	67%
Grade 1-2	63%	43%	54%	40%	55%	44%	50%	33%
Total cases	79	63	13	15	11	9	2	6

FL: Follicular lymphoma, pos.: Positive, neg.: Negative, R: Rearrangement, MBR: Major breakpoint region, ABR: Alternative breakpoint region.

Table 3

Comparison of conventional cytogenetics and FISH analysis for *BCL6* rearrangement in t(14;18)-negative FL cases.

case	Age	Gender	Diagnosis	Cytogenetic abnormality involving chromosome 3	FISH-*BCL6*-MBR	FISH-*BCL6*-ABR
1	73	F	FL-1	No	Neg.	**Pos.** *
2	69	M	FL-2	No	Neg.	**Pos.**
3	41	F	FL-3A	Add(3)(q12)	Neg.	**Pos.**
4	63	F	FL-3A	+3	Neg.	**Pos.**
5	82	F	FL-3A	No	Neg.	**Pos.**
6	67	M	FL-3A(30%) DLBCL(70%)	+3	Neg.	**Pos.** *
7	61	F	FL-1	No	**Pos.**	Neg.
8	55	F	FL-2	No	**Pos.**	**NR** **
9	66	F	FL-2	No	**Pos.**	**NR** **
10	28	F	FL-2	t(3;14)(q29;q11.2)	**Pos.**	Neg.
11	82	F	FL-3A(30%) DLBCL(70%)	No	**Pos.**	**NR** **
12	55	F	FL-3A(50%) DLBCL(50%)	t(3;14)(q27;q32)	**Pos.**	Neg.
13	70	F	FL-3A(50%), DLBCL(50%)	t(3;22)(q27;q11.2)	**Pos.**	Neg.
14	58	F	FL-3A(70%) DLBCL(30%)	+3	**Pos.**	Neg.
15	50	F	FL-3B(10%) DLBCL(90%)	t(3;22)(q27;q11.2)	**Pos.**	Neg.
16	68	M	FL-2	t(3;9)(q27;p21)	Neg.	NR**
17	63	M	FL-3A	t(3;14)(q29;q32)	Neg.	Neg.
18	70	F	FL-3A	+3	Neg.	Neg.
19	58	M	FL-3A	+3	Neg.	Neg.
20	53	F	FL-3A	+3	Neg.	Neg.
21	71	F	FL-3A	+3	Neg.	NR**

FL: Follicular lymphoma, DLBCL: Diffuse large B-cell lymphoma, pos.: Positive, neg.: Negative, MBR: Major breakpoint region, ABR: Alternative breakpoint region.

* One BCL6 signal is deleted

** NR - No results