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SHORT COMMUNICATION

A novel fusion 5′*AFF3*/3′*BCL2* originated from a t(2;18)(q11.2;q21.33) translocation in follicular lymphoma

L Impera[1], F Albano[2], C Lo Cunsolo[3], S Funes[3], P Iuzzolino[3], F Laveder[4], I Panagopoulos[5], M Rocchi[1] and CT Storlazzi[1]

[1]*Department of Genetics and Microbiology, University of Bari, Bari, Italy;* [2]*Department of Pathology, Section of Hematology, University of Bari, Bari, Italy;* [3]*Dipartimento di Medicina di Laboratorio, Ospedale S. Martino, Belluno, Italy;* [4]*Dipartimento di Discipline Mediche, Ospedale S. Martino, Belluno, Italy and* [5]*Department of Clinical Genetics, University Hospital, Lund, Sweden*

Follicular lymphoma is the second most frequent type of non-Hodgkin's lymphoma in adults. The basic molecular defect consists of the t(14;18)(q32;q21) translocation, juxtaposing the B-cell lymphoma protein 2 gene *BCL2* to the immunoglobulin heavy chain locus *IGH@*, and leading to the antiapoptotic BCL2 protein overproduction. Variations in the t(14;18) are rare and can be classified into two categories: (i) simple variants, involving chromosomes 18 and 2, or 22, in which the fusion partner of *BCL2* is the light-chain *IGK@* or *IGL@*; (ii) complex variant translocations occurring among chromosomes 14, 18 and other chromosomes. We report a follicular lymphoma case showing *BCL2* overexpression, detected by immunohistochemistry and real-time quantitative PCR, consequently to the formation of a novel fusion gene between the 5′ of the lymphoid nuclear transcriptional activator gene *AFF3* at 2q11.2, and the 3′ of *BCL2*. This case shows evidence, for the first time, of *BCL2* overexpression consequently to the fusion of *BCL2* to a non-IG partner locus.
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Follicular lymphoma (FL) is the second most frequent type of non-Hodgkin's lymphoma in adults (de Jong, 2005). The t(14;18)(q32;q21) translocation juxtaposes the B-cell lymphoma protein 2 gene (*BCL2*), at 18q21, to the immunoglobulin heavy chain locus (*IGH@*) at 14q32. This rearrangement leads to the overproduction of the antiapoptotic BCL2 protein, the basic molecular defect in FL.

Variations in the t(14;18) are rare and can be classified into two categories: (i) simple variants, involving chromosome 18 and either chromosome 2 or 22, in which the fusion partner of *BCL2* is the light-chain *IGK@* (2p11.2) or *IGL@* (22q11.2); (ii) complex variant translocations, involving chromosomes 14, 18 and one or two other chromosomes (Bentley *et al*., 2005). Such variants can be considered as biologic equivalents of the t(14;18) and, therefore, more than 90% of indolent FLs express high levels of BCL2 protein (de Jong, 2005).

Here we describe a FL case showing *BCL2* overexpression, detected by immunohistochemistry and real-time quantitative PCR. This case is the result of the fusion of the 3′ end of *BCL2* with the 5′ of *AFF3 (AF4/FMR2 family member 3 isoform 1,* also known as *LAF4)* at 2q11.2. The chimeric gene was investigated by RT–PCR and fluorescent *in situ* hybridization analyses.

A 65-year-old female patient was referred to our hospital because of persistent submandibular and multiple cervical lymphadenopathy. The patient did not complain of fatigue, night sweats or significant weight loss. Laboratory findings showed elevated lactate dehydrogenase and hypergammaglobulinemia. Other biochemical values were in the normal range. A total body computerized axial tomographic scan showed enlargement of multiple submandibular, cervical, axillary and mediastinal lymph nodes. Submandibular lymph node biopsy was performed. Immunophenotyping showed positivity for CD10 and CD20. No evidence of lymphoma involvement was found in the bone marrow. On the basis of clinical, radiological, histological and immunological findings, FL stage II-A, grade 1, FLIPI score 3, was diagnosed. The patient received six cycles of rituximab, cyclophosphamide, vincristine and prednisone (R-CVP). Staging after polychemotherapy showed complete remission.

Classical cytogenetic analysis, performed on the patient's tumor cells, revealed a karyotype 47,XX,t(2;18)(q11;q21),+11,i(12)(q10)[8] (Figure 1a and data not shown) that was confirmed by multicolor-fluorescent *in situ* hybridization (Figure 1a and data not shown). Fluorescent *in situ* hybridization experiments, using appropriate bacterial artificial chromosome clones, revealed that the breakpoints were encompassed by the clones RP11-321A19 (chr2:100,004,992-100,203,010), containing the 5′ portion of *AFF3*, and RP11-28F1 (AC021803)

Correspondence: Dr CT Storlazzi, Department of Genetics and Microbiology, University of Bari, Via Amendola 165/A, P.zza Giulio Cesare, Bari 70126, Italy.
E-mail: c.storlazzi@biologia.uniba.it
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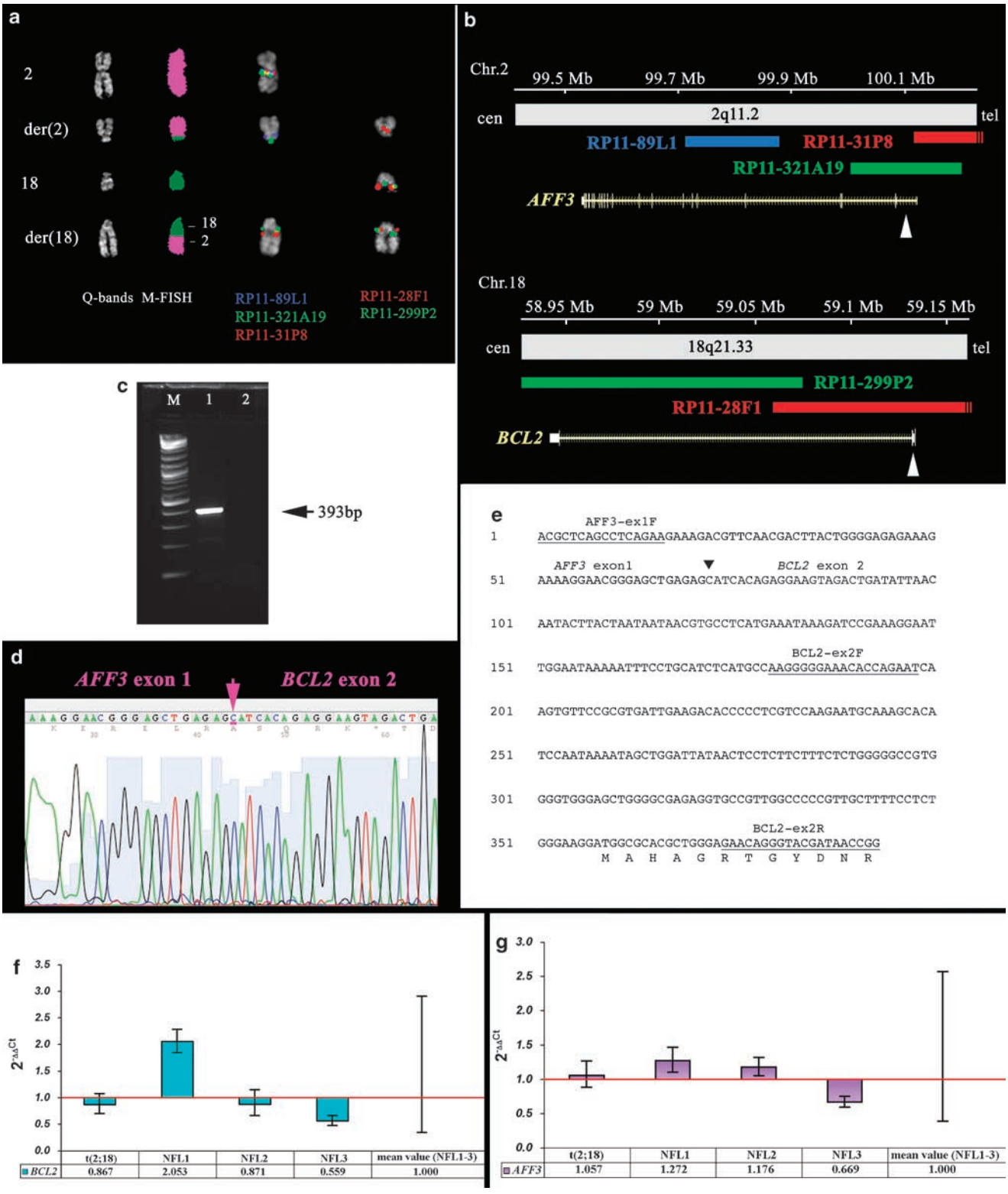

(chr18:59,058,635-59,215,681), spanning the 5′ end of *BCL2*. Both of the fluorescent *in situ* hybridization probes showed splitting signals on normal and derivative chromosomes 2 and 18 (Figures 1a and b).

RT–PCR with an *AFF3* forward primer, specific for exon 1 of the transcript in form 1 (accession number: NM_002285), and a *BCL2* reverse primer within the coding sequence of exon 2 of the transcript variant α (accession number: NM_000633), yielded a single band

Figure 1 (**a**) Q-banding, multicolor-fluorescent *in situ* hybridization (FISH) analysis [performed using the 24-colour SpectraVysion probe (Abbott, Abbott Park, IL, USA)], and FISH analyses performed on the case under study. Each column shows normal and derivative chromosomes 2 and 18 relative to the FISH experiments with probes listed at the bottom of the figure (columns 3 and 4). Whole chromosome paints of chromosomes 2 and 18 were pseudocolored in purple and dark green, respectively (column 2). (**b**) Maps of the breakpoint regions in chromosome bands 2q11.2 (top) and 18q21.33 (bottom). White arrowheads indicate the breakpoint regions mapped by FISH experiments. (**c**) RT–PCR experiment on the cDNA of the patient (lane 1) and of a normal control (lane 2), using the primer set AFF3-ex1F (5′ ACGCTCAGCCTCAGAAGAAA 3′) + BCL2-ex2R (5′ CCGGTTATCGTACCCTGTTC 3′). A chimeric transcript of 393 bp is evident in lane 1. M: 2-Log DNA Ladder (New England Biolabs, Milan, Italy) (**d**) Partial sequence chromatogram showing the fusion junction (purple arrowhead) on the chimeric *AFF3*/*BCL2* transcript. The nucleotide micro-homology at the fusion junction is underlined in purple. (**e**) Complete nucleotide sequence of *AFF3*/*BCL2* chimeric transcript. The sequence of the primers used in PCR experiments is underlined. The arrowhead indicates the *AFF3*/*BCL2* transcript junction. (**f**) and (**g**) real-time quantitative (RQ) PCR results obtained using 1 × PlatinumSYBR Green qPCR SuperMix-UDG (Invitrogen, Carlsbad, CA, USA), and primer sets specifically designed for *BCL2* [BCL2-ex2F; BCL2-ex2R (Figure 1e)] and *AFF3* (AFF3ex6F: 5′ CAGGGGGAGGAGAGTAGATC 3′; AFF3ex7R: 5′AAGTGGTGGAAGCCAGGTC 3′) coding sequences. 28S RNA was used as the reference gene and the mean value of the three follicular lymphoma (FL) control samples (positive for *IGH@*/*BCL2* rearrangement) was utilized for calibration. The gene expression level variation was estimated by comparing the values of $2^{-\Delta\Delta Ct}$ (relative amount of cDNA) for the Ct values in the patient with t(2;18), and three cases of nodal FLs, with relative value of the calibrator. (**f**) The results showed comparable *BCL2* transcriptional levels between the patient with t(2;18) and the mean Ct value of the nodal FL controls [mean value (NFL1–3)]. (**g**) RQ-PCR results showing no statistically significant *AFF3* mis-expression in our patients versus the control FL samples.

of 393 bp (Figure 1c). The sequencing of the RT–PCR product revealed the occurrence of a 5′*AFF3*/3′*BCL2* fusion transcript, in which the whole *AFF3* exon 1 was fused to the entire exon 2 of *BCL2* (Figures 1d and e). The micro-homology of one nucleotide (C) occurred at the fusion junction (Figures 1d and e). The fusion breakpoint mapped before the start of *AFF3* coding sequence (*AFF3* exon 2) and preserved the *BCL2* ATG initiation codon, which begins at nt 87 of *BCL2* exon 2 (NM_000633) (Figure 1e). RT–PCR for the reciprocal *BCL2*/*AFF3* fusion product, using *BCL2* exon 1 forward and *AFF3* exon 2 reverse primers, yielded negative results (data not shown). Immunohistochemical analysis, using a specific *BCL2* antibody, showed the nuclear overexpression of the BCL2 protein in the neoplastic follicular cells (Figure 2b).

The expression level of *BCL2* and *AFF3* was determined by real-time quantitative PCR, using proper primer combinations for *BCL2* and *AFF3* (Figures 1f and g). This analysis revealed that the *BCL2* transcript showed comparable expression levels in our patient with respect to the *IGH@*/*BCL2*-positive FL cases (Figure 1f). In addition, there was no statistically significant mis-expression of the *AFF3* transcripts in the case with the t(2;18) rearrangement versus the other three FLs without the translocation (Figure 1g).

The overall data reported here demonstrate a novel chromosomal rearrangement in FL, that is, a t(2;18)(q11.2;p21.33) translocation, leading to the over-expression of *BCL2*. To the best of our knowledge, this is the first case in which *BCL2* fuses with a gene other than Ig loci. Mahmoodi *et al*. (2004) described a novel t(16;18)(p13;q21.3) translocation in a *BCL2*-positive FL case, but they did not identify the candidate partner gene at 16p13.

The mechanism responsible for the transcriptional deregulation of an oncogene through its juxtaposition to an ectopic promoter is known as 'promoter swapping.' It has been reported in several tumors (pleomorphic adenoma of the salivary glands, lipoblastoma, aneurysmal bone cyst, teratoma). Similarly, in a case of primary splenic FL we have recently documented the upregulation of *SOX5*, one of the Sry (sex determining region Y)-box (SOX) genes. After the juxtaposition of *SOX5* coding sequence to the *P2RY8* (G-protein coupled purinergic receptor P2Y8) promoter sequence,

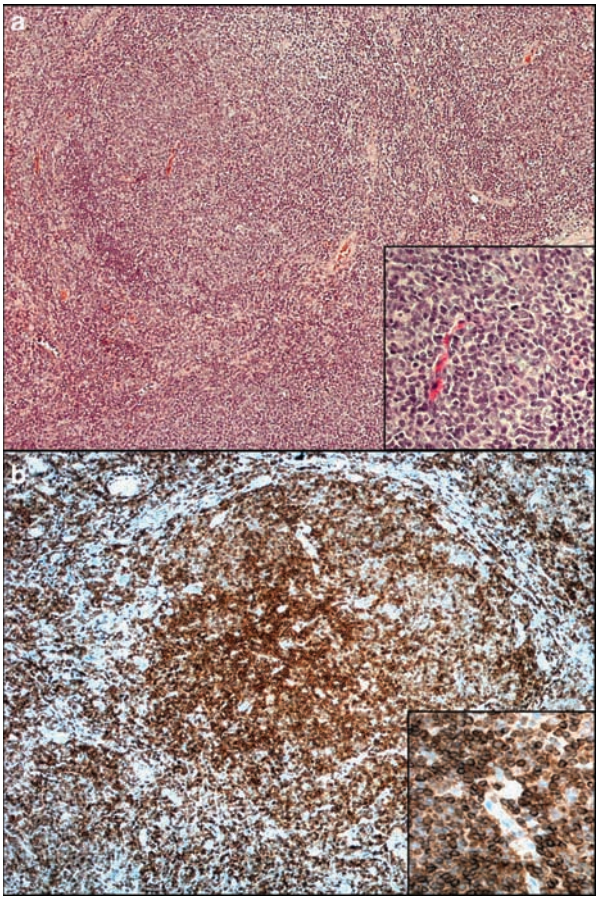

Figure 2 (**a**) Pictures, at different magnification (× 10 and × 40), of histological samples from the patient's tumor (hematoxylin and eosin stain). (**b**) Immunohistochemistry analysis with anti-BCL2 monoclonal antibody (clone bcl-2/100/D5, Novocastra Laboratories, Ltd, Newcastle upon Tyne, UK), showing nuclear reactivity in the majority of follicular lymphomatous cells (× 10 and × 40 original magnifications).

it was highly active in lymph nodes and in lymph (Storlazzi *et al*., 2007). The *AFF3* promoter is reported to be highly expressed in lymphoid tissues (Hiwatari *et al*., 2003). Rearrangements of the *BCL2* gene are recurrent in B-cell tumors. In t(14;18) cases, the breakpoints are mapped within specific regions located at the 3′ end of the gene (MBR, mcr, icr, 3′BCL2, 5′ mcr) outside its coding region (Weinberg *et al*., 2007). Conversely, simple variant translocations with *IGL* loci showed breakpoints clustering at the 5′ end of the gene, that is, from 378 to 2312 bp upstream of the *BCL2* translational initiation site (Yonetani *et al*., 2001). This case showed a *BCL2* breakpoint within the first intron, exactly at the same location as one FL case (case no. 61) with 5′-*BCL2*/*IGLκ* reported in Yonetani *et al*. (2001).

The partner *AFF3* gene encodes a lymphoid nuclear protein of 1227 amino acids with transactivation potential and is thought to have a role in early lymphoid development (Hiwatari *et al*., 2003). The AFF3 is a member of a family of proteins including AFF1 (4q21.3–22.1, also known as AF4), AFF2 (Xq28, alias FMR2) and AFF4 (5q31.1, alias AF5q31), which have various similarities: size of transcripts, protein size of approximately 1300 amino acids and nuclear localization of the proteins. AFF1 and AFF4 were also found to be involved in B-cell acute lymphoblastic leukemia (ALL), as fusion partners of the *MLL* gene. Similarly, *AFF3* has been implicated in tumorigenesis, as it was reported to be the 3′ fusion partner of *MLL (5)* and *RUNX1* (Chinen *et al*., 2008) in pediatric ALL, respectively, with B-ALL and T-ALL phenotype. In both chimeras, the breakpoint disrupting *AFF3* was located within the region homologous to the transactivation domain of AFF1 and AFF4 (exons 5–7), leading to the retention of this oncogenic domain in all the AFF3 fusion proteins (Hiwatari *et al*., 2003; Chinen *et al*., 2008). In this case, the *AFF3* breakpoint was located within the 5′UTR region, that is, outside its coding region. It is conceivable that the *AFF3* promoter is potentially able to activate the expression of *BCL2*, which is usually over-represented in *IGs*/*BCL2*-positive FL cases.

In conclusion, the FL case we reported shows overexpression of *BCL2* resulting from its fusion with a non-IG partner. Identification of *AFF3* as the partner gene was greatly facilitated by the accurate molecular cytogenetic analysis performed by appropriate bacterial artificial chromosome clones.

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