Pathol. Oncol. Res. (2016) 22:233–243
DOI 10.1007/s12253-015-9972-1

REVIEW

A Novel Non-Immunoglobulin (*non-Ig*)/*BCL6* Translocation in Diffuse Large B-Cell Lymphoma Involving Chromosome 10q11.21 Loci and Review on Clinical Consequences of *BCL6* Rearrangements

Marie Jarosova[1] · Eva Kriegova[2] · Petra Schneiderova[2] · Regina Fillerova[2] · Vit Prochazka[1] · Michaela Mikesova[1] · Patrik Flodr[3] · Karel Indrak[1] · Tomas Papajik[1]

Received: 18 May 2015 / Accepted: 19 August 2015 / Published online: 30 August 2015

Abstract *BCL6* rearrangements (3q27) are the most common chromosomal abnormalities in diffuse large B-cell lymphoma (DLBCL), with numerous immunoglobulin (*Ig*) and *non-Ig* genes as partners. In DLBCL, the translocations occur predominantly in the "major breakpoint region" encompassing the first noncoding exon and a part of the first intron of *BCL6*; few cases with "alternative breakpoint cluster" located 245–285 kb 5′ *BCL6* were also described. The regulatory sequences of known *Ig* and *non-Ig* partners replace the 5′ untranslated region of the *BCL6* in the same transcriptional orientation. Contrary to *Ig*/*BCL6* fusions typical by high *BCL6* gene expression, in *non-Ig*/*BCL6* translocations were observed unexpectedly low *BCL6* mRNA levels. From the clinical point of view, the survival rate of DLBCL patients with *non-Ig* partners is inferior to those with *Ig/BCL6* translocations, suggesting that *non-Ig/BCL6* fusion is a poor prognostic indicator. Hereby we provide comprehensive information about known *non-Ig* translocation partners and clinical consequences of *BCL6* rearrangements in DLBCL. Moreover, we describe a novel reciprocal translocation t(3;10) in refractory patient with DLBCL with the breaking points at 5′ untranslated region of *BCL6* and 5′ untranslated region of the *RASGEF1A* gene on chromosome 10q11.21 loci; this rearrangement was associated with low *BCL6* and *RASGEF1A* gene expressions. Our patient harbouring dual chromosomal rearrangement involving *BCL2* and *BCL6* genes relapsed three-times and died soon; thus, further supporting the notion that *non-Ig/BCL6* fusion is a poor prognostic indicator of DLBCL. There is evidence of prognostic value of *BCL6* rearrangements also in rituximab era.

Keywords Diffuse large B-cell lymphoma · *BCL6* rearrangements · *BCL2* rearrangements · Non-immunoglobulin gene translocations · Complex chromosomal changes

Marie Jarosova and Eva Kriegova contributed equally to this work.

✉ Marie Jarosova
marie.jarosova@fnol.cz

[1] Department of Hemato-Oncology, Faculty of Medicine and Dentistry, Palacky University Olomouc and the University Hospital Olomouc, Hnevotinska 3, 77900 Olomouc, Czech Republic

[2] Department of Immunology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Hnevotinska 3, 77900 Olomouc, Czech Republic

[3] Department of Clinical and Molecular Pathology, Faculty of Medicine and Dentistry, Palacky University Olomouc and the University Hospital Olomouc, Hnevotinska 3, 77900 Olomouc, Czech Republic

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin's lymphomas. This malignancy is characterized by wide variability in clinical outcome and high genetic heterogeneity [1, 2]. The most common chromosomal abnormality in DLBCL is a 3q27 translocation affecting *BCL6* gene, occurring in up to 45 % of patients [3–6].

The *BCL6* gene (3q27) is a proto-oncogene expressed in the course of B-cell differentiation in the germinal centre (GC) [7]. This zinc-finger transcription factor functions as a sequence-specific transcriptional repressor [8–10]. *BCL6* is commonly translocated in DLBCL with diverse partners, including one of three immunoglobulin gene (*IGH*, *IGK*, *IGL*) loci [11–13] or diverse *non-Ig* chromosomal loci [3, 5, 6, 14]. The translocations affecting the *BCL6* gene occur in DLBCL

predominantly in the major breakpoint region (MBR) encompassing the first noncoding exon and a part of the first intron of *BCL6* [3, 12]; few cases with alternative breakpoint cluster (ABR) located 245–285 kb 5′ *BCL6* were also described [15]. As a result, many types of regulatory sequences of known *Ig* as well as *non-Ig* genes replace the 5′ untranslated region of the *BCL6* in the same transcriptional orientation and the rearranged *BCL6* is presumed to be under the control of the replaced promoter activity [3]. From the clinical point of view, there is evidence about the influence of 3q27 translocation on the etiopathogenesis of DLBCL. Moreover, the survival rate of DLBCL patients with *non-Ig* partners is inferior to that of those with *Ig/BCL6* translocation, suggesting that *non-Ig/BCL6* fusion is a poor prognostic indicator of DLBCL [16–18].

Here, we review current knowledge about the *BCL6* rearrangements in DLBCL and spectrum of known *non-Ig* fusion partners involved in *non-Ig/BCL6* rearrangements and clinical consequences of *BCL6* translocations in DLBCL. Additionally, we describe of a novel *non-Ig/BCL6* translocation involving chromosome 10q11.21 loci found in refractory postransplant patient with DLBCL.

Case Description

A 52-year old male was referred to the Department of Haemato-Oncology, University Hospital in Olomouc, with severe B-symptoms including weight loss of 20 kg and abdominal pain caused by a large abdominal tumour mass in June 2006. Laparoscopic biopsy of the small mesenteric lymph node revealed anaplastic subtype DLBCL with a fraction of Hodgkin- and Reed-Sternberg-like neoplastic cells and neoplastic lymphoid cells bearing CD20 and CD30 positivity and Bcl2 positivity. Trephine biopsy and flow cytometry of the bone marrow did not show lymphoma infiltration. Staging was done using a whole-body FDG-PET/CT fusion camera (Siemens Biograph 16); PET-positive enlarged lymph nodes were detected in the mediastinal, retroperitoneal and mesenteric areas. Finally, the Ann Arbor clinical stage was IIIB; the International Prognostic Index (IPI) and age-adjusted IPI were intermediate-high and high, respectively.

Our patient was treated with intensive rituximab (R) - anthracycline-containing sequential protocol [19], followed by up-front autologous stem cell transplantation (ASCT) after BEAM 200 conditioning in February 2007. Treatment response was assessed on day +100 (May 2007) after ASCT using PET/CT and classified as PET-negative complete remission (CR).

At first relapse in September 2010, FDG-PET/CT revealed PET-positive generalized peripheral, mediastinal, retroperitoneal, mesenteric and inguinal lymphadenopathy with maximum lymph node size of 38 mm, PET-positive splenomegaly and FDG bone marrow uptake. Biopsy of the PET-positive inguinal lymph node confirmed tumour cells expressing CD20, *BCL2* and CD10 (Fig. 1). Based on *BCL6* gene rearrangement and the presence of complex karyotypic changes, the patient was classified as being in the ABC DLBCL prognostic subgroup.

The patient was treated with one cycle of platinum-based salvage therapy (EDHAP), with accompanying severe sepsis. Subsequent therapy with one CHOP-like and one R-Dexa regimen was accompanied by prolonged neutropenia despite the application of pegylated G-CSF. Bone marrow examination excluded therapy-related myelodysplastic syndrome. Lymphoma infiltration was not detectable. PET-positive enlarged lymph nodes were detected in all previously involved areas and response to therapy was reported as stable.

The patient was followed up until subsequent progression of lymphoma in August 2012. The neoplastic cells bore Bcl2 positivity, CD19, CD20, CD22, FMC7 antigens, but were CD10 negative. Due to severe allergic reaction to rituximab, the patient was treated with six cycles of the CHOP regimen (from August 2012 to January 2013). The applied chemotherapy brought about short-term lymphoma control and after 3 months, lymphoma progressed again. Therefore, the patient was shifted to palliative care. In February 2015, after 96-months after ASCT and after three relapses/progressions, the patient died.

Cytogenetic Analysis

At the time of diagnosis, standard cytogenetic analysis was performed on the lymph node and bone marrow samples. In the lymph node, karyotype 79–88, XXY,-Y,−1,−3,−6,−7,−9,−14,−15,−22[cp5]/46,XY [5] was confirmed. In the bone marrow, only normal karyotypes were found (46,XY [10]). Due to the limited amount and quality of lymph node material, only PCR-examination of *BCL2* rearrangement and FISH with LSI IGH/BCL2 DC DF probe (Abbott Molecular, Illinois, USA) were performed with positive result for *BCL2/IgH* rearrangement in the minor cluster region.

At the time of first relapse in September 2010, conventional cytogenetic analysis of the lymph node showed complex karyotype with 49–50 chromosomes in the 9 metaphases karyotyped. In the bone marrow, only normal karyotypes (46,XY [27]) were found. To determine chromosomal and gene rearrangements, FISH was used with the following set of probes: LSI BCL6 DC, LSI IGH/BCL2 DC DF, LSI TP53, CEP 17 (Abbott Molecular), ON 1q21/SRD (1p36), ON 6q23/CEP6 and ON RET (10p11) Break probe (Kreatech, Amsterdam, Netherlands), RET probe (Agilent, Santa Clara, USA) and BAC probes (RP11-124O11, RP11-168L22) (BlueGnome, Cambridge, UK); at least 200 interphase cells were evaluated. Other techniques applied included multicolor FISH (mFISH) with the 24XCyte Human Multicolor probe set

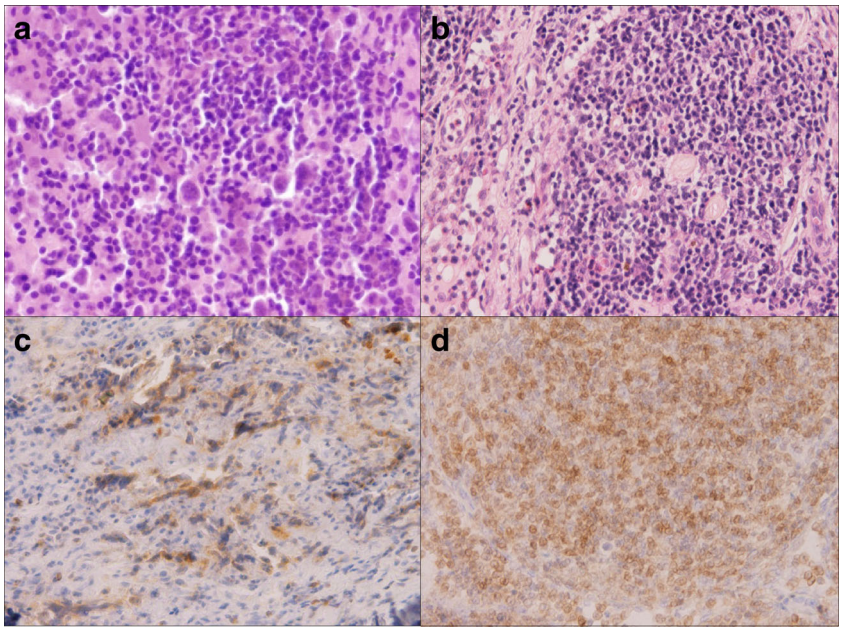

Fig. 1 The histological features of formalin-fixed paraffin-embedded lymph nodes taken at the time of (**a**) diagnosis and (**b**) relapse stained with H&E. Immunohistochemistry confirmed Bcl-2 positive expression in neoplastic cells (clone 100, Biogenex (Dako) at (**c**) the time of diagnosis and as well at (**d**) the time of 1. relapse. Magnification 200×

(MetaSystems, Altlussheim, Germany) and array comparative genomic hybridization (arrayCGH) with 4x44K microarray (BlueGnome) in accordance with the manufacturer's instructions. The arrayCGH result was evaluated with BlueFuse Multi software (BlueGnome).

The arrayCGH method confirmed gains in the region 1q21.1qter and on chromosomes 5, 12 and 17, and loss of 6q12q27. Based on the cytogenetic, FISH, mFISH and arrayCGH analyses, the following complex karyotype in three pathological clones were determined:

$$48, XY, +X, t(3;10)(q27;q11.21), der(6)t(1;6)(q21.1;q12), +12, t(14;18)(q32;q21)[4]/50, XY, +X, t(3;10)(q27;q11.21), +5, der(6)t(1;6)(q21.1;q12), +12, t(14;18)(q32;q21), +17[3]/53, XY, +X, +Y, t(3;10)(q27;q11.21), +5, der(6)t(1;6)(q21.1;q12), +11, +12, t(14;18)(q32;q21), +17, +22[2].$$

These findings showed clonal evolution of the clone with 48 chromosomes. This clonal evolution was represented by the required gains of chromosomes 5 and 17 in one and 5, 17, 11, 22 and chromosome Y in the last clone. In all these clones, FISH and mFISH showed two reciprocal translocations: t(14;18)(q32;q21) and t(3;10) with *BCL2* and *BCL6* gene rearrangements, respectively (Fig. 2). As the red signal of *BCL6* probe covering 5′ *BCL6* region was detected on chromosome 10, we started mapping the breakpoint on chromosome 10 with RET probes. Whereas the RET probe (Agilent) was located on both chromosomes 10, the RET break-part probe (Kreatech) shows normal fusion signal on chromosome 10 and smaller green/red fusion signal on derivative chromosome 10, and the larger green signal being located on the derivative chromosome 3 (Fig. 2e). The FISH results with BAC probes confirmed the breakpoint located in the 5′ non-coding region of *RASGEF1A* gene (Fig. 3). Finally, the breakpoint on chromosome 10 was mapped to the 10q11.21 region. This breakpoint as well as the gene candidate represent the first observation in a DLBCL case, as shown in Table 1, where the spectrum of known *non-Ig*/BCL6 translocations reported in DLBCL is summarized.

Gene Expression Profiling of *BCL2*, *BCL6, RASGEF1A*

To assess the gene expression level of *BCL2*, *BCL6, RASGEF1A* in the lymph node from our DLBCL patients with t(3;10), we performed quantitative RT-PCR and compared the mRNA expression of *BCL2*, *BCL6, RASGEF1A* in our patient with lymph nodes of 1) two DLBCL cases with *BCL6* rearrangements such as t(3;14) and t(3;22), and 2) patients with DLBCL without *BCL6* rearrangements (DLBCL, n=11); and with peripheral blood from 3) healthy control subjects (C, n=18).

Total RNA from the lymph nodes and peripheral mononuclear cells was isolated with mirVana miRNA kit (Ambion, Austin, USA) and reverse transcription was performed with anchored dT primers (Roche Applied Science, Indianapolis, USA) according to the manufacturer's recommendation.

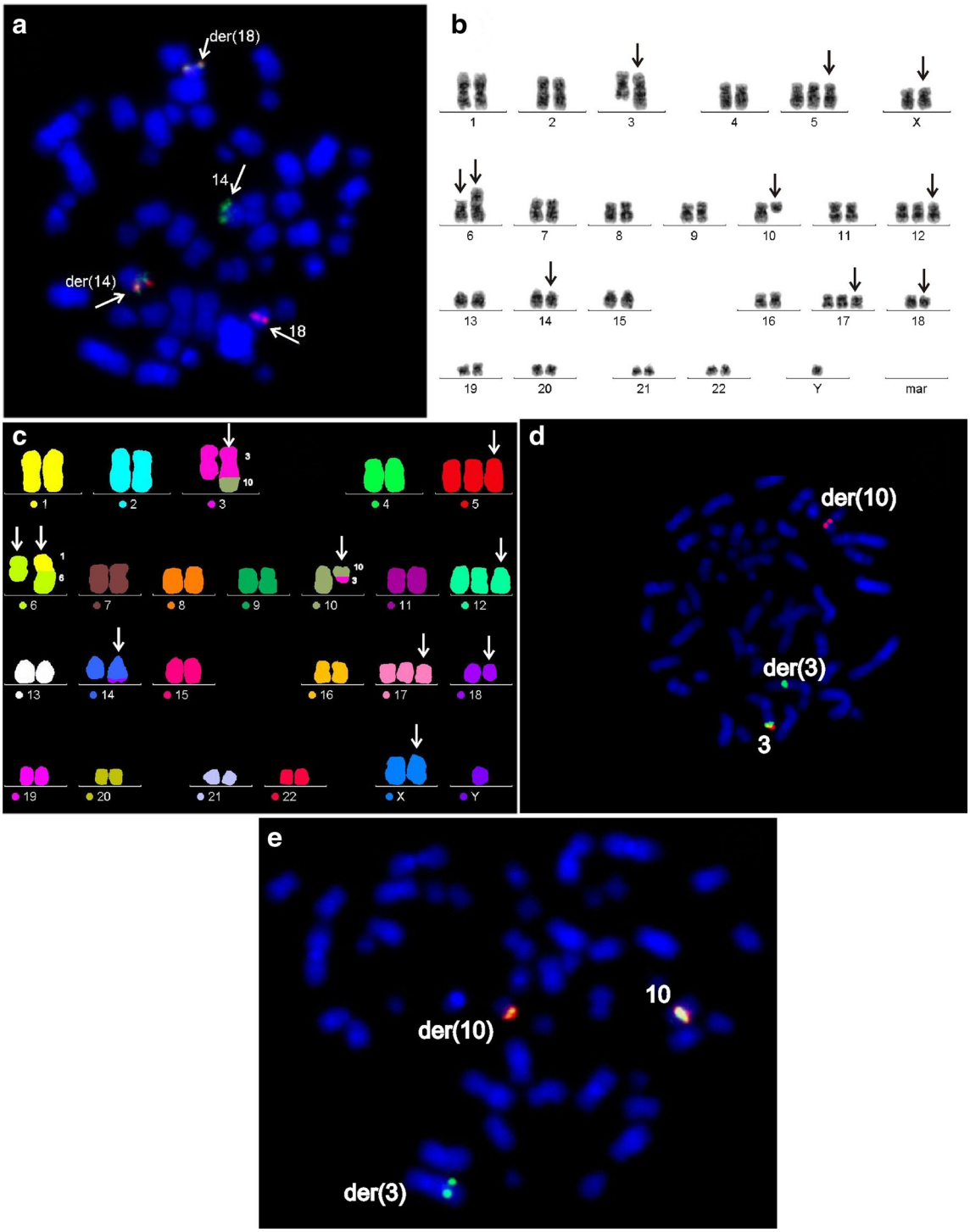

Fig. 2 Cytogenetic and molecular cytogenetic analyses of tumour tissue of our DLBCL patient. **a** FISH result with LSI BCL2/IgH Dual Colour, Dual Fusion Translocation probe (Abbott Molecular) confirming t(14;18) with fusions on der(14) and der(18). **b** Karyogram (G-banding technique) documenting complex chromosome aberrations detected in one of the three malignant clones (changes are indicated by *arrows*). **c** mFISH complex karyotype (changes are indicated by *arrows*). **d** FISH with LSI *BCL6* Dual Colour Break Apart probe (Abbott Molecular) confirming a break of *BCL6* gene and location the 5′ untranslated region of *BCL6* gene (*red signal*) on derivative chromosome 10. **e** FISH with the *RET* (10q11) Dual Colour Break Probe (Kreatech) confirming the break and location of a green/red signal *RET* on derivative chromosome 10 and larger green signal of probe on derivative chromosome 3

Quantitative PCR was performed using TaqMan probes (*BCL2:* Hs00608023_m1, *BCL6*: Hs00153368_m1, Life Technologies; *RASGEF1A:* Hs.PT.58.3776983, *PGK1:* Hs.PT.39a.1406561, Integrated DNA Technologies) on

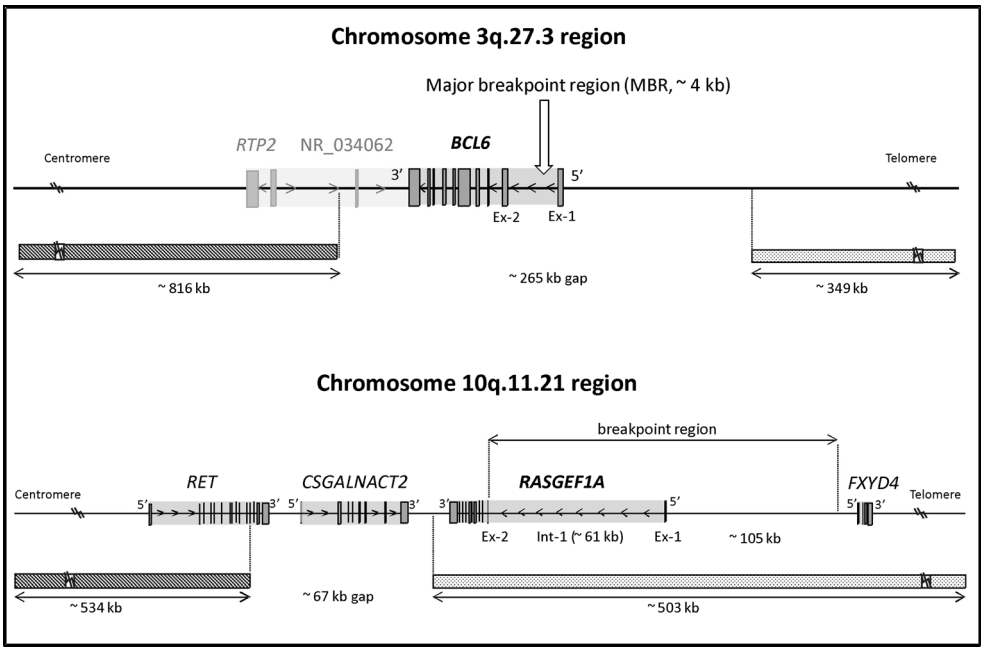

Fig. 3 Diagram of positions of FISH break-apart probes for *BCL6* rearrangements at chromosome 3q27 and those for chromosome 10q11.21 loci

Rotor-GeneQ (QIAGEN Inc., Valencia, CA) as described elsewhere [62]; a second-derivative method was used for calculations (RotorGene Software 6.1.81, QIAGEN Inc.); *PGK1* as a reference gene [63]. Data analysis was performed using GraphPad Prism 5.01 (GraphPad Software, La Jolla, CA, USA).

Our qRT-PCR analysis revealed that novel *non-Ig*/BCL6 translocation involving chromosome 10q11.21 loci in our DLBCL patient was associated with low mRNA expression of *BCL6* and *RASGEF1A* genes comparing to i) DLBCL patient with t(3;22), ii) patients with DLBCL without *BCL6* rearrangements and iii) healthy control subjects (Fig. 4). Additionally, we confirmed upregulated gene expression of *BCL2* in our DLBCL patient carrying also *BCL2/IgH* rearrangement.

Role of *BCL6* in Normal Cells and in B-Cell Lymphomagenesis

To gain deeper knowledge about the consequences of *BCL6* gene rearrangements, we need to clarify the role of *BCL6* in normal cells and in B-cell lymphomagenesis. A direct target of *BCL6* in germinal centre (GC) B-cells is the anti-apoptotic proto-oncogene *BCL2*, thus an important function of *BCL6* in facilitating apoptosis of GC B-cells is the suppression of *BCL2* [64]. Further, *BCL6* controls BCR signalling through repression of the SYK phosphatase, PTPROt; thus, being involved in a *BCL6*-dependent pro-survival pathway in B-cell lymphomagenesis [65]. *BCL6* is also required for GC B-cells to proliferate and tolerate the DNA damage that occurs as a by-product during *Ig* affinity maturation [66]. *BCL6* mediates these effects by direct binding and repressing the replication checkpoint and DNA damage sensor encoding gene *ATR,* as well as the key checkpoint genes *CHEK1, TP53, CDKN1A, CDKN2A* and *p14ARF* [67–70]. Next, downregulation of *BCL6* is a prerequisite for GC B-cells to mature toward plasma cells, because *BCL6* is a repressor of *PRDM1*, a master regulator of plasma cell differentiation [67, 71]. Nevertheless, *BCL6* contains the negative regulatory regions within the first exon and intron of the *BCL6* gene and can, therefore, autoregulate its own expression [72]. However, the exact role of *BCL6* in DLBCL pathogenesis is still limited because only few genes have been functionally characterized as direct targets of *BCL6* transrepression activity.

BCL6 Gene Rearrangements in DLBCL

BCL6 gene rearrangements belong to the most common aberrations observed in DLBCL [3–7, 73]. Most frequently, the *BCL6* gene is frequently involved in reciprocal translocations with *IgH* gene and rarely also with *IgL* and *IgK* genes [11–13]. Additionally, more than 20 *non-Ig* fusion partners have been identified in *non-Ig*/*BCL6* fusions to date. According to the published data, the most frequent partner chromosomes involved in *non-Ig*/*BCL6* translocations in DLBCL are chromosomes 6 and 12; however, almost all chromosomes were identified in *non-Ig*/*BCL6* fusions, except chromosomes 5, 15, 17, 20, 21, X and Y. Regarding the involved genes, the majority of *non-Ig* fusion genes are involved in cell cycle control and genome stability [3]. In all yet reported *BCL6* translocations, normal regulatory sequences of *BCL6* are replaced with those of the partner gene, because the translocated gene partners are in the same transcriptional direction [18]. This process, termed "promoter substitution", is hypothesized to deregulate *BCL6* expression; thus, the changes in *BCL6* together with fusion partner gene

Table 1 Fusion partners in *non-Ig/BCL6* rearrangements reported in DLBCL

Translocation	Chromosomal locus partners	Gene partner	References
t(1;3)	(p34;q27)		[20, 21]
	(q32;q27)		[22]
	(q25;q27)	*GAS5*	[21, 23]
t(2;3)	(q21;q27)		[24]
	(p12;q27)		[25, 26]
	(q33;q27)		[27]
	(p11;q27)		[18, 28]
	(p23;q27)	*ALK*	[29]
t(3;3)	(q11;q27)		[30]
	(q25;q27)	*MBNL1*	[31]
	(q29;q27)	*TFRC*	[13]
	(q27-q28;q27)	*ST6GAL1*	[31]
	(q28;q27)	*EIF4A2*	[12]
t(3;4)	(q27;q25)		[32]
	(q27;q32)		[33]
	(q27;p13)	*RHOH*	[34, 35]
t(3;6)	(q27;p25)		[36]
	(q27;p21.2)	*PIM1*	[18]
	(q27;p12)	*HSPCB*	[37]
	(q27;q15)	*SNHG5*	[38]
	(q27;q21)	*SFRS3*	[39]
	(q27;p21.33)	*HIST1H4I*	[40, 41]
t(3;7)	(q27;p12)	*IKZF1*	[42, 43]
	(q27;q13)	*ZNFN1A1*	[13, 44]
	(q27;q21)		[45]
	(q27;q32)	*FRA7H*	[46]
t(3;8)	(q27;q24)	*MYC*	[47–50]
t(3;9)	(q27;p13)		[33]
	(q27;q12)	*GRHPR*	[31]
t(3;10)	(q27;q22)		[51]
	(q27;q11.21)	*RASGEF1A*	this study
t(3;11)	(q27;p14)		[52]
	(q27;q23.1)	*POU2AF1*	[53]
t(3;12)	(q27;q24)		[54]
	(q27;q13)		[28]
	(q27;p11)		[28]
	(q27;p13.31)	*GAPDH*	[55]
	(q27;q23)	*NACA*	[13]
	(q27;p12.1)	*LRMP*	[31]
t(3;13)	(q27;q14.3)	*LCP1*	[56]
t(3;14)	(q27;q32.33)	*HSPCA*	[13]
t(3;16)	(q27;p13)	*CIITA*	[28, 57, 58]
	(q27;p11)	*IL21R*	[59]
t(3;18)	(q27;q21)		[50, 60]
t(3;19)	(q27;q13)	*NAPA*	[61]

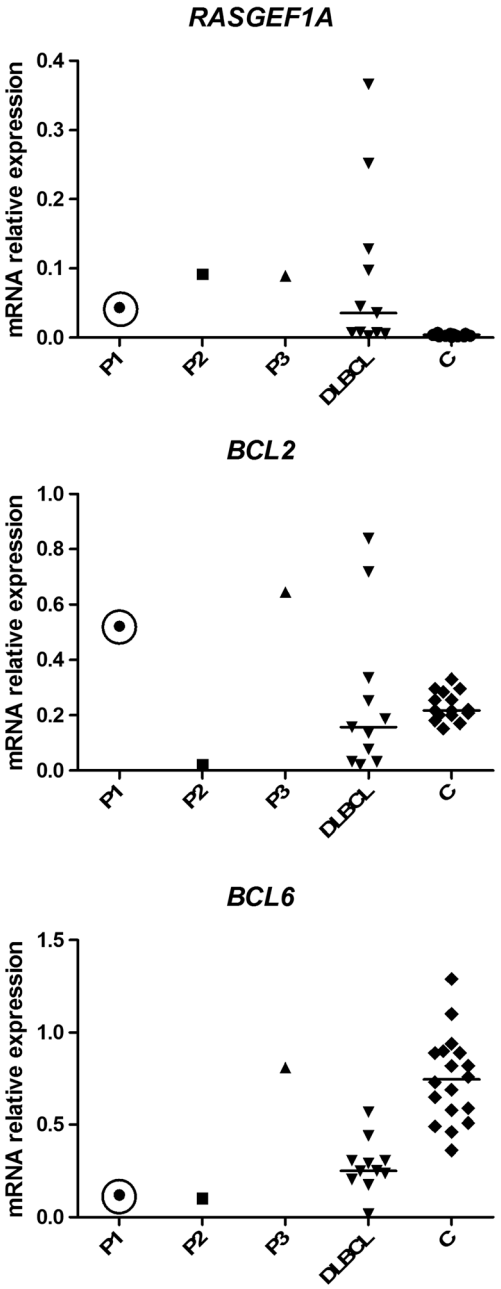

Fig. 4 Relative mRNA expression of *BCL2*, *BCL6*, and *RASGEF1A* (ration target gene expression/reference gene *PGK1*) in lymph node from DLBCL patients 1) with *BCL6* rearrangements (reported case P1 with t(3;10); P2 with t(3;14); P3 with t(3;22)) and 2) without *BCL6* rearrangements (DLBCL, $n=11$); and in the peripheral blood from 3) healthy control subjects (C, $n=18$)

expression may influence the biology of DLBCL [15]. Nevertheless, although the *BCL6* rearrangements are predominantly associated with DLBCL, this genetic aberration may be present also in other types of lymphoma such as follicular [74] and mantle cell lymphomas [75].

In this study we included also a description of a novel *non-Ig/BCL6* translocation involving chromosome 10q11.21 loci in a refractory posttransplant DLBCL patient. According to the FISH results, the 5′ region of *BCL6* on chromosome 3 was

replaced by 5′ non-coding region of the *RASGEF1A* gene from the chromosome loci 10q11.21, both fusion genes oriented in the same transcriptional direction. *RASGEF1A* (RasGEF domain family, member 1A) encodes a putative Ras guanine nucleotide exchange factor domain-containing protein [76]. The animal studies showed that *RASGEF1A,* an evolutionary conserved gene, is paternally imprinted in mice and regulates longevity as well as embryogenesis [77]. Besides delays in aging, *RASGEF1A* deficient mice possessed enhanced tumor free survival, and protection against oxidative stress [78]. In humans is *RASGEF1A* probably not imprinted and serves as an *in vivo* activator for *H-RAS* and members of the *R-RAS* and *RAC* subfamilies [79], regulator of glucose metabolism and insulin - insulin like growth factor signaling [80] and/or is involved in cell-cell adhesion [76, 81]. Moreover, *RASGEF1A* regulates proliferation and metastatic behavior of human alveolar rhabdomyosarcomas [82]. In our DLBCL cohort, we observed high interindividual variability in gene expression of *RASGEF1A*, whereas our case with t(3;10) belonged to those with lower *RASGEF1A* expression. The exact role of this gene in DLBCL should be further explored in future studies. This is the first observation of t(3;10)(q27;q11.21) in DLBCL, the only single case of t(3;10) reported in DLBCL involved other loci (q27;q22) [51]. Reciprocal translocation t(3;10)(q27;q11.21) was already reported in follicular lymphoma; however, no link to patient outcome and break-point region was given [18].

To obtain more insights into the break-point region in *BCL6* rearrangements, Lu et al. [83] analyzed a large set of *BCL6* chromosomal translocations to *Ig* as well as non-*Ig* partners and showed that *BCL6* breaks occur at different regions depending on the fusion partner. The *BCL6* breaks in *non-Ig* rearrangements occur at CpG/CGC sites in addition to WGCW and the *Ig*/*BCL6* translocations prefer known activation-induced cytosine deaminase (AID) hotspots, such as WGCW and WRC (W=A/T, R=A/G) [83]. Interestingly, there are many similarities between *BCL6* and *IGVH* somatic mutations, suggesting that the *Ig*-somatic mutation machinery also targets *BCL6*, thereby predisposing to chromosomal breaks and subsequent translocations [1]. Similar mechanism is reported also in *BCL2*, where *BCL2* mutations commonly occur in DLBCL patients with *BCL2*/*IgH* rearrangements as a result of somatic hypermutation normally occurring at the *IgH* locus [84]. The theory of the driving role of somatic mutations in *non-Ig* genes for *non-Ig*/*BCL6* fusions is supported by common observation of these translocations in treated patients, as was shown also in our case. Further research in this direction is needed.

Clinical Consequences of *BCL6* Translocations in DLBCL

Although there is no consensus on the effect of *BCL6* translocation on the clinical outcome, studies on *Ig/BCL6* rearrangements commonly point to the fact that high *BCL6* expression at mRNA and/or protein levels is a favourable prognostic indicator of DLBCL [3, 85, 86]. One may suggest that this may be through the repressory control of anti-apoptotic *BCL2* and other known *BCL6* pro-survival target genes. On the other hand, enhanced *BCL6* levels may perturb the molecular network that controls the differentiation of GC B-cells to *Ig*-secreting plasma cells, thereby predisposing B-cells to neoplastic transformation [67]. Contrary, in *non-Ig*/*BCL6* translocations were *BCL6* mRNA levels unexpectedly low and inferior survival in DLBCL patients with *non-Ig*/*BCL6* translocations comparing to those with *Ig*/*BCL6* fusions was reported [3, 7, 87]. In line with observations in other *non-Ig/BCL6* translocations, the t(3;10) translocation in our case was associated with low mRNA expression of the *BCL6* as well as its fusion partner *RASGEF1A*.

Lymphomas with recurrent chromosomal breakpoints activating multiple oncogenes, including *BCL6* and/or *BCL2* and/or *MYC* are often referred as “Dual Hit” lymphomas (DHL). DHL are highly aggressive lymphomas with generally poor response to first line and salvage treatment. Limited data is available to guide therapeutic decisions, and despite aggressive measures including high dose chemotherapy followed by ASCT, outcome is sadly poor [17, 20, 88]. Similarly, our patient with *non-Ig*/*BCL6* fusion with low gene expression of *BCL6* and combination with rearrangement of *BCL2,* a repressional target of *BCL6*, belonged to the high-risk category with poor prognosis. He relapsed several times after aggressive treatment; thus, further supporting the suggestion that *non-Ig/BCL6* fusion and its combination with high expression of *BCL2* are poor prognostic indicators of DLBCL. In line with the observation of others [4], we suggest that there is potential value for *BCL6* rearrangement and together with the activation of *BCL2* as adverse predictive biomarkers in DLBCL also in the rituximab era.

Conclusions

Reports on genetic variability and rearrangements involving *BCL6*, a gene most commonly affected in DLBCL, and the clinical consequence of these genetic changes may contribute to the understanding of the role of *BCL6* in etiopathogenesis of DLBCL and also open new therapeutic options for DLBCL patients.

Acknowledgments This work was supported by the grant of the Ministry of Health of the Czech Republic IGA MZ CR NT11103.

Compliance with Ethical Standards The authors declare that they have no conflict of interest. Written informed consent was duly obtained from the patient for the publication of this report and its accompanying images. All examinations were performed in accordance with the current

version of the Helsinki Declaration and the study was approved by the Ethics Committee of the University Hospital in Olomouc.

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in the study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

1. Zhang J, Grubor V, Love CL, Banerjee A, Richards KL, Mieczkowski PA, Dunphy C, Choi W, Au WY, Srivastava G, Lugar PL, Rizzieri DA, Lagoo AS, Bernal-Mizrachi L, Mann KP, Flowers C, Naresh K, Evens A, Gordon LI, Czader M, Gill JI, Hsi ED, Liu Q, Fan A, Walsh K, Jima D, Smith LL, Johnson AJ, Byrd JC, Luftig MA, Ni T, Zhu J, Chadburn A, Levy S, Dunson D, Dave SS (2013) Genetic heterogeneity of diffuse large B-cell lymphoma. Proc Natl Acad Sci U S A 110(4):1398–1403
2. Tirado CA, Chen W, García R, Kohlman KA, Rao N (2012) Genomic profiling using array comparative genomic hybridization define distinct subtypes of diffuse large B-cell lymphoma: a review of the literature. J Hematol Oncol 5:54
3. Ohno H (2006) Pathogenetic and clinical implications of non-immunoglobulin; BCL6 translocations in B-cell non-Hodgkin's lymphoma. J Clin Exp Hematop 46(2):43–53
4. Shustik J, Han G, Farinha P, Johnson NA, Ben Neriah S, Connors JM, Sehn LH, Horsman DE, Gascoyne RD, Steidl C (2010) Correlations between BCL6 rearrangement and outcome in patients with diffuse large B-cell lymphoma treated with CHOP or R-CHOP. Haematologica 95(1):96–101
5. Flodr P, Látalová P, Tichý T, Kubová Z, Papajík T, Šváchová M, Vrzalíková K, Radová L, Jarošová M, Murray PG (2014) Diffuse large B-cell lymphoma—the history, current view and new perspectives. Neoplasma 61(5):491–504
6. Nedomova R, Papajik T, Procházka V, Indrak K, Jarosova M (2013) Cytogenetics and molecular cytogenetics in diffuse large B-cell lymphoma (DLBCL). Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 157(3):239–247
7. Lossos IS, Czerwinski DK, Alizadeh AA, Wachser MA, Tibshirani R, Botstein D, Levy R (2004) Prediction of survival in diffuse large-B-cell lymphoma based on the expression of six genes. N Engl J Med 350(18):1828–1837
8. Chang CC, Ye BH, Chaganti RS, Dalla-Favera R (1996) BCL-6, a POZ/zinc-finger protein, is a sequence-specific transcriptional repressor. Proc Natl Acad Sci U S A 93(14):6947–6952
9. Barros P, Lam EW, Jordan P, Matos P (2012) Rac1 signalling modulates a STAT5/BCL-6 transcriptional switch on cell-cycle-associated target gene promoters. Nucleic Acids Res 40(16): 7776–7787
10. Siggs OM, Beutler B (2012) The BTB-ZF transcription factors. Cell Cycle 11(18):3358–3369
11. Ueda Y, Nishida K, Miki T, Horiike S, Kaneko H, Yokota S, Misawa S, Abe T, Kashima K, Taniwaki M (1997) Interphase detection of BCL6/IgH fusion gene in non-Hodgkin lymphoma by fluorescence in situ hybridization. Cancer Genet Cytogenet 99(2): 102–107
12. Yoshida S, Kaneita Y, Aoki Y, Seto M, Mori S, Moriyama M (1999) Identification of heterologous translocation partner genes fused to the BCL6 gene in diffuse large B-cell lymphomas: 5′-RACE and LA - PCR analyses of biopsy samples. Oncogene 18(56):7994–7999
13. Akasaka H, Akasaka T, Kurata M, Ueda C, Shimizu A, Uchiyama T, Ohno H (2000) Molecular anatomy of BCL6 translocations revealed by long-distance polymerase chain reaction-based assays. Cancer Res 60(9):2335–2341
14. Jardin F, Ruminy P, Bastard C, Tilly H (2007) The BCL6 proto-oncogene: a leading role during germinal center development and lymphomagenesis. Pathol Biol 55(1):73–83
15. Iqbal J, Greiner TC, Patel K, Dave BJ, Smith L, Ji J, Wright G, Sanger WG, Pickering DL, Jain S, Horsman DE, Shen Y, Fu K, Weisenburger DD, Hans CP, Campo E, Gascoyne RD, Rosenwald A, Jaffe ES, Delabie J, Rimsza L, Ott G, Müller-Hermelink HK, Connors JM, Vose JM, McKeithan T, Staudt LM, Chan WC (2007) Distinctive patterns of BCL6 molecular alterations and their functional consequences in different subgroups of diffuse large B-cell lymphoma. Leukemia 21(11):2332–2343
16. Barrans SL, O'Connor SJ, Evans PA, Davies FE, Owen RG, Haynes AP, Morgan GJ, Jack AS (2002) Rearrangement of the BCL6 locus at 3q27 is an independent poor prognostic factor in nodal diffuse large B-cell lymphoma. Br J Haematol 117(2):322–332
17. Akasaka T, Ueda C, Kurata M, Akasaka H, Yamabe H, Uchiyama T, Ohno H (2000) Nonimmunoglobulin (non-Ig)/BCL6 gene fusion in diffuse large B-cell lymphoma results in worse prognosis than Ig/BCL6. Blood 96(8):2907–2909
18. Keller CE, Nandula S, Vakiani E, Alobeid B, Murty VV, Bhagat G (2006) Intrachromosomal rearrangement of chromosome 3q27: an under recognized mechanism of BCL6 translocation in B-cell non-Hodgkin lymphoma. Hum Pathol 37(8):1093–1099
19. Procházka V, Papajík T, Faber E, Raida L, Kapitáňová Z, Langová K, Prouzová Z, Jarošová M, Indrák K (2014) Soluble interleukin-2 receptor level predicts survival in patients with follicular lymphoma treated with CHOP chemotherapy in the rituximab era. Leuk Lymphoma 55(7):1584–1590
20. Niitsu N, Nakamine H, Hagiwara Y, Tanae K, Miura I (2008) The t(1;3)(p34;q27) translocation: a nonrandom BCL6 rearrangement in diffuse large B cell lymphoma. Ann Hematol 87(2):151–153
21. Nakamura Y, Takahashi N, Kakegawa E, Yoshida K, Ito Y, Kayano H, Niitsu N, Jinnai I, Bessho M (2008) The GAS5 (growth arrest-specific transcript 5) gene fuses to BCL6 as a result of t(1;3)(q25;q27) in a patient with B-cell lymphoma. Cancer Genet Cytogenet 182(2):144–149
22. Bloomfield CD, Arthur DC, Frizzera G, Levine EG, Peterson BA, Gajl-Peczalska KJ (1983) Nonrandom chromosome abnormalities in lymphoma. Cancer Res 43(6):2975–2984
23. Chenevix-Trench G, Brown JA, Tyler GB, Behm FG (1988) Chromosome analysis of 30 cases of non-Hodgkin's lymphoma. Med Oncol Tumor Pharmacother 5(1):17–32
24. Martín-Subero JI, Odero MD, Hernandez R, Cigudosa JC, Agirre X, Saez B, Sanz-García E, Ardanaz MT, Novo FJ, Gascoyne RD, Calasanz MJ, Siebert R (2005) Amplification of IGH/MYC fusion in clinically aggressive IGH/BCL2-positive germinal center B-cell lymphomas. Genes Chromosomes Cancer 43(4):414–423
25. Jerkeman M, Johansson B, Akerman M, Cavallin-Ståhl E, Kristoffersson U, Mitelman F (1999) Prognostic implications of cytogenetic aberrations in diffuse large B-cell lymphomas. Eur J Haematol 62(3):184–190
26. Utsumi S, Miura I, Takahashi N, Ohshima A, Nimura T, Hashimoto K, Chubachi A, Saito M, Takatsu H, Yamaguchi A et al (1995) Bone and brain involvement in a case of diffuse large B cell lymphoma associated with t(2;3)(p12;q27). Int J Hematol 62(4):247–252

27. Dave BJ, Weisenburger DD, Higgins CM, Pickering DL, Hess MM, Chan WC, Sanger WG (2004) Cytogenetics and fluorescence in situ hybridization studies of diffuse large B-cell lymphoma in children and young adults. Cancer Genet Cytogenet 153(2):115–121
28. Sanchez-Izquierdo D, Siebert R, Harder L, Marugan I, Gozzetti A, Price HP, Gesk S, Hernandez-Rivas JM, Benet I, Solé F, Sonoki T, Le Beau MM, Schlegelberger B, Dyer MJ, Garcia-Conde J, Martinez-Climent JA (2001) Detection of translocations affecting the BCL6 locus in B cell non-Hodgkin's lymphoma by interphase fluorescence in situ hybridization. Leukemia 15(9):1475–1484
29. Van Roosbroeck K, Cools J, Dierickx D, Thomas J, Vandenberghe P, Stul M, Delabie J, De Wolf-Peeters C, Marynen P, Wlodarska I (2010) ALK-positive large B-cell lymphomas with cryptic SEC31A-ALK and NPM1-ALK fusions. Haematologica 95(3): 509–513
30. Yoshioka T, Miura I, Kume M, Takahashi N, Okamoto M, Ichinohasama R, Yoshino T, Yamaguchi M, Hirokawa M, Sawada K, Nakamura S (2005) Cytogenetic features of de novo CD5-positive diffuse large B-cell lymphoma: chromosome aberrations affecting 8p21 and 11q13 constitute major subgroups with different overall survival. Genes Chromosomes Cancer 42(2): 149–157
31. Akasaka T, Lossos IS, Levy R (2003) BCL6 gene translocation in follicular lymphoma: a harbinger of eventual transformation to diffuse aggressive lymphoma. Blood 102(4):1443–1448
32. Hashimoto K, Miura I, Chyubachi A, Saito M, Miura AB (1995) Correlations of chromosome abnormalities with histologic and immunologic characteristics in 49 patients from Akita, Japan with non-Hodgkin lymphoma. Cancer Genet Cytogenet 81(1):56–65
33. Wlodarska I, Stul M, De Wolf-Peeters C, Hagemeijer A (2004) Heterogeneity of BCL6 rearrangements in nodular lymphocyte predominant Hodgkin's lymphoma. Haematologica 89(8):965–972
34. Pasqualucci L, Neumeister P, Goossens T, Nanjangud G, Chaganti RS, Küppers R, Dalla-Favera R (2001) Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. Nature 412(6844):341–346
35. Dallery E, Galiègue-Zouitina S, Collyn-d'Hooghe M, Quief S, Denis C, Hildebrand MP, Lantoine D, Deweindt C, Tilly H, Bastard C et al (1995) TTF, a gene encoding a novel small G protein, fuses to the lymphoma-associated LAZ3 gene by t(3;4) chromosomal translocation. Oncogene 10(11):2171–2178
36. Chaganti SR, Rao PH, Chen W, Dyomin V, Jhanwar SC, Parsa NZ, Dalla-Favera R, Chaganti RS (1998) Deregulation of BCL6 in non-Hodgkin lymphoma by insertion of IGH sequences in complex translocations involving band 3q27. Genes Chromosomes Cancer 23(4):328–336
37. Yeh YM, Chang KC, Chen YP, Kao LY, Tsai HP, Ho CL, Wang JR, Jones D, Chen TY (2010) Large B cell lymphoma presenting initially in bone marrow, liver and spleen: an aggressive entity associated frequently with haemophagocytic syndrome. Histopathology 57(6):785–795
38. Tanaka R, Satoh H, Moriyama M, Satoh K, Morishita Y, Yoshida S, Watanabe T, Nakamura Y, Mori S (2000) Intronic U50 small-nucleolar-RNA (snoRNA) host gene of no protein-coding potential is mapped at the chromosome breakpoint t(3;6)(q27;q15) of human B-cell lymphoma. Genes Cells 5(4):277–287
39. Chen W, Itoyama T, Chaganti RS (2001) Splicing factor SRP20 is a novel partner of BCL6 in a t(3;6)(q27;p21) translocation in transformed follicular lymphoma. Genes Chromosomes Cancer 32(3): 281–284
40. Kurata M, Maesako Y, Ueda C, Nishikori M, Akasaka T, Uchiyama T, Ohno H (2002) Characterization of t(3;6)(q27;p21) breakpoints in B-cell non-Hodgkin's lymphoma and construction of the histone H4/BCL6 fusion gene, leading to altered expression of Bcl-6. Cancer Res 62(21):6224–6230
41. Akasaka T, Miura I, Takahashi N, Akasaka H, Yonetani N, Ohno H, Fukuhara S, Okuma M (1997) A recurring translocation, t(3;6)(q27;p21), in non-Hodgkin's lymphoma results in replacement of the 5′ regulatory region of BCL6 with a novel H4 histone gene. Cancer Res 57(1):7–12
42. Katsura Y, Ohta I, Yoshida C, Ohtani H, Komeno T (2011) Diffuse large B-cell lymphoma carrying both t(3;7)(q27;p12) and t(8;14)(q24;q32). Intern Med 50(8):905–908
43. Ichinohasama R, Miura I, Funato T, Sato I, Suzuki C, Saito Y, Decoteau JF, Myers JB, Kadin ME, Sawai T, Ooya K (1998) A recurrent nonrandom translocation (3;7)(q27;p12) associated with BCL-6 gene rearrangement in B-cell diffuse large cell lymphoma. Cancer Genet Cytogenet 104(1):19–27
44. Hosokawa Y, Maeda Y, Ichinohasama R, Miura I, Taniwaki M, Seto M (2000) The Ikaros gene, a central regulator of lymphoid differentiation, fuses to the BCL6 gene as a result of t(3;7)(q27;p12) translocation in a patient with diffuse large B-cell lymphoma. Blood 95(8):2719–2721
45. Kumari P, Mukherjee G, Rao CR, Devi MG, Biswas S, Appaji L, Arunakumari BS, Padma M, Bapsy PP, Sundareshan TS (2003) Cytogenetic study of non-Hodgkin lymphoma from South India. histologic and geographic correlations. Cancer Genet Cytogenet 141(1):14–19
46. Schneider B, Nagel S, Kaufmann M, Winkelmann S, Bode J, Drexler HG, MacLeod RA (2008) T(3;7)(q27;q32) fuses BCL6 to a non-coding region at FRA7H near miR-29. Leukemia 22(6): 1262–1266
47. Bertrand P, Bastard C, Maingonnat C, Jardin F, Maisonneuve C, Courel MN, Ruminy P, Picquenot JM, Tilly H (2007) Mapping of MYC breakpoints in 8q24 rearrangements involving non-immunoglobulin partners in B-cell lymphomas. Leukemia 21(3): 515–523
48. Johnson NA, Savage KJ, Ludkovski O, Ben-Neriah S, Woods R, Steidl C, Dyer MJ, Siebert R, Kuruvilla J, Klasa R, Connors JM, Gascoyne RD, Horsman DE (2009) Lymphomas with concurrent BCL2 and MYC translocations: the critical factors associated with survival. Blood 114(8):2273–2279
49. Wang HY, Bossler AD, Schaffer A, Tomczak E, DiPatri D, Frank DM, Nowell PC, Bagg A (2007) A novel t(3;8)(q27;q24.1) simultaneously involving both the BCL6 and MYC genes in a diffuse large B-cell lymphoma. Cancer Genet Cytogenet 172(1):45–53
50. Bacher U, Haferlach T, Alpermann T, Kern W, Schnittger S, Haferlach C (2011) Several lymphoma-specific genetic events in parallel can be found in mature B-cell neoplasms. Genes Chromosomes Cancer 50(1):43–50
51. Nordgren A, Sørensen AG, Tinggaard-Pedersen N, Blennow E, Larsson C, Lagercrantz S (2000) New chromosomal breakpoints in non-Hodgkin's lymphomas revealed by spectral karyotyping and G-banding. Int J Mol Med 5(5):485–492
52. Iqbal J, Gupta S, Chen QH, Brody JP, Koduru P (2007) Diffuse large B-cell lymphoma with a novel translocation involving BCL6. Cancer Genet Cytogenet 178(1):73–76
53. Galiéque Zouitina S, Quief S, Hildebrand MP, Denis C, Lecocq G, Collyn-d'Hooghe M, Bastard C, Yuille M, Dyer MJ, Kerckaert JP (1996) The B cell transcriptional coactivator BOB1/OBF1 gene fuses to the LAZ3/BCL6 gene by t(3;11)(q27;q23.1) chromosomal translocation in a B cell leukemia line (Karpas 231). Leukemia 10(4):579–587
54. Mikraki V, Jhanwar SC, Filippa DA, Wollner N, Chaganti RS (1992) Distinct patterns of chromosome abnormalities characterize childhood non-Hodgkin's lymphoma. Br J Haematol 80(1):15–20
55. Montesinos-Rongen M, Akasaka T, Zühlke-Jenisch R, Schaller C, Van Roost D, Wiestler OD, Siebert R, Deckert M (2003) Molecular characterization of BCL6 breakpoints in primary diffuse large B-cell lymphomas of the central nervous system identifies GAPD as novel translocation partner. Brain Pathol 13(4):534–538

56. Laï JL, Daudignon A, Kerckaert JP, Galiègue-Zouitina S, Detourmignies L, Morel P, Bauters F, Fenaux P (1998) Translocation (3;13)(q27;q14): a nonrandom and probably secondary structural change in non-Hodgkin lymphomas. Cancer Genet Cytogenet 103(2):140–143
57. Johnson NA, Al-Tourah A, Brown CJ, Connors JM, Gascoyne RD, Horsman DE (2008) Prognostic significance of secondary cytogenetic alterations in follicular lymphomas. Genes Chromosomes Cancer 47(12):1038–1048
58. Au WY, Gascoyne RD, Viswanatha DS, Skinnider BF, Connors JM, Klasa RJ, Horsman DE (1999) Concurrent chromosomal alterations at 3q27, 8q24 and 18q21 in B-cell lymphomas. Br J Haematol 105(2):437–440
59. Ueda C, Akasaka T, Kurata M, Maesako Y, Nishikori M, Ichinohasama R, Imada K, Uchiyama T, Ohno H (2002) The gene for interleukin-21 receptor is the partner of BCL6 in t(3;16)(q27;p11), which is recurrently observed in diffuse large B-cell lymphoma. Oncogene 21(3):368–376
60. Ichinohasama R, Miura I, Takahashi N, Sugawara T, Tamate E, Endoh K, Endoh F, Naganuma H, DeCoteau JF, Griffin JD, Kadin ME, Ooya K (2000) Ph-negative non-Hodgkin's lymphoma occurring in chronic phase of Ph-positive chronic myelogenous leukemia is defined as a genetically different neoplasm from extramedullary localized blast crisis: report of two cases and review of the literature. Leukemia 14(1):169–182
61. Yunis JJ, Oken MM, Theologides A, Howe RB, Kaplan ME (1984) Recurrent chromosomal defects are found in most patients with non-Hodgkin's-lymphoma. Cancer Genet Cytogenet 13(1):17–28
62. Kriegova E, Arakelyan A, Fillerova R, Zatloukal J, Mrazek F, Navratilova Z, Kolek V, du Bois RM, Petrek M (2008) PSMB2 and RPL32 are suitable denominators to normalize gene expression profiles in bronchoalveolar cells. BMC Mol Biol 9:69
63. Falkenberg VR, Whistler T, Murray JR, Unger ER, Rajeevan MS (2011) Identification of Phosphoglycerate Kinase 1 (PGK1) as a reference gene for quantitative gene expression measurements in human blood RNA. BMC Res Notes 4:324
64. Saito M, Novak U, Piovan E, Basso K, Sumazin P, Schneider C, Crespo M, Shen Q, Bhagat G, Califano A, Chadburn A, Pasqualucci L, Dalla-Favera R (2009) BCL6 suppression of BCL2 via Miz1 and its disruption in diffuse large B cell lymphoma. Proc Natl Acad Sci U S A 106(27):11294–11299
65. Juszczynski P, Chen L, O'Donnell E, Polo JM, Ranuncolo SM, Dalla-Favera R, Melnick A, Shipp MA (2009) BCL6 modulates tonic BCR signaling in diffuse large B-cell lymphomas by repressing the SYK phosphatase, PTPROt. Blood 114(26):5315–5321
66. Basso K, Dalla-Favera R (2012) Roles of BCL6 in normal and transformed germinal center B cells. Immunol Rev Immunol Rev 247(1):172–183
67. Basso K, Saito M, Sumazin P, Margolin AA, Wang K, Lim WK, Kitagawa Y, Schneider C, Alvarez MJ, Califano A, Dalla-Favera R (2010) Integrated biochemical and computational approach identifies BCL6 direct target genes controlling multiple pathways in normal germinal center B cells. Blood 115(5):975–984
68. Ci W, Polo JM, Cerchietti L, Shaknovich R, Wang L, Yang SN, Ye K, Farinha P, Horsman DE, Gascoyne RD, Elemento O, Melnick A (2009) The BCL6 transcriptional program features repression of multiple oncogenes in primary B cells and is deregulated in DLBCL. Blood 113(22):5536–5548
69. Ranuncolo SM, Polo JM, Dierov J, Singer M, Kuo T, Greally J, Green R, Carroll M, Melnick A (2007) Bcl-6 mediates the germinal center B cell phenotype and lymphomagenesis through transcriptional repression of the DNA-damage sensor ATR. Nat Immunol 8(7):705–714
70. Phan RT, Dalla-Favera R (2004) The BCL6 proto-oncogene suppresses p53 expression in germinal-centre B cells. Nature 432(7017):635–639
71. Tunyaplin C, Shaffer AL, Angelin-Duclos CD, Yu X, Staudt LM, Calame KL (2004) Direct repression of prdm1 by Bcl-6 inhibits plasmacytic differentiation. J Immunol 173(2):1158–1165
72. Kikuchi M, Miki T, Kumagai T, Fukuda T, Kamiyama R, Miyasaka N, Hirosawa S (2000) Identification of negative regulatory regions within the first exon and intron of the BCL6 gene. Oncogene 19(42):4941–4945
73. Beà S, Colomo L, López-Guillermo A, Salaverria I, Puig X, Pinyol M, Rives S, Montserrat E, Campo E (2004) Clinicopathologic significance and prognostic value of chromosomal imbalances in diffuse large B-cell lymphomas. J Clin Oncol 22(17):3498–3506
74. Jardin F, Gaulard P, Buchonnet G, Contentin N, Leprêtre S, Lenain P, Stamatoullas A, Picquenot JM, Duval C, Parmentier F, Tilly H, Bastard C (2002) Follicular lymphoma without t(14;18) and with BCL-6 rearrangement: a lymphoma subtype with distinct pathological, molecular and clinical characteristics. Leukemia 16(11):2309–2317
75. Miura I, Ohshima A, Chubachi A, Nimura T, Komatsuda A, Utsumi S, Saito M, Machii T, Nakamura S, Seto M, Miura AB (1997) BCL6 rearrangement in a patient with mantle cell lymphoma. Ann Hematol 74(5):247–250
76. Yaman E, Gasper R, Koerner C, Wittinghofer A, Tazebay UH (2009) RasGEF1A and RasGEF1B are guanine nucleotide exchange factors that discriminate between Rap GTP-binding proteins and mediate Rap2-specific nucleotide exchange. FEBS J 276(16):4607–4616
77. Ratajczak MZ, Kucia M, Liu R, Shin DM, Bryndza E, Masternak MM, Tarnowski M, Ratajczak J, Bartke A (2011) RasGrf1: genomic imprinting, VSELs, and aging. Aging (Albany NY) 3(7):692–697
78. Borrás C, Monleón D, López-Grueso R, Gambini J, Orlando L, Pallardó FV, Santos E, Viña J, Font de Mora J (2011) RasGrf1 deficiency delays aging in mice. Aging (Albany NY) 3(3):262–276
79. Ura K, Obama K, Satoh S, Sakai Y, Nakamura Y, Furukawa Y (2006) Enhanced RASGEF1A expression is involved in the growth and migration of intrahepatic cholangiocarcinoma. Clin Cancer Res 12(22):6611–6616
80. Font de Mora J, Esteban LM, Burks DJ, Nunez A, Garces C, Garcia-Barrado MJ, Iglesias-Osma MC, Moratinos J, Ward JM, Santos E (2003) Ras-GRF1 signaling is required for normal beta-cell development and glucose homeostasis. Embo J 22(12):3039–3049
81. Pannekoek WJ, Linnemann JR, Brouwer PM, Bos JL, Rehmann H (2013) Rap1 and Rap2 antagonistically control endothelial barrier resistance. PLoS One 8(2):e57903
82. Tarnowski M, Schneider G, Amann G, Clark G, Houghton P, Barr FG, Kenner L, Ratajczak MZ, Kucia M (2012) RasGRF1 regulates proliferation and metastatic behavior of human alveolar rhabdomyosarcomas. Int J Oncol 41(3):995–1004
83. Lu Z, Tsai AG, Akasaka T, Ohno H, Jiang Y, Melnick AM, Greisman HA, Lieber MR (2013) BCL6 breaks occur at different AID sequence motifs in Ig-BCL6 and non-Ig-BCL6 rearrangements. Blood 121(22):4551–4554
84. Lohr JG, Stojanov P, Lawrence MS, Auclair D, Chapuy B, Sougnez C, Cruz-Gordillo P, Knoechel B, Asmann YW, Slager SL, Novak AJ, Dogan A, Ansell SM, Link BK, Zou L, Gould J, Saksena G, Stransky N, Rangel-Escareño C, Fernandez-Lopez JC, Hidalgo-Miranda A, Melendez-Zajgla J, Hernández-Lemus E, Schwarz-Cruz y Celis A, Imaz-Rosshandler I, Ojesina AI, Jung J, Pedamallu CS, Lander ES, Habermann TM, Cerhan JR, Shipp MA, Getz G, Golub TR (2012) Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. Proc Natl Acad Sci U S A 109(10):3879–3884

85. Lossos IS, Jones CD, Warnke R, Natkunam Y, Kaizer H, Zehnder JL, Tibshirani R, Levy R (2001) Expression of a single gene, BCL-6, strongly predicts survival in patients with diffuse large B-cell lymphoma. Blood 98(4):945–951
86. Ueda C, Akasaka T, Ohno H (2002) Non-immunoglobulin/BCL6 gene fusion in diffuse large B-cell lymphoma: prognostic implications. Leuk Lymphoma 43(7):1375–1381
87. Ueda C, Uchiyama T, Ohno H (2002) Immunoglobulin (Ig)/BCL6 versus non-Ig/BCL6 gene fusion in diffuse large B-cell lymphoma corresponds to a high- versus low-level expression of BCL6 mRNA. Blood 99(7):2624–2625
88. Cheema FN, Agloria M, Koshy N, Hildebrandt GC (2014) Double hit lymphoma—a case of unusual response after sequential aggressive chemotherapy and review of the literature. Clin Med Insights Case Rep 7:117–121