BIRC3 alterations in chronic and B-cell acute lymphocytic leukemia patients

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Abstract. Deletions within chromosome 11q22-23, are considered among the most common chromosomal aberrations in chronic lymphocytic leukemia (CLL), and are associated with a poor outcome. In addition to the ataxia telangiectasia mutated (*ATM*) gene, the baculoviral IAP repeat-containing 3 (*BIRC3*) gene is also located in the region. *BIRC3* encodes a negative regulator of the non-canonical nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) protein. Disruption of *BIRC3* is known to be restricted to CLL fludarabine-refractory patients. The aim of the present study was to determine the frequency of copy number changes of *BIRC3* and to assess its association with two known predictors of negative CLL outcome, *ATM* and tumor protein 53 (*TP53*) gene deletions. To evaluate the specificity of *BIRC3* alterations to CLL, *BIRC3* copy numbers were assessed in 117 CLL patients in addition to 45 B-cell acute lymphocytic leukemia (B-ALL) patients. A commercially available multiplex ligation dependent probe amplification kit, which includes four probes for the detection of *TP53* and four probes for *ATM* gene region, was applied. Interphase-directed fluorescence *in situ* hybridization was used to apply commercially available probes for *BIRC3*, *ATM* and *TP53*. High resolution array-comparative genomic hybridization was conducted in selected cases. Genetic abnormalities of *BIRC3* were detected in 23/117 (~20%) of CLL and 2/45 (~4%) of B-ALL cases. Overall, 20 patients with CLL and 1 with B-ALL possessed a *BIRC3* deletion, whilst 3 patients with CLL and 1 with B-ALL harbored a *BIRC3* duplication. All patients with an *ATM* deletion also carried a *BIRC3* deletion. Only 2 CLL cases possessed deletions in *BIRC3*, *ATM* and *TP53* simultaneously. Evidently, the deletion or duplication of *BIRC3* may be observed rarely in B-ALL patients. *BIRC3* duplication may occur in CLL patients, for which the prognosis requires additional studies in the future. The likelihood that *TP53* deletions occur simultaneously with *BIRC3* and/or *ATM* aberrations is low. However, as *ATM* deletions may, but not always, associate with *BIRC3* deletions, each region should be considered in the future diagnostics of CLL in order to aid treatment decisions, notably whether to treat with or without fludarabine.

Introduction

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia observed in people aged >50 years in Western countries. CLL is characterized by a heterogeneous clinical course, with a time to progression ranging from months to decades (1). The presence of cytogenetic abnormalities is a hallmark of CLL. The most common recurrent aberrations in CLL affect chromosomes 11q, 13q, 14q, 17p and the whole of chromosome 12. Certain abnormalities, including deletions in 11q22.3, the ataxia telangiectasia mutated (*ATM*) gene (10-20% of all CLL cases), and 17p13.1, the tumor protein 53 (*TP53*) gene (5-10% of all CLL cases), are associated with a poor clinical outcome. Therefore, the detection of these aberrations is important for identifying high-risk patients, who suffer from rapid disease progression and a decreased overall survival time (2). Other frequent chromosomal aberrations in CLL are associated with a good (deletions in 13q14 or 14q32.33) or intermediate (trisomy 12) prognosis (1,3-5).

CLL is considered to be an insidious disease. Certain CLL patients, particularly patients with a good prognosis, survive for several years without requiring treatment; however, another

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subgroup of patients experience an aggressive disease course and have a short life expectancy, despite aggressive treatment (6). The latter group tends to exhibit a particular lack of response to fludarabine-based regimens, which are generally considered to be the first line of treatment for CLL (6). In a large fraction of these patients, the molecular basis of the aggressive clinical course remains unclear; however, in ~40% of patients, the molecular basis is hypothesized to be due to *TP53* disruption (7). In addition, activation of the nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) pathway is considered to be a mechanism of resistance to disease eradication (7).

From a clinical perspective, CLL cases may be divided into three major clinical phases: i) newly diagnosed CLL; ii) progressive CLL; and iii) relapsed or fludarabine-refractory CLL. *TP53* abnormalities are observed in 40-50% of relapsed and fludarabine-refractory CLL cases and the deletion of 11q22-23 occurs in 25-30% of relapsed or fludarabine-refractory CLL patients (8). In a large previous study, 637 patients were classified into four risk groups according to a multivariate analysis of overall survival, which was based on genomic abnormalities and the mutational status of *TP53*, baculoviral IAP repeat-containing 3 (*BIRC3*), translocation-associated notch homolog 1 and splicing factor 3B subunit 1. Notably, the high-risk group was composed of patients that exhibited disruption to *TP53* and/or *BIRC3* (9).

In CLL, deletions within the long arm of chromosome 11 may be highly variable in size. The deletion may be distinguished as the more common 'classical or large deletion' or an 'atypical or small deletion', which is uncommon and more frequently associated with *ATM* mutations. This variation indicates that other genes may contribute to the pathobiology of 11q deletions in CLL, and one of the genes that is hypothesized to be involved is *BIRC3* (10). *BIRC3* disruption, mutations or deletions are rarely detected in CLL at diagnosis (4% of patients), but are detected in 24% of fludarabine-refractory CLL patients. In a previous study, fludarabine-sensitive patients did not exhibit *BIRC3* mutations initially, which suggests that *BIRC3* disruption may be specifically associated with a chemo-refractory CLL subtype (7). Therefore, *BIRC3* disruption may be added to the panel of cytogenetic abnormalities, as it may be helpful in the early identification of relapsed and fludarabine-refractory CLL patients. Affected patients should be considered for other treatment regimens, including cyclin-dependent kinase inhibitor, Bruton's tyrosine-kinase inhibitor, B-cell lymphoma 2 inhibitor or and alemtuzumab/corticosteroids (8,10). *BIRC3* abnormalities provide a molecular rationale for using NF-κB inhibitors, which remain under development (7).

Materials and methods

Patients and sample preparation. The present study included 117 CLL patients, and 45 B-cell acute lymphocytic leukemia (B-ALL) patients that were diagnosed according to standard criteria (11). The samples were obtained with the informed consent from the corresponding patients and according to the institutional Ethical Committee guidelines. For CLL cases, DNA was extracted from lymphocytes using the Gentra® Puregene® Blood kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. For B-ALL cases, DNA was derived from cytogenetically prepared cells, as previously described (2), which were fixed in methanol/acetic acid (dilution, 3:1) (Table I).

Interphase fluorescence in situ hybridization (iFISH) analysis. iFISH analyses were performed as previously described (2), using the following commercially available probes: LSI p53/LSI ATM (in 17p13.1 and 11q22.3), CEP 3 (D3Z1 in 3p11.1-q11.1), CEP 4 (D4Z1 in 4p11-q11), CEP 7 (D7Z1 in 7p11.1-q11.1), CEP 11 (D11Z1 in 11p11.11-q11), CEP 16 (D16Z2 in 16p11.1-q11.1), CEP 17 (D17Z1 in 17p11.1-q11.1) and CEP 18 (D18Z1 in 18p11.1-q11.1), all from Vysis (Abbott GmbH & Company, KG, Wiesbaden, Germany); and ZytoLight® SPEC *BIRC3/MALT1* DualColor Dual Fusion probe (in 11q22.2 and 18q21.32) from ZytoVision GmbH (Bremerhaven, Germany). For each iFISH analysis, 100-200 interphase nuclei were examined per patient and probe.

Multiplex ligation-dependent probe amplification (MLPA) analysis. MLPA was performed using the SALSA MLPA probemix P377-A1 for Hematological Malignancies kit (MRC-Holland, Amsterdam, Netherlands). The P377-A1 probemix kit contains 52 probes for 37 genes. The *TP53* and *ATM* genes were assessed by four probes each; however, probes for the *BIRC3* gene were not included in the kit (2). MLPA was successfully performed on 85/117 CLL samples and 32/45 B-ALL samples. MLPA was not successful for the remaining samples due to fragmentation of DNA.

Array-comparative genomic hybridization (aCGH). aCGH was performed using the Agilent SurePrint G3 Human Genome Microarray 180 K (Agilent Technologies, Santa Clara, CA, USA), as previously described (12). aCGH was applied in a total of 3 CLL patients that carried a *BIRC3* duplication and in 1 B-ALL patient that possessed a *BIRC3* deletion.

Results

Gene copy numbers. *BIRC3* gene copy number variations were detected in 23/117 (~20%) of CLL and 2/45 (~4%) of B-ALL cases, as summarized and detailed in Fig. 1. *BIRC3* deletions were identified in 20 cases of CLL (cases C-1 to C-20) and in 1 case of B-ALL (case A-1). *ATM* deletion was detected in the identical 20 CLL and 1 B-ALL cases. Therefore, all patients with a *BIRC3* deletion also possessed an *ATM* deletion. However, in cases C-1, C-8, C-10, C-13 and C-20 the detected clone sizes with deletions in *BIRC3* and *ATM* were extremely varied from one another. In case C-1, the clone with the *ATM* deletion was 8x smaller compared with that with the *BIRC3* deletion, whereas in cases C-8, C-10, C-13 and C-20, the clone with the *BIRC3* deletion was 2-3x smaller than that with the *ATM* deletion. A *BIRC3* duplication was identified in 1 case of B-ALL (case A-2) and in 3 CLL patients (cases C-21 to C-23) (Table II).

With regard to *TP53* abnormalities, 3 patients with B-ALL possessed a *TP53* deletion in the absence of any aberrations in *BIRC3*. *TP53* deletions were present in 11 CLL patients, 7 of which possessed no associated *BIRC3* aberrations and, notably, 2 of which were accompanied by *BIRC3* and *ATM* amplification.

BIRC3 duplication. In total, 3 CLL patients harbored a *BIRC3* duplication (cases C-21 to C-23), 2 of which (C-21 and C-22) were accompanied by *ATM* and *MALT1* duplications

Table I. Gender, age and cytogenetic results of the B-ALL and CLL cases used in the present study.

Case no.	Gender	Age, years	DNA extracted from	Cytogenetic results
A-1	Male	84	BM	46,XY,-9,t(9;22)(q34;q11),del(11)(q),+mar[cp3]/46,XY[5]
A-2	Male	23	BM	Hyperdiploid/46,XY
A-3	Male	34	BM	46,XY
A-4	Male	19	BM	46,XY
A-5	Female	76	BM	45,X,-X[14]/46,XX[2]
C-1	Male	73	BM	46-47,XY,del(11)(q22q2?3),add(17)t(17;?)(p11.2;?)[cp5]/
				45-46,XY,del(11)(q22q2?3),del(17)(p11.2)[cp4]/
				43-46,XY,del(11)(q22q2?3)[cp2]/
				46,XY[7]
C-2	Female	50	B	n.a.
C-3	Female	39	BM	43-46,XY,del(11)(q2?2q2?4)[cp5]/
				45-46,XY,del(11)(q2?2q2?4),del(15)(q1?1q2?3)[cp11]/
				46,XY[1]
C-4	Male	64	BM	46,XY
C-5	Male	43	BM	46,XY
C-6	Male	67	BM	46,XY
C-7	Male	77	BM	46,XY,del(11)(q?21),add(20)(p13)[7]/
				45,X,-Y[10]/ 46,XY[3]
C-8	Male	53	BM	46,XY
C-9	Male	59	BM	n.a.
C-10	Male	73	BM	45,XY,der(2)t(2;13)(q?37;q?14),?del(6)(p?23),
				del(11)(q?21)der(12)t(12;13)(q?24;q?22),-13[cp4]/
				46,XY[19]
C-11	Male	72	B	n.a.
C-12	Female	73	BM	46,XX,add(11)(q?22)[3]/ 46,XX[12]
C-13	Male	54	B	46,XY
C-14	Male	68	BM	46,XY
C-15	Male	53	BM	46,XY
C-16	Male	75	BM	n.a.
C-17	Female	67	BM	46,XX[18]
				45,X,-X[1]
C-18	Male	74	BM	n.a.
C-19	Male	65	BM	46,XY
C-20	Male	77	B	45-46,XY,del(11)(q?22q?23)[cp14]
				46,XY[5]
C-21	Male	83	BM	47,XY,-11,+12,+mar[cp3]/
				47,XY,del(5)(p1?3),-11,+12,-17,+mar1,+mar2[cp6]/
				46,XY[9]
C-22	Male	72	BM	46,XY
C-23	Male	59	B	46,XY
C-24	Female	66	B	n.a.
C-25	Female	71	B	46,XX
C-26	Male	65	BM	46,XY,?t(3;?)(p21;?),add(17)(p?12)ort(17;?),-8,+mar[cp7]
				46,XY[9]
C-27	Female	74	B	46,XX
C-28	Female	74	BM	46,XX,i(17)(q10)[1]/ 46,XX,+12,i(17)(q10),-21[9]/
				46,XX,t(3;?)(q2?9;?)[4],-7[4],+12[4],i(17)(q10)[4][cp4]/
				46,XX[4]
C-29	Female	90	B	n.a.
C-30	Male	56	BM	n.a.
C-31	Female	65	BM	46,XX

A-, B-ALL case; C-, CLL case; BM, cell pellet in Carnoys fixative from bone marrow; B, blood; n.a., data not available.

Table II. Summary of MLPA and iFISH results of *TP53*, *ATM*, *BIRC3* and *MALT1* in all studied cases.

	TP53 (%)		ATM (%)		BIRC3 (%)	MALT1 (%)
Case no.	MLPA	iFISH	MLPA	iFISH	iFISH	iFISH
A-1	N	N	D	D (76.5)	D (75.0)	N
A-2	n.a.	A (100.0)	n.a.	A (100.0)	A (100.0)	A (100.0)
A-3	D	D (8.5)	N	N	N	N
A-4	D	D (10.0)	N	N	N	N
A-5	D	D (10.0)	N	N	N	N
A-6 to A-33	N	N	N	N	N	N
A-34 to A-45	n.a.	N	n.a.	N	N	N
C-1	D	D (86.0)	D	D (11.0)	D (80.0)	N
C-2	D	D (21.0)	N	D (23.0)	D (22.0)	N
C-3	N	N	D	D (98.0)	D (90.0)	N
C-4	N	N	D	D (23.5)	D (30.0)	N
C-5	N	N	D	D (24.0)	D (25.0)	N
C-6	N	N	D	D (88.0)	D (85.0)	N
C-7	N	N	D	D (90.0)	D (80.0)	N
C-8	N	N	D	D (77.0)	D (50.0)	N
C-9	N	N	D	D (98.0)	D (75.0)	N
C-10	N	N	D	D (87.0)	D (60.0)	N
C-11	N	N	D	D (95.0)	D (90.0)	N
C-12	N	N	D	D (83.0)	D (80.0)	N
C-13	N	N	D	D (93.0)	D (25.0)	N
C-14	N	N	N	D (33.0)	D (15.0)	N
C-15	N	N	N	D (12.0)	D (13.0)	N
C-16	n.a.	N	n.a.	D (80.0)	D (78.0)	N
C-17	n.a.	N	n.a.	D (10.0)	D (9.0)	N
C-18	n.a.	N	n.a.	D (73.0)	D (64.0)	N
C-19	n.a.	N	n.a.	D (9.0)	D (10.0)	N
C-20	n.a.	N	n.a.	D (96.0)	D (42.0)	N
C-21	D	D (16.0)	N	A (50.0)	A (50.0)	A (50.0)
C-22	D	D (40.0)	N	A (40.0)	A (40.0)	A (40.0)
C-23	N	N	N	N	A (36.0)	N
C-24	D	D (19.0)	N	N	N	N
C-25	D	D (36.0)	N	N	N	N
C-26	D	D (89.0)	N	N	N	N
C-27	D	D (77.0)	N	N	N	N
C-28	D	D (95.0)	N	N	N	N
C-29	N	D (11.5)	N	N	N	N
C-30	n.a.	D (86.5)	n.a.	N	N	N
C-31	N	N	N	N	N	A (75.0)
C-32 to C-91	N	N	N	N	N	N
C-92 to C-117	n.a.	N	n.a.	N	N	N

Data are expressed as type of change to copy number (% of cells with aberration). *TP53*, tumor protein 53; *ATM*, ataxia telangiectasia mutated; *BIRC3*, baculoviral IAP repeat-containing 3; *MALT1*, mucosa-associated lymphoid tissue lymphoma translocation gene 1; MLPA, multiplex ligation-dependent probe amplification; iFISH, interphase fluorescence *in situ* hybridization; A-, B-ALL case; C-, CLL case; N, no aberration; D, deletion; A, amplification; n.a., data not available.

in addition to *TP53* deletion. To study these cases in greater depth, iFISH was performed using centrometric probes for chromosomes (CEP) 3, 4, 7, 11, 16 and 18. For these chromosomes, 3 signals were detected in 11% (case C-21) and 25% (case C-22) of the cells, and 4 signals were detected in 29% (case C-21) and 25% (C-22) of the cells, respectively (Fig. 2).

The third CLL case (C-23) with *BIRC3* duplication was associated with normal copy numbers of *TP53* and *ATM*; the
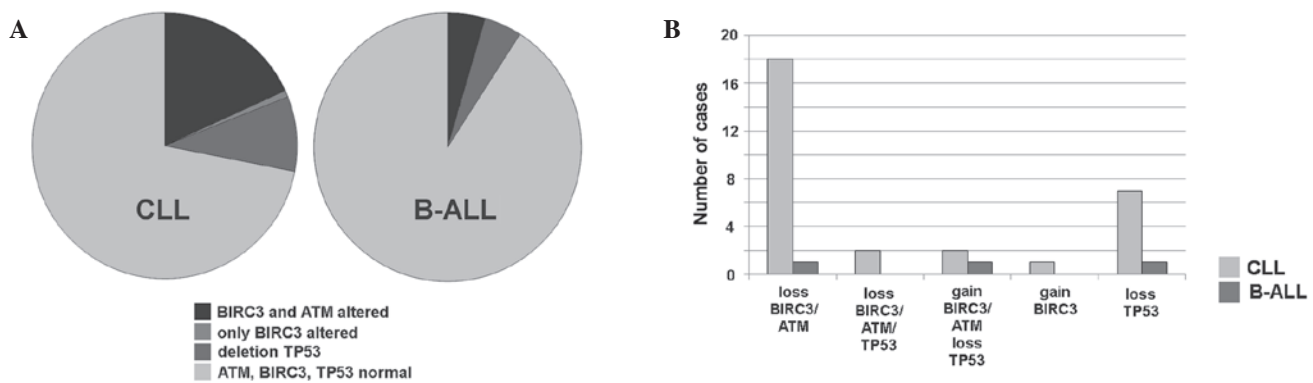
Figure 1. The distribution and of *BIRC3*, *ATM* and *TP53* aberrations in CLL and B-ALL patients are summarized. (A) In CLL and B-ALL, the majority of patients did not show alterations in the three genes. Distribution of the loss and gain of copy numbers in the two patient groups is depicted. (B) Combinations of loss and gain of the three genes were identified in the patients with CLL and B-ALL, with alterations from part (A). *BIRC3*, baculoviral IAP repeat-containing 3; *ATM*, ataxia telangiectasia mutated; *TP53*, tumor protein 53; CLL, chronic lymphocytic leukemia; B-ALL, B-cell acute lymphocytic leukemia.
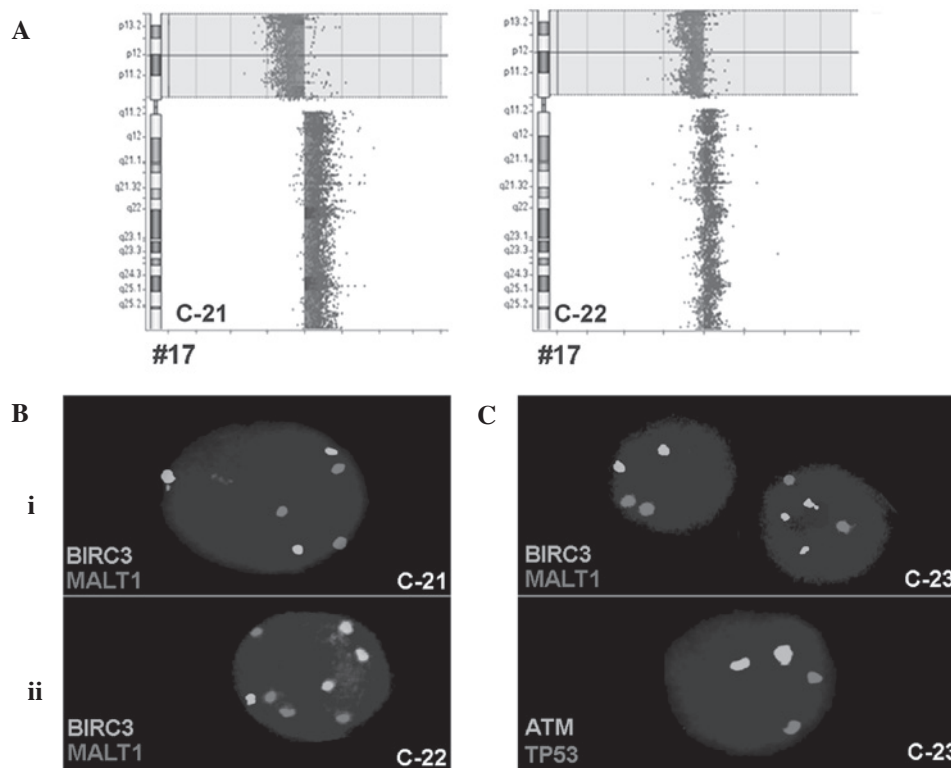
Figure 2. (A) Array comparative genomic hybridization confirmed the deletion in *TP53*, which was detected initially using iFISH and multiplex ligation dependent probe amplification for CLL cases C-21 and C-22. The whole short arm was deleted and the long arm was possibly duplicated due to an isochromosome 17 formation, at least in case C-21. (B) Examples for gain of copy numbers for *BIRC3* and *MALT1* in the 2 cases by iFISH: i) C-21, an example of 3 copies and ii) C-22, an example of 4 copies. (C) iFISH results of the CLL case C-23. *BIRC3* had 3 copies in certain cells; however *ATM*, *MALT1* and *TP53* exhibited only 2 copies each, in all cells. *TP53*, tumor protein 53; CLL, chronic lymphocytic leukemia; iFISH, interphase fluorescence *in situ* hybridization; *BIRC3*, baculoviral IAP repeat-containing 3; *MALT1*, mucosa-associated lymphoid tissue lymphoma translocation gene 1; *ATM*, ataxia telangiectasia mutated.

centromeric probes for chromosomes 11 and 17 only revealed 2 signals each (Fig. 2).

Based on the aCGH results for cases C-21, C-22 and C-23, the *TP53* deletion in C-21 and C-22 was confirmed; however, *BIRC3* was normal in all 3 patients (Fig. 2). Therefore, cases C-21 and C-22 had a mixture of a malignant triploid and tetraploid cell clones and a deletion in *TP53*. Case C-23 demonstrated the selective gain of copy numbers for *BIRC3*, without *ATM* involvement, in 36% of the cells; however, this finding was not detectable using aCGH.

MALT1 duplication. The patient with a *MALT1* duplication (case C-31) possessed an acquired trisomy of chromosome 18, which was confirmed using MLPA and iFISH. The probes for the deleted in colorectal cancer gene on 18q21.2 and RNA (guanine-7-)methyltransferase gene on 18p11.22 revealed a duplication by MLPA, which was confirmed by iFISH in 75% of the cells.

B-ALL patients. Regarding B-ALL patients, 1 case revealed a deletion in *BIRC3* along with *ATM* (case A-1), and another case
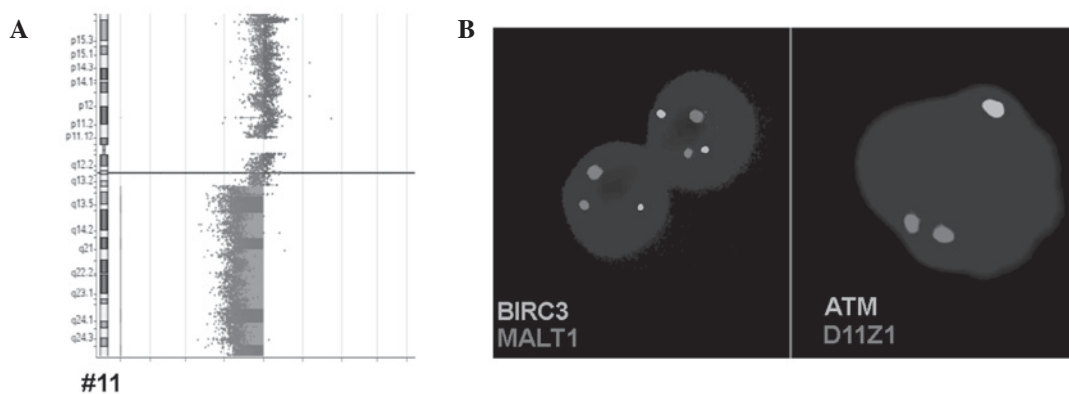
Figure 3. Results obtained for B-ALL case A-1 by array comparative genomic hybridization and iFISH are summarized. (A) Deletion in 11q22-q23, initially detected by iFISH, resulted in loss of the whole long arm of a chromosome 11. (B) Examples for heterozygote deletions of ATM and BIRC3 detected by iFISH are depicted. iFISH, interphase fluorescence *in situ* hybridization; ATM, ataxia telangiectasia mutated; BIRC3, baculoviral IAP repeat-containing 3; MALT1, mucosa-associated lymphoid tissue lymphoma translocation gene 1; D11Z1, CEP 11 probe.

was identified as possessing a triploid/hyperdiploid karyotype in the iFISH analysis using CEP 11, 17 and 18 (case A-2, result not shown), as was observed in the cases C-21 and C-22.

The *BIRC3* deletion in B-ALL case A-1 was confirmed by aCGH, which reveled that the deletion in the long arm of chromosome 11 covered between chr11:67,773,863 and 134,945,165 (GRCh37/hg19) (Fig. 3). The *ATM* and *BIRC3* genes are located between positions 102,188,181 and 108,239,826.

Discussion

The *BIRC3* gene, located on 11q22.2, is ~6 Mb centromeric to the *ATM* gene locus and is considered to be a negative regulator of the non-canonical NF-κB signaling pathway (13,14). *BIRC3* cooperates with tumor necrosis factor receptor-associated factors 2 and 3, in the same protein complex that negatively regulates the mitogen-activated protein kinase 14, a serine-threonine kinase and central activator of non-canonical NF-κB signaling (7). In addition, a frequent aberration associated with *BIRC3* is the recurrent t(11;18)(q21;q21) translocation, which involves the mucosa-associated lymphoid tissue lymphoma translocation gene 1 (*MALT1*), located on 18q21.32. This type of alteration appears in mucosa-associated lymphoid tissue (MALT) lymphoma (15).

The present study regarding *BIRC3* copy number variations in 117 CLL and 45 B-ALL patients has revealed several major findings that, to the best of our knowledge, have not been previously reported. Firstly, *BIRC3* duplications were detected in 3 cases of CLL, and 2 of these were associated with *ATM* and *MALT1* duplications, in addition to *TP53* deletions; BIRC3 amplification was detected in these 2 cases as a result of a hyperdiploid cell clone and has been previously reported in cases of CLL, but is not a frequent event (16,17). In addition, 1 B-ALL patient possessed a duplication of *BIRC3* due to partial hyperdiploidy, which is more common in B-ALL compared with CLL, and is associated with good prognosis in pediatric patients (18). A CLL-case with a *BIRC3* duplication possessed normal *ATM* and *TP53* copy numbers; however, the duplication was not detected by aCGH, most likely due to the low sensitivity of aCGH for mosaic detection, despite being

present in 36% of the cells. Previous studies on the interaction of *BIRC3* with the NF-κB pathway indicate that *BIRC3* duplication may lead to the inactivation of tumor suppressor activity (19-21).

As the predominant morphological feature of CLL is the accumulation of small B lymphocytes (1), B-ALL patients were chosen to be the second group to be tested for *BIRC3*-alterations in the present study. Therefore the second important finding of the present study is the detection of a *BIRC3* deletion in 1 of the 45 studied B-ALL cases. The aCGH for case A-1 revealed the deletion of almost all of the long arm of chromosome 11, and the most frequent aberrations associated with chromosome 11 in B-ALL patients are structural abnormalities in band 11q23, which harbors the myeloid/lymphoid leukemia gene (22).

Chromosomal deletions involving 11q have been reported in certain subtypes of hematological malignancies, including B-cell CLL, and are associated with a poor prognosis in mantle cell lymphomas or T-cell prolymphocytic leukemia (23). Therefore, the prognosis for the B-ALL patient in the present study may be poor or extremely poor. Additional studies are required to determine the role of *BIRC3* in the prognosis of B-ALL patients.

The disruption of *BIRC3* is specifically restricted to chemo-refractory cases in progressive CLL patients, and may selectively associate with fludarabine-refractory patients with normal *TP53* (7). Therefore, another notable finding of the present study is that *BIRC3* abnormalities were associated with *TP53* deletion in only 4/117 CLL cases. According to previous studies, the frequency of *BIRC3* disruption is low at diagnosis; however, *BIRC3* disruptions tend to accumulate among refractory CLL and emerge over time. Patients harboring a *BIRC3* disruption typically experience an aggressive disease course, even compared with other clinically aggressive groups (14,24). This aspect of the disease was not the focus of the present study. However, examining *BIRC3* duplication cases for the presence of mutations may be interesting for future study.

The application of a *BIRC3* probe in iFISH increased the diagnostic yield in CLL and ALL cases. As in MLPA or aCGH by *BIRC3*-directed iFISH, only gain or loss of copy numbers can be registered. However, to the best of our knowledge, *BIRC3* activation by a translocation has not previously been

reported. Therefore, routine iFISH and MLPA testing in CLL and ALL should be enhanced by a probe specific for *BIRC3*. iFISH is superior to MLPA as it is able to detect low level mosaics in the probe sample, however, MLPA is more cost-effective (2). A combination of the two approaches, by performing MLPA followed by iFISH in selected cases may be the best compromise if corresponding MLPA sets are available (2).

In conclusion, the hypothesis by Rose-Zerilli *et al* (25) that *BIRC3* deletions are always associated with *ATM* deletions is questioned at least for a small percentage of cases. As screening of the *BIRC3* gene is not routinely undertaken for CLL patients (2,26), the results of the present study suggest that screening may be considered as necessary in the future, particularly to aid in making the correct treatment decisions; particularly, whether to treat with or without fludarabine.

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