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Discontinuous deletions at 11q23 in B cell chronic lymphocytic leukemia

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Loss of genomic material in 11q is one of the most common structural chromosome aberrations in B cell chronic lymphocytic leukemia (B-CLL). In order to characterize the deletions of 11q23 in B-CLL, we performed fluorescence *in situ* hybridization (FISH) with eight YAC (yeast artificial chromosome) probes on peripheral leukocytes of 30 patients. These YACs form a contig spanning 7.8 Mb at 11q23.1-q23.3. We found deletions in nine out of 30 cases (30%) and five of them had discontinuous deletions in this region. The region represented by YAC 755b11 (1.6 Mb in size) was involved in all cases with deletions, supporting the hypothesis that this region might contain a novel gene of pathogenic importance to B-CLL. A more distal region represented by YAC 785e12 (760 kb in size) was deleted frequently and specifically. Whether there is another novel gene of pathogenic importance to B-CLL and what is its potential relationship to the deletions in the region represented by YAC 755b11, are issues that require further studies.
Keywords: B cell chronic lymphocytic leukemia; fluorescence *in situ* hybridization; yeast artificial chromosome; deletions at 11q23

Introduction

B cell chronic lymphocytic leukemia (B-CLL) comprises 90% of chronic lymphocytic leukemias in the United States and Europe.[1] Clonal chromosome aberrations are detected in approximately 40–50% of B-CLL cases by conventional chromosome banding analysis. This figure may not reflect the real situation due to the low spontaneous mitotic activity of leukemic B cells and their poor response to mitogens.[2] By using optimized mitogen stimulation, the frequency of clonal chromosome aberrations in B-CLL rose to 79%.[3] In a recent study, chromosome aberrations were detected in 81% of all cases by fluorescence *in situ* hybridization (FISH).[4]

Loss of genomic material in the long arm of chromosome 11 (11q) has been identified as a recurring abnormality in various types of lymphoproliferative disorders. Previous studies have shown that 11q deletions belong to the most common structural chromosome aberrations in B-CLL.[3,5–8] In a compilation of data from the Catalog of Chromosome Aberrations in Cancer,[9] some of the most common structural aberrations resulting in loss of chromosome material in the categories of 'lymphoproliferative disorders' and 'non-Hodgkin's lymphomas' were deletions affecting region 11q21–25, and chromosome band 11q23 was the most frequently affected area. 11q22–24 is a frequently deleted region in a variety of other neoplasias as well, such as breast,[10] ovarian,[11] cervical,[12] and colorectal carcinomas,[13] malignant melanomas[14] and paragangliomas.[15]

The recurring loss of material from chromosome bands 11q21-q25 suggests that a novel tumor suppressor gene is located in this genomic region. More direct evidence of the existence of a functional tumor suppressor gene was obtained from studies using the chromosome 11 microcell hybrid technique. Cell growth was suppressed when the whole or a part of chromosome 11 from normal human cells was introduced into tumor cells.[16,17] These studies suggested that a tumor suppressor gene was located in 11q13-qter.

In a recent study on B-CLL, the commonly deleted region in 11q14-q23 was narrowed down to a region represented by YACs (yeast artificial chromosome) 755b11, 975h6 and 888h8. Screening at other loci along chromosome 11q14–23 failed to reveal any aberration outside this consensus region.[18]

In order to further characterize the deletions around this consensus area, we performed interphase FISH on 30 B-CLLs by using eight YAC probes, which formed a contig at 11q23.1-q23.3.

Materials and methods

Patients

Peripheral blood specimens were collected from 30 CLL patients referred to the CLL out-patient clinic of the Tampere University Hospital. Twenty-two of the patients were men and eight were women; the age of the patients ranged from 48 to 79 years (median, 64 years). The diagnosis and staging of CLL were based on standard clinical, morphological, and immunophenotyping criteria.[19–22] All patients had the B-CLL phenotype. The 'CLL scores'[23] ranged from 3 to 5. Table 1 shows the clinical–hematological details at sampling. Peripheral blood from two healthy persons was used as control.

Standard cytogenetics

Mononuclear cells from peripheral blood were isolated and cultured with optimal mitogen stimuli as described elsewhere,[3] and studied by standard G-banding.

Fluorescence in Situ hybridization (FISH)

DNA probes: Based on a YAC contig and *Not*I restriction map developed by Arai *et al*, eight YAC clones (793d9, 755b11, 913g9, 957e4, 771d4, 939b12, 785e12 and 911f2) were purchased from CEPH-Généthon (Paris, France). These YACs constitute a contig of 7.8 Mb in size spanning region 11q23.1-q23.3 (Figure 1). All the YAC probes hybridized only to 11q22-q23 and none of them showed chimerism by FISH. YAC 953a4 (CEPH-Généthon), mapped to 11p13, was used as the chromosome copy number control. The identities of the YACs were confirmed by PCR with their corresponding markers[24] (Figure 1). The yeast was grown in AHC media for

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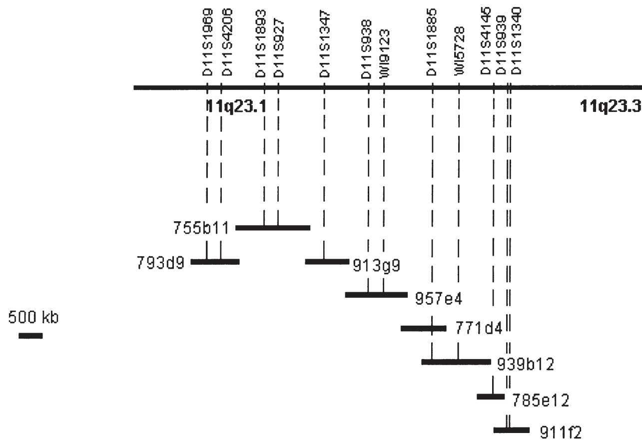

Figure 1 YAC contig spanning the region 11q23.1-q23.3. The YACs were tested by PCR using the markers listed above.

4–6 days and DNA was extracted from the yeast by using a glass bead-phenol procedure.[25]

FISH: The test and control DNA were labeled by nick translation with biotin-14-dATP (Gibco BRL, Paisley, UK) and digoxigenin-11-dUTP (Boehringer Mannheim, Mannheim, Germany), respectively. Dual-color FISH was performed when probes 793d9, 755b11 and 913g9 were tested on the samples. To simplify the procedure, the remaining experiments were carried out by using single color FISH. We consider this to be reliable because the complete karyotypes of all CLL samples were available and none of them showed copy number aberrations involving chromosome 11. In addition, dual-color FISH with probes 755b11 and 785e12 labeled with biotin-14-dATP and digoxigenin-11-dUTP, respectively, was performed on case Nos. 3, 9, 18 and 30. Similarly, dual-color FISH with probes 939b12 and 911f2 labeled with biotin-14-dATP and digoxigenin-11-dUTP, respectively, was performed on case Nos. 3, 9 and 30. The hybridization mixture contained approximately 1–2 μg labeled DNA, 25 μg human Cot-1 DNA (Gibco BRL), and 25 μg herring sperm DNA (Sigma Chemical, Bornem, Belgium) dissolved in 50% formamide and 10% dextran sulfate in $2 \times$ SSC. The hybridization mixture was denatured at 75°C for 5 min.

Slides for FISH were prepared as described elsewhere.[18] To remove excess cytoplasm, the slides were pre-treated with pepsin (0.01 mg/ml in 0.01 M HCl; Sigma, St Louis, MO, USA) for 7 min and dehydrated in 70%, 85% and 100% ethanol for 5 min each. The slides were denatured in 70% formamide/$2 \times$ SSC (pH = 7) at 65°C for 2 min, followed by dehydration in 70%, 85% and 100% ethanol for 2 min each. After applying the denatured hybridization mixture on to the slides, the slides were incubated at 37°C for 2 nights.

Detection and analysis: Test probes labeled with biotin were visualized by tetra-rhodamine-isothiocyanate[26] (TRITC; Vector Laboratories, Burlingame, CA, USA) and control probes labeled with digoxigenin were visualized by fluorescein-isothiocyanate (FITC; Sigma) in dual-color FISH. Only slides with high hybridization efficiency, indicated by two signals of the control probe in more than 90% of the nuclei, were analyzed. In single-color FISH, all the probes were visualized by FITC. Preparations showing clear and strong signals in more than 90% of the nuclei were analyzed. From each preparation a minimum of 200 morphologically intact and non-overlapping nuclei and metaphase spreads were scored. Images were captured using an Olympus fluorescence microscope (Tokyo, Japan) connected to a cooled charge coupled device (CCD) camera and the ISIS digital image analysis system (Metasystems, Altlussheim, Germany).

Controls: Two normal blood samples were tested by using all of the probes. In addition, controls of the chromosome copy number provided by YAC probe 953a4 located at 11p13 were included in every hybridization experiment. The cut-off level was defined by the mean value plus three times of the standard deviation (s.d.) of the frequency of control cells exhibiting only one signal (mean 7.8%; s.d. 3.3%; cut-off level 17.7%).

Results

Standard karyotyping results are listed in Table 1. Deletions of 11q were identified in six cases (Nos. 1, 2, 9, 11, 18 and 20). Five out of six cases with 11q deletions had other chromosome abnormalities (Table 1). There were no numerical abnormalities or translocations involving chromosome 11 in any of the cases studied. In addition to the six cases mentioned above, FISH study revealed three other cases (Nos. 3, 25 and 30) with 11q23 deletions (Table 1). The percentage of cells showing deletions ranged from 22% to 93% (mean, 77%). Five out of these nine cases showed discontinuous deletions (Table 2).

The genomic region represented by probes 793d9, 755b11 and 913g9 was deleted in all cases with deletions. Another region, which is represented by YAC 785e12 located about 4 Mb distal from 755b11, was deleted in all but one of the cases (No. 2). The area between these two regions, represented by YACs 957e4, 771d4 and 939b12, was deleted disproportionately and discontinuously. In case Nos. 3, 9 and 30, the region represented by YAC 785e12 was deleted while both of the regions flanking it were not.

In the two cases (Nos. 3 and 9) with discontinuous deletions, two signals were seen at their normal positions at 11q when probes 771d4, 939b12 and 911f2 were hybridized to the samples. The same was true when case No. 18 was hybridized with probes 771d4 and 939b12. Dual-color FISH on metaphase cells from case Nos. 3, 9, 18 and 30 with probes 755b11 and 785e12 showed that the deletions of these regions affected the same chromosome. Dual-color FISH on interphase cells from case Nos. 3, 9 and 30 with probes 939b12 and 911f2 showed no split signals, which suggests that no translocation breakpoint was involved in these regions.

Discussion

In the present study, we showed that deletions at 11q23.1-q23.3 were discontinuous in B-CLL. The regions represented by YACs 793d9, 755b11 and 913g9 were deleted in all cases with deletions. A more distal region represented by YAC 785e12 was also frequently and specifically deleted.

The genomic region represented by probe 755b11 was involved in all cases with deletions. The importance of this region is highlighted by recent findings which showed that in

Table 1 Clinical and hematological data of 30 B-CLL patients at sampling

Patient No.	*Sex/Age*	*FAB diagnosis*[a]	*Immuno-phenotype score*[b]	*Stage (Binet)*[c]	*Progression*[d]	*Blood lymphocytes ($\times 10^9$/l)*	*Karyotype*	*Deletion found by FISH*
1	M/54	CLL	5	A	S	63	46,XY,**del(11)(q14q24)**[1]/46,idem,del(13)(q?14q?32)[1]/46,idem, t(3;10)(p13-21;q25-26),del(13)(q?14q?32)[6]/46,XY[6]	**Yes**
2	M/64	CLL	5	A	T	46	46,XY,del(7)(q32q36),**del(11)(q21q23)**[6]/46,idem, del(6)(q?21q?24)[4]/46,XY[7]	**Yes**
3	M/72	CLL	4(Igκ)	B	F	84	47,XY,+12[2]/46,XY[25]	**Yes**
4	M/68	CLL	ND	A	T	7	46,XY[20]	No
5	F/61	CLL	ND	A	S	86	46,XX,del(6)(q?22)[2]/46,XX[18]	No
6	F/50	CLL	4(Igλ)	A	S	130	46,XX[20]	No
7	M/58	CLL/PL	4(Igλ)	C	T	310	46,XY,+der(12)t(12;15)(p12;q13-15), der(14;17)(q10;q10)[17]/46, idem, add(1)(q21)[2]/46,XY[1]	No
8	M/69	CLL	5	A	I	51	46,XY,del(13)(q?14q?22[2]/46,XY[18]	No
9	F/69	CLL	5	C	T	88	46,XX,**del(11)(q13q23)**, del(13)(q12q21)[7]/46,XX[9]	**Yes**
10	M/65	CLL	5	C	T	132	46,XY,del(13)(q?14q?22)[2]/46,XY[18]	No
11	M/68	CLL	3(Igλ, CD5)	C	F	114	46,XY,+8,del(6)(q?23), **del(11)(q?14)**, del(13)(q?14q?22),–20[cp9]/46,XY[5]	**Yes**
12	F/67	CLL/PL	4(Igλ)	C	T	188	45–46,XX,add(1)(p33–35),del(5)(q?31),–10,–17, +mar, inc[cp10]/46,XX[10]	No
13	M/68	CLL	5	A	I	81	46,XY,del(13)(q?14q?22)[2]/46,XY[18]	No
14	M/57	CLL	5	A	F	120	46,XY[20]	No
15	F/73	CLL/mix	3(Igκ, CD23)	C	F/T	79	47,XX,+12[5]/46,XX[15]	No
16	M/55	CLL/PL	4(CD5)	A	F/T	36	48,XY,+6,add(11)(p?15),+12[4]/46,XY[8]	No
17	M/53	CLL/PL	5	B	I	69	46,XY[20]	No
18	M/71	CLL/mix	5	B	S	89	46,XY,**del(11)(q?22q?24)**[2]/46,XY[18]	**Yes**
19	M/54	CLL/PL	4(Igλ)	B	I	59	46,XY,del(6)(q?23)[2]/46,XY[18]	No
20	M/62	CLL	3(CD23, Igλ)	B	S	59	46,XY,**del(11)(q?13)**, del(13)(q?14)[2]/46,XY[15]	**Yes**
21	F/79	CLL/mix	4(Ig23)	B	T	97	46,XX[15]	No
22	M/54	CLL/mix	4(Igκ)	B	T	216	47,XY,+12[2]/46,XY[10]	No
23	M/48	CLL	5	A	S	93	46,XY[20]	No
24	M/66	CLL	5	A	I	67	46,XY[20]	No
25	M/67	CLL	ND	C	F/T	178	46,XY[20]	**Yes**
26	M/78	CLL	4(CD5)	B	F	224	46,XY,t(4;11)(p?15;q?21)[3]/46,XY[17]	No
27	F/70	CLL	4(Igλ)	A	F	69	43–44,XX,–4,del(6)(q?22),–16,–17,–18, +2mar[11]/46,XX[4]	No
28	M/59	CLL	5	A	I	98	46,XY[20]	No
29	M/71	CLL	3(Igκ, CD22)	A	F	134	46,XY[20]	No
30	F/77	CLL	5	C	F	206	46,XX[2]	**Yes**

[a]According to Bennett *et al* (1989).
[b]According to Matutes *et al* (1994). A score of five means CD22⁻, CD23⁺, CD5⁺, FMC7⁻, and undetectable or weak Igκ/λ. The marker causing a deviation from score 5 is indicated in parentheses: Igκ/λ means moderate–strong Igκ/λ. CD22 means CD22-positive. CD23 means CD23-negative. CD5 means CD5-negative.
[c]According to Binet *et al* (1981).
[d]S (slow), blood lymphocytes increased less than 20% within a year; F (fast), lymphocyte doubling time 1 year; I (intermediate), between S and F; T, chemotherapy given afterwards; if alone, natural disease progression non-evaluable.

mantle cell lymphomas the minimal common region of the deletions at 11q22-q23 was the region represented by YAC probe 755b11 only.[18,27] Two translocation breakpoints have also been found in this region.[18] In our study, case No. 3 had a deletion frequency of 63% in this region, while the flanking regions had deletion frequencies of only 42% and 31%, respectively. This suggests that some population of the cells had deletions only in this region. In addition to our results, other studies also support the idea that the region harbors a novel gene that has pathogenic significance in B-CLL.[18]

The region represented by YAC 785e12 was deleted in eight out of the 30 cases studied. The importance of this region can be seen in case Nos. 3, 9 and 30, where the region represented by 785e12 was deleted while the flanking regions remained intact. Furthermore, our FISH results on interphase cells suggested that the deletion of this region was not caused by translocations. In previous studies on breast carcinomas[28] and cervical carcinomas,[12] loss of heterozygosity for marker APOC-3, which is located in the region represented by 785e12, was found to occur at frequencies as high as 45% and 43%, respectively, suggesting the existence of a tumor suppressor gene. However, in our study, one case with 11q deletions did not involve the region represented by YAC 785e12. Similarly, Stilgenbauer *et al*[18] have reported that seven out of 18 CLL cases in their study had deletions in regions other than the region represented by YAC 785e12. One explanation why this region was not found deleted in all cases with 11q deletions might be that a part of this region could have been deleted but was beyond the resolution limit of FISH. The deletion of this region might also be a late event

Table 2 Deletion mapping at 11q23 on 9 B-CLL cases.

YAC known gene	793d9	755b11 FDX1	913g9 NCAM CRYA2	957e4 PLZF DRD2	771d4	939b12	785e12 APO C3	911f2 APO C3
Case No. 1	82	89	79	93	83	88	83	85
11	90	78	77	89	86	93	89	87
25	65	93	84	83	93	75	88	69
18	68	81	69	66	10	8	74	77
20	74	83	77	17	87	84	86	22
30	61	81	81	82	75	17	88	13
9	65	67	65	76	10	8	63	4
3	42	63	31	50	10	5	61	6
2	77	83	82	23	7	7	3	2

The extent of deletions is indicated by shading. The numbers are the percentages of cells with deletions.

in the disease progression and may not be seen at the early stages of the disease.

The remaining regions were deleted discontinuously and disproportionately. Discontinuous deletion of 11q23 has not been reported in B-CLL previously. In our study, the deletions of two regions represented by YACs 755b11 and 785e12 were found to affect the same chromosome, which might suggest that the deletions of these two regions are concomitant. The regions not deleted had the two signals at their normal positions. Therefore, it would be unlikely that one allele would have been lost while the other one was duplicated to give the false negative results. Concomitant gains and losses of alleles situated in the immediate vicinity have previously been found in sarcomas.[29] It has been shown that different genes act as independent targets of selection for amplification or deletion, which might support the hypothesis that 11q23 harbors at least two genes that act as targets of selection for deletion. One of them may reside within the region represented by YAC 755b11 and the other within the region represented by YAC 785e12. These genes might be important in the pathogenesis of B-CLL.

Chromosome region 11q22–23 is a gene-rich area, containing several genes related to hematologic malignancies, such as the *MLL* gene, the *ATM* (ataxia-telangiectasia mutated) gene, the *RDX* (a cytoskeletal protein that shows homology to the *NF2* tumor suppressor gene product) gene, the *NCAM* gene, the *MRE11* (meiotic recombination (*S. cerevisiae*) 11 homolog) gene, the *IL1BC* (interleukin 1 beta convertase) gene, and the *PLZF* (promyelocytic leukemia zinc finger) gene. However, none of these genes are located in either of our two critical regions. The *PPP2R1B* gene, which encodes the β isoform of the A subunit of the serine/threonine protein phosphatase 2A (PP2A), has been identified as a putative human tumor suppressor gene.[30] This gene resides very close to the region represented by YAC 755b11. Its exact location and its possible role in the pathogenesis of B-CLL are under investigation. To our knowledge, no other tumor suppressor gene is located in this area.

In summary, we have demonstrated that deletions at 11q23.1–23.3 in B-CLL are discontinuous. Our results support the hypothesis that the region represented by YAC 755b11(1.6 Mb in size) may contain a novel gene of pathogenic significance in B-CLL. A more distal region represented by YAC probe 785e12 (760 kb in size) was shown to be frequently and specifically deleted. Whether this region contains another pathogenically important gene to B-CLL and how it may relate to the deletions in the region represented by YAC 755b11 are issues that deserve to be studied further.

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