A

B

C

Fig. 1. Representative array CGH profiles of individual tumors and genome-wide frequencies of copy number alterations. Whole genomic profiles are shown for three representative cases of DLBCL (*A* and *B*, CD5$^+$; *C*, CD5$^-$). Log$_2$ ratios were plotted for all clones based on their chromosome position, with *vertical dotted bars* representing the separation of chromosomes. Clones are ordered from chromosome 1 to 22 and X within each chromosome on the basis of the Sanger Center Mapping Position, February 2004 version. *A*, regions of copy number gain: 1q21.2-q24.3, 2p15.1-p221, 7p2.3-ptel, 7p11.2-p21.1, 7q21.11-q31.1, 8p11.23, 8q24.13-qtel, 11q22.3-qtel, 13q21.32-q32.2, 13q34-qtel, 15, 17q, 19q13.43-qtel, 20, and 21; regions of copy number loss: 1p36.21-p36.32, 2p22.3-ptel, 4p15.1-ptel, 6p22.3-p25.3, 7p21.3, 7q31.33, 8p12-ptel, 8q12.1, 13q34-qtel, 14q22.2-q21.3, and 17p. *B*, regions of copy number gain: 3p14.2-qtel, 7, 9ptel-q32.2, 12p11.2-ptel, 15q24.3-qtel, and 18; regions of copy number loss: 1p35.1-ptel, 9p21, 15q14-q21.1, and 17p. *C*, regions of copy number gain: 1p36, 3, 6p, 7q21.11-qtel, 11, 16p13.3, 17cen-q23.2, and 18; regions of copy number loss: 3p14.2, 6p22.3-p25.3, 6q14.1-qtel, 9q22.33, 15q26.2-qtel, and 17p. Log$_2$ ratio of −2.01 for the single BAC, RP11-48E21, suggests homozygous loss at 3p14.2 locus.

(defined as log$_2$ ratio +0.2 to +1.0), as well as regions suggestive of heterozygous loss/deletion (defined as log$_2$ ratio −1.0 to −0.2).

The entire tumor set comprised an average copy number gain of 311.1 Mb or 10.9% of the genome (6.8 regions) and an average copy number loss of 174.4 Mb or 6.8% of the genome (5.9 regions). The CD5$^+$ group showed a larger average fraction of copy number gain (370.9 Mb, 13.4%) than the CD5$^-$ group (311.1Mb, 10.9%), whereas the former (110.5 Mb, 3.8%) contained a smaller average fraction of copy number loss than the latter (174.4 Mb, 6.8%). The average number of total alterations consisted of 8.1 regions of gain and 5.4 regions of loss in the CD5$^+$ group and 6.0 regions of gain and 6.4 regions of loss in the CD5$^-$ group.

We defined region of gain or loss as (*a*) continuously ordered three clones showing gain or loss or (*b*) clones showing high copy number gain (log$_2$ ratio > +1.0) or homozygous loss (log$_2$ ratio < −1.0; *e.g.*, a gain of 2p15, a loss of 3p14.2 and a loss of 9p21). Recurrent region was defined as a region seen in >20% of cases. The most frequently

gained or lost BAC/PACs in each of the recurrent regions are listed in Tables 1 and 2.

In the CD5$^+$ group (25 cases), recurrent regions of gain were 1q21.2-q32.3, 1q42.2-q42.3, 2p15-p16.1, 3, 5p13.2-p13.1, 6p22.3-p25.3, 7p22.2-q31.1, 8q24.13-q24.22, 11q22.1-q25, 12, 13q21.1-q34, 16p13.3-q21, 17q23.2-q24.3, 18, and 19p13.13-q13.43, and X and recurrent regions of loss were 1p36.23-p36.32, 1p36.13-p36.21, 1p34.3-p35.1, 1q43-q44, 3p14.2, 6q14.1-q27, 8p23.3-p21.2, 9p21, 15q13.1-q14, and 17p11.2-p13.3.

In the CD5$^-$ group (41 cases), recurrent regions of gain were 1q21.2-q31.1, 1q32.1-q32.2, 3p25.2-q29, 5p13.1-p13.2, 5p13.2-p14.1, 6p12.3-p25.3, 7, 8q24.13-q24.21, 9p24.2-p13.2, 11q23.2-q24.2, 12q13.2-q21.2, 16p13.3, 18, and X and recurrent regions of loss were 1p36.23-p36.32, 3p14.2, 6q12-q25.2, 6q27, 9p21, 15q15.2-q21.1, and 17p11.2-p13.3.

Regions of gain observed in >20% of cases in both the CD5$^+$ and CD5$^-$ groups were 1q21.2-q31.1, 1q32.1-q32.2, 3p25.2-q29, 5p13.1-

Table 1 *Most frequently gained clones of CD5+ and/or CD5− DLBCL*

Clone name	Cytogenetic position	Genes*	CD5+ (n = 25) %†	CD5− (n = 41) %	All (n = 66) %
RP11-367J7‡,§	1q23.1	SPAP1	36	29.3	31.8
RP11-23I7§	1q31.1	MDM4	24	24.4	25.8
RP5-956O18‡	1q42.2	PGBD5	20	22.7	21.2
RP11-17D23‡	2p15	USP34	24	17.1	19.7
RP11-861A13§	3q12.1	CD47	48	24.4	33.3
RP11-211G3‡	3q27.3	BCL6	56	36.6	43.9
RP11-317I23‡,§	5p13.2	SLC1A3	20	22.0	21.2
RP11-233K4§	6p25.3	MUM1	24	29.3	27.3
RP3-431A14‡	6p21.31	CDKN1A	29	26.6	33.3
RP11-514K1‡,§	7q21.2	CDK6	24	41.5	34.8
RP1-80K22‡,§	8q24.2	MYC	20	24.4	22.7
RP11-39K24§	9p24.1	JAK2	12	26.6	21.2
RP11-758H14§	11q23.3	MLL	40	31.7	34.8
RP11-1007G5‡	11q24.3	ETS1	56	29.3	39.3
RP11-100C20‡	12p12.1	BCAT1	44	17.1	25.8
RP11-181L23§	12q13.3	GLI	32	24.4	21.2
RP11-571M6§	12q14.1	CDK4	32	24.4	21.2
RP11-450G15§	12q15	MDM2	32	24.4	27.2
RP11-335P18‡	13q21.32	PCDH9	32	12.2	19.7
RP11-461N23‡	13q32.3	EBI2	32	17.1	21.2
RP11-473M20§	16p13.3	NK4	36	65.9	56.1
RP11-548B6‡	16p12.1	PKC beta	28	7.3	15.1
RP11-371B4‡	17q23.2	APPBP2	20	4.9	10.6
RP11-28F1§	18q21.1	BCL2	36	41.5	39.4
RP11-676J15‡	18q22.3	NETO1	44	38.8	47.0
RP11-43B2‡	19q13.33	FUT1	56	17.1	31.8
RP11-50I11‡	19q13.41	CD37	56	14.6	30.3
RP11-45K21‡	19q13.43	NALP13	56	26.8	37.9

* Genes contained in clones.
† % of cases with copy number gain.
‡ Most frequently gained clones of recurrent regions in CD5+ DLBCL.
§ Most frequently gained clones of recurrent regions in CD5− DLBCL.

p13.2, 6p21.1-p25.3, 7p22.2-q31.1, 8q24.13-q24.21, 11q23.2-q24.3, 12q13.2-q21.2, 16p13.3, 18, and X and regions of loss were 1p36.31-p36.32, 3p14.2, 6q14.1-q25.2, 6q27, 9p21, and 17p11.2-p13.3.

Among the recurrent regions, the gain of 2p15 (BAC, RP11-17D23), loss of 3p14.2 (BAC, RP11-48E21), and loss of 9p21.1 (BAC, RP11-149I2) showed genomic aberrations by single or two continuous clones. Three of 13 cases with gain of 2p15 showed high-level copy number gain ($\log_2$ ratio > +1.0) at a restricted position of the genome (~2 Mb resolution level) encompassing two continuously ordered BACs, RP11-17D23 (500 kb centromeric to the *REL* gene) and RP11-511I11 (1.8 Mb centromeric to BAC, RP11-17D23).

Eighteen of the 66 cases (28%) showed loss of 3p14.2. Among the clones contained in 3p14.2, BAC, RP11-48E21 (including *FHIT*) only was lost in 13 of the 18 cases (7 cases of CD5+ and 11 cases of CD5− DLBCL), with no surrounding BACs showing any obvious copy

number losses (Fig. 2A). Among the 13 cases with a single loss, 2 cases showed homozygous loss at BAC, RP11-48E21 ($\log_2$ ratio < −1.0). 3p14.2 was also deleted in the cell line OCI-LY13.2 established from a patient with aggressive malignant lymphoma (18). Fluorescence *in situ* hybridization analyses results were consistent with those of the array CGH (Fig. 2, B and C). Similarly, the BAC clone RP11-149I2, including *INK4a/ARF*, only was lost in 9 of the 32 cases that showed loss of 9p21 among a total of 66 DLBCL cases, and 2 of the 9 cases showed homozygous loss.

Finally, we analyzed X chromosomes for male patients only (10 cases in the CD5+ group and 25 cases in the CD5− group). Two CD5+ cases and three CD5− cases showed low-grade copy number gains throughout the entire X chromosome but no high-grade amplification. Heterozygous losses were found at Xq21 in two cases in the CD5+ group, whereas no homozygous loss region was found at chromosome X.

Table 2 *Most frequently lost clones of CD5+ and/or CD5− DLBCL*

Clone name	Cytogenetic position	Genes*	CD5+ (n = 25) %†	CD5− (n = 41) %	All (n = 66) %
RP11-37J18‡,§	1p36.32		40	27	31.8
RP11-473A10‡	1p36.13	NM_018125	24	22	22.7
RP5-1125N11‡	1p35.2	LAPTM5	28	22	24.2
RP11-439E19‡	1q44	NM_016009	24	0	9.1
RP11-48E21‡,§	3p14.2	FHIT	28	26.8	28.7
RP11-329G3‡,§	6q21	PREP	44	36.6	39.4
RP11-421D16§	6q27	SMOC2	24	19.5	21.2
RP11-240A17‡	8p23.3	DLGAP2	36	7.3	18.1
RP11-149I2‡,§	9p21.3	INK4a/ARF	40	31.7	34.8
RP11-2C7‡	15q13.2	ATP10A	24	17.1	19.7
RP11-164J23§	15q21.1		16	29.3	24.2
RP11-199F11‡	17p13.1	TP53	36	26.8	30.3
RP11-187D20§	17p12	NM_182567	20	39	30.3

* Genes contained in clones.
† % of cases with copy number loss.
‡ Most frequently lost clones of recurrent regions in CD5+ DLBCL.
§ Most frequently lost clones of recurrent regions in CD5− DLBCL.
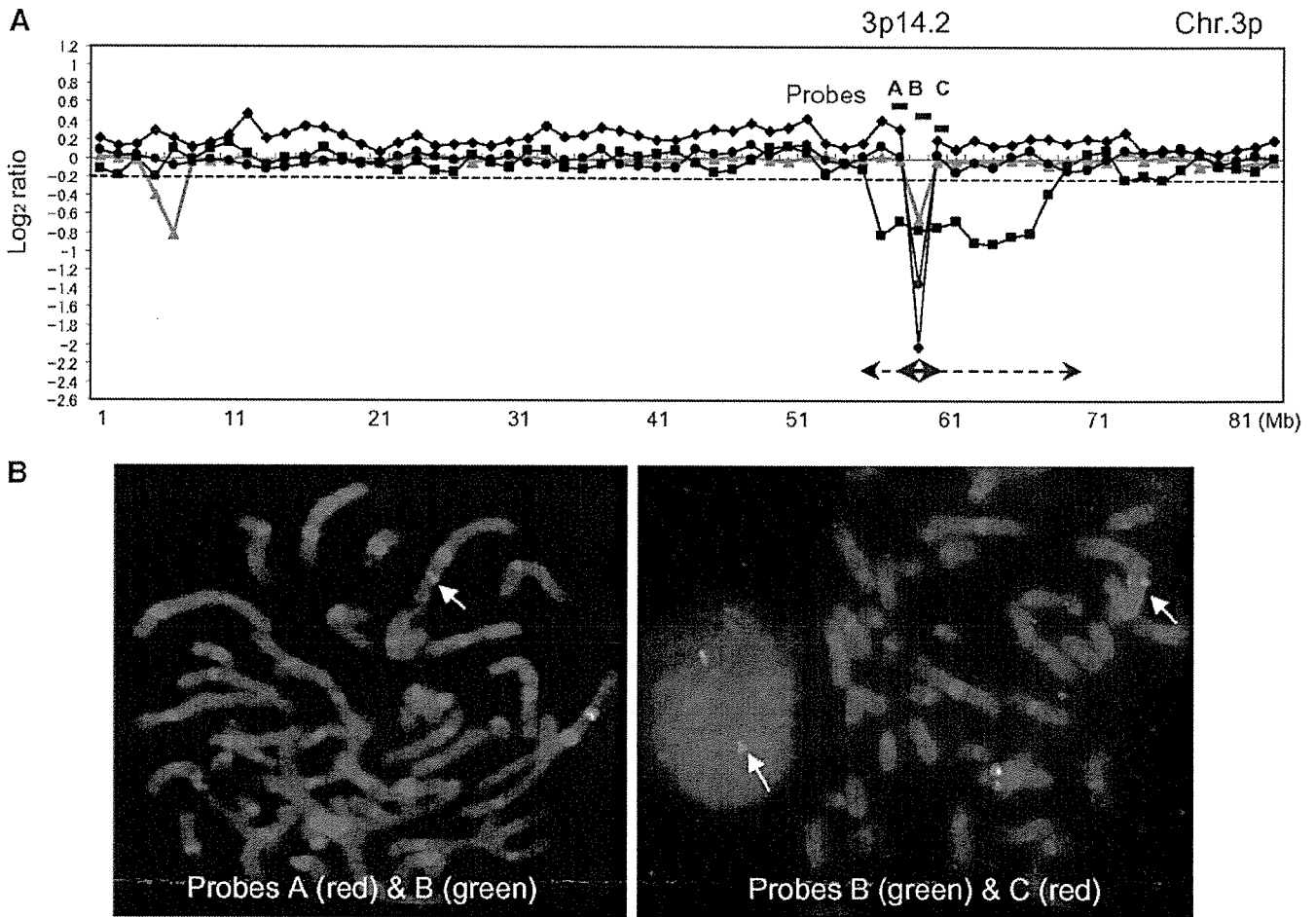
A

3p14.2   Chr.3p

B

Fig. 2. Genomic profile of chromosome 3p and fluorescence *in situ* hybridization (FISH). *A*, four representative individual genomic profiles of 3p. Minimum common loss of 3p14.2 is indicated by the *thick arrow* (BAC, RP11-48E21 locus). The *three black lines* represent individual profiles of each DLBCL case and the *red line* the individual profile of the malignant lymphoma cell line OCI-LY13.2. Physical distances from telomere to centromere are indicated below the *square box* (Mb). Positions of probes used in FISH analyses in *B* and *C* are also presented as *small bold horizontal lines*. Probe A: BAC, RP11-391P4; probe B: BAC, RP11-48E21; probe C: BAC, RP11-611H10. Probe A is 1.6 Mb telomeric to probe B, whereas probe C is 1.2 Mb centromeric to probe B. The *thin arrow* indicates region of loss in one case. BAC, RP11-48E21, contains the *FHIT* tumor suppressor gene. *B*, dual-color FISH analysis with probes A and B of the OCI-LY13.2 cell line. A metaphase chromosome has two pairs of red signals (probe A, *red*) and one pair of green signals (probe B, *green*), indicating heterozygous loss of probe B. *C*, dual-color FISH analysis with probes B and C of the OCI-LY13.2 cell line. As in the case of probes A and B, a metaphase and an interphase chromosome have two pairs of red signals (probe C, *red*) and one pair of green signals (probe B. *green*), indicating heterozygous loss of probe B (*green*).

Genomic Copy Number Changes Characteristic of CD5⁺ DL-BCL. We next compared the frequency of gain and loss of clones in the CD5$^+$ and CD5$^-$ groups. Screening on a single-clone basis for candidate clones showed that 48 clones were more frequently ($P < 0.05$) gained or lost in the CD5$^+$ than in the CD5$^-$ group. Among these 48 clones, 6 of 10p14-p15.3, 3 of 12p12, 3 of 16p12, and 9 of 19q13.32-q13.43 were continuous clones in terms of the whole genome mapping position according to the Ensemble Genome Data Resources of the Sanger Center Institute (January 2004 version). The remaining 27 clones showed individual $P < 0.05$, *i.e.*, with no neighboring clones showing such significance.

Twenty clones were identified as being lost significantly more frequently in the CD5$^+$ group than in the CD5$^-$ group. Among these 20 clones, 3 clones of 1q43, 6 of 1q43-q44, 2 of 8p23.3, and 5 of 8p23.1-p23.2 were continuous, and the remaining 4 clones showed individual $P < 0.05$ with no neighboring clones showing such significance. In the CD5$^-$ group, 10 gained clones and 6 lost clones showed individual $P < 0.05$ with no neighboring clones showing such significance.

Clones screened on a single-clone basis as described above were subsequently subjected to multiple comparison corrections to find

clones statistically relevant in terms of differences in frequency between the CD5$^+$ and the CD5$^-$ groups. Because

$$\prod_{i\,=\,n}^{n\,+\,k} p_i$$

values of continuous clones with gains of 10p14-p15.3 and 19q13.32-q13.43 and losses of 1q43-q44 and 8p23.1-p23.2 were $<4.2 \times 10^{-6}$, these regions of gain and loss were determined to be characteristic of the CD5$^+$ group. No significantly frequently occurring region of gain or loss was found in the CD5$^-$ group after multiple comparison corrections had been performed. Regions characteristic of CD5$^+$ DLBCL and the BACs they contained are listed in Table 3.

Genomic Copy Number Changes Affecting Prognosis of DL-BCL. The next step was the analysis, using the Kaplan-Meier method and log-rank test, of the probabilities of survival of the cases that had been stratified according to the presence or absence of one of the specific genomic gains or losses. All clones that showed aberrant copy number changes in either the CD5$^+$ or the CD5$^-$ group were sub-

Table 3 *List of BACs characteristic of CD5⁺DLBCL and statistic analysis*

Clone name*	Gain/Loss	Chromosome band	Mb†	Genes‡	CD5⁺ (n = 25) %§	CD5⁻ (n = 41) %	All (n = 66) %	Fisher's P¶
RP11-362D13	Gain	10p15.3	2.2		16	0	6.1	0.0176
RP11-453F1	Gain	10p15.2	3.3		16	0	6.1	0.0176
RP11-154P11	Gain	10p15.1	4.3		16	0	6.1	0.0176
RP11-445P17	Gain	10p15.1	5.1	AKR1C4	16	0	6.1	0.0176
RP11-563J2	Gain	10p15.1	6.3		16	0	6.1	0.0176
RP11-379F12	Gain	10p14	8.1	GATA3	16	0	6.1	0.0176
RP11-124P12	Gain	19q13.32	52.2	NPAS1	40	17.1	25.8	0.0476
RP11-3N16	Gain	19q13.33	53.2	LIG1	48	29.3	28.8	0.0113
RP11-43B2	Gain	19q13.33	53.9	FUT1	56	17.1	31.8	0.0022
RP11-50I11	Gain	19q13.33	54.5	CD37	56	14.6	30.3	0.0007
RP11-265B5	Gain	19q13.33	55.1	IL4I1	48	19.5	31.8	0.0051
RP11-256B9	Gain	19q13.41	57.5	ZNF137	44	14.6	25.8	0.0046
RP11-158G19	Gain	19q13.42	59.1	MYDM	44	14.6	25.8	0.0046
RP11-705C4	Gain	19q13.42	60.7	IL11	48	19.5	30.3	0.0259
RP11-45K21	Gain	19q13.43	61.2	NALP13	56	26.8	37.9	0.0216
RP11-420P11	Gain	19q13.43	63.2	NM_023926	44	22.0	30.3	0.0964
RP11-435F13	Loss	1q44	238.1	RGS7	24	0	9.1	0.0019
RP11-269F20	Loss	1q44	240.6	AKT3	20	0	7.6	0.0059
RP11-424N15	Loss	1q44	241.5	ADSS	20	0	7.6	0.0059
RP11-74P14	Loss	1q44	242.9	Q96QW3	20	0	7.6	0.0059
RP11-439E19	Loss	1q44	243.7	NM_016002	24	0	9.1	0.0019
RP11-152M6	Loss	1q44	243.9	NM_015431	20	0	7.6	0.0059
RP11-18D5	Loss	8p23.3	0.3	NM_181648	28	4.9	13.6	0.0214
RP11-240A17	Loss	8p23.3	1.2	DLGAP2	36	7.3	18.1	0.0066
RP11-82K8	Loss	8p23.3	2.1	MYOM2	36	14.6	22.7	0.0685
RP11-29A2	Loss	8p23.2	5.1		32	9.8	18.2	0.0449
RP11-728L1	Loss	8p23.2	5.6		32	9.8	18.2	0.0449
RP11-18L2	Loss	8p23.1	8.6	MFHSA1	24	2.4	10.6	0.0099
RP11-24I4	Loss	8p23.1	10.0	MSRA	32	9.8	18.2	0.0449
RP11-254E10	Loss	8p23.1	11.2	LOC157740	32	7.3	16.7	0.0154

* List of BAC/PACs from p telomere to q telomere for each chromosome number.
† On the basis of Ensembl genome mapping position.[8]
‡ Genes contained in clones.
§ % of cases with copy number gain or loss.
¶ P values of Fisher's exact test between frequency of CD5⁺ cases and those of CD5⁻.

jected to screening, followed by multiple comparison corrections for the clones that showed $P < 0.05$ in screening with the log-rank test.

In the CD5⁺ group, 252 clones (98 gained and 154 lost clones) showed $P < 0.05$ for prognosis according to log-rank test results on a single-clone basis. Eighteen of the lost clones were continuous at 1p34.3-p36.21, and multiple comparison corrections showed them to have deleterious effects on survival. Forty-four of the gained clones were of 13q. Among the 44 clones, 29 at 13q21.1-q31.3 and 15 at 13q31.3-q34 were continuous and were identified by multiple comparison corrections as being linked to poor prognosis. The remaining 190 gained or lost clones fell short of statistical significance.

In the CD5⁻ group, 252 clones (197 gained and 55 lost clones) showed $P < 0.05$ for prognosis on a single-clone basis. Thirty-four of these clones were of 5p. Among the 34 clones, 19 clones at 5p14.2-p15.33 and 5 clones at 5p12-p13.2 were continuous, and multiple comparison corrections indicated they had favorable impacts on survival. The remaining 218 gained and lost clones fell short of statistical significance. CD5⁺ DLBCL cases with gain of 13q21.1-q34 showed significantly inferior survival than did CD5⁺ cases without such gain (Fig. 3A). In contrast, gain at the corresponding region did not affect survival of CD5⁻ DLBCL cases. Similarly, CD5⁺ DLBCL cases with loss of 1p34.3-p36.21 showed significantly inferior survival than did CD5⁺ cases without such loss (Fig. 3B), whereas loss at the corresponding region did not affect survival of CD5⁻ DLBCL cases. Conversely, CD5⁻ DLBCL cases with a gain of 5p showed superior overall survival (Fig. 3C) to those without such gain, but a gain of 5p had no impact on survival of CD5⁺ DLBCL cases. A list of BAC/PACs associating with prognosis of CD5⁺ or CD5⁻ DLBCL and prognostic data can be found in Supplementary Data 1.

DISCUSSION

Array CGH methods have been successfully used for the high-resolution analysis of genomic alterations not only in a variety of solid tumors (7–9, 25, 26) but also in hematological malignancy (27–30). In this study, we used our own array CGH (10) to analyze 70 cases of DLBCL and identified regions of genomic gain and loss of DLBCLs that were relevant to clinical subtypes and patient survival.

Array CGH detected aberrations of several loci that were undetectable by chromosomal CGH (also known as conventional CGH). Loss of 3p14.2 was one such aberration. This lost locus was detected by array CGH in 18 of 66 DLBCL cases but in none by chromosomal CGH. The responsible regions for 13 of these 18 cases were covered by a single BAC, RP11-48E21, which included the *FHIT* tumor suppressor gene. This clearly demonstrates that array CGH is more sensitive than chromosomal CGH. These findings also suggest that array CGH is a useful tool for identifying and narrowing down to the target genes. It should be noted, however, that the efficacy of array CGH is limited to identification of copy number changes of genome, and it can therefore not detect chromosomal translocations, mutations, and epigenetic events that could affect gene expressions. The use of the array CGH method in combination with other newly developed technologies such as microarray analysis of transcripts and SKY analysis of chromosomes may further facilitate our understanding of molecular events underlying DLBCL.

We reported previously that chromosomal CGH analyses of patient samples identified six recurrent regions of gain (3q, 6p, 11q21-q24, 12q, 13q22-q32, and 18q) and four recurrent regions of loss (1p, 6q, 17p, and 19p; ref. 17). Array CGH analysis of the same set of patient samples found several novel recurrent regions of gain such as 1q32, 5p13, 7p22-q31, 8q24.13-q24.21, and 16p13, as well as recurrent
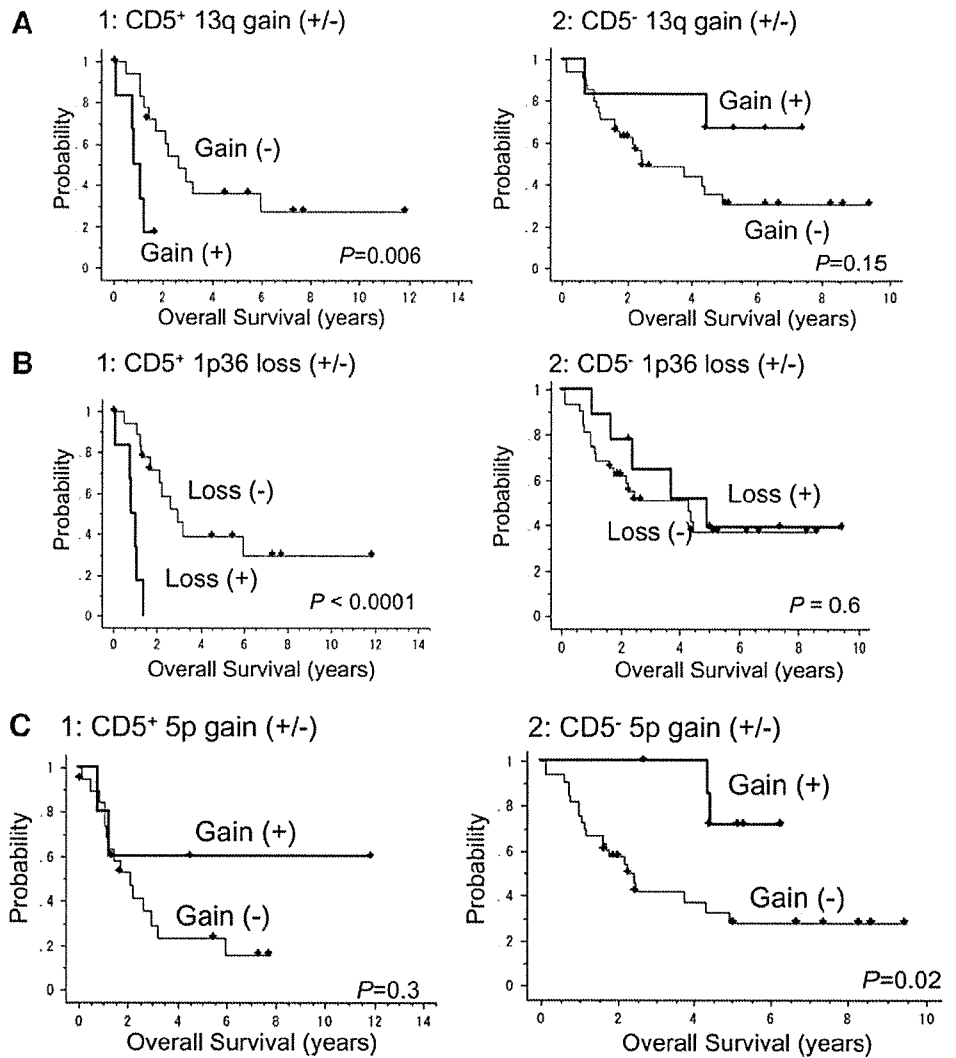
Fig. 3. Kaplan-Meier survival curves for CD5⁺ and CD5⁻ DLBCL cases in the presence or absence of 13q gain, 1p36 loss, and 5p gain. Overall survivals for the CD5⁺ and CD5⁻ groups. *Horizontal lines:* overall survival (years); *vertical lines:* probability. *A*, survival curves for CD5⁺ cases (*panel 1*) according to the presence (6 cases) or absence (19 cases) of gain of 13q21.1-q31.3 and survival curves for CD5⁻ cases (*panel 2*) according to the presence (6 cases) or absence (35 cases) of gain of 13q21.1-q31.3. *B*, survival curves for CD5⁺ cases (*panel 1*) according to the presence (6 cases) or absence (19 cases) of loss at 1p36.13-p36.21 and survival curves for CD5⁻ cases (*panel 2*) according to the presence (9 cases) or absence (32 cases) of loss at 1p36.13-p36.12. *C*, survival curves for CD5⁺ cases (*panel 1*) according to the presence (4 cases) or absence (21 cases) of gain at 5p14.2-p15.33 and survival curves for CD5⁻ cases (*panel 2*) according to the presence (7 cases) or absence (34 cases) of gain at 5p14.2-p15.33.

regions of loss such as 3p14.2 and 9p21. However, loss of 19p detected by chromosomal CGH could not be confirmed in array analysis. The reasons for this apparent discrepancy between chromosomal CGH and array CGH are not yet entirely clear. One possibility is that chromosome 19 contains blocks of heterochromatin that are difficult to assess with chromosomal CGH.

It is therefore possible that the discrepancy is due to the unreliable results obtained with chromosomal CGH for these regions. Other researchers reported that the use of chromosomal CGH methods resulted in the identification of 1q21-q23, 2p12-p16, 3q26-q27, 7q11, 8q24, 9q34, 11cen-q23, 12p, 12cen-q13, 13q32, 16p12, 16q21, 18q21-q22, and 22q12 as recurrent regions of gain and 1p34-ptel, 6q23-qtel, 8p22-ptel, 17p12, and 22q as recurrent regions of loss (31–33). Our array CGH could not identify 9q34 and 22q12 as recurrent regions of gain. Gains of 9q34 and 22q12 were found in only 5 cases (7.6%) and 6 cases (9.1%), respectively, of a total of 66 DLBCL cases. This discrepancy could be due to differences in the patient samples analyzed and in the sensitivity of the methods used.

Array CGH analysis has been applied by Martinez-Climent *et al.* (29) to follicular lymphoma and DLBCL transformed from follicular lymphoma. They reported that 14 regions of gain and 9 regions of loss were acquired as a result of the transformation. Among these 23 regions, gains of 4p12-pter, 9q13-q31, and 17q21 and losses of 4q21-q23, 5q21-q23, 9q31-qter, 11q24-q25, and 13q14-q21 were not

found as recurrent genomic aberrations in our study of *de novo* DLBCL. Conversely, we found genomic alterations such as gains of 11q24, 13q13, and 16p13 and loss of 3p14 in our study of *de novo* DLBCL but not in the reported cases of transformed DLBCL. These findings suggest *de novo* DLBCL and transformed DLBCL are different in terms of the genes responsible for lymphomagenesis.

Array CGH analysis identified largely identical genomic aberration patterns in the CD5⁺ and in CD5⁻ groups. However, gains of 10p14-p15.3 and 19q13.32–13.43 and losses of 1q43-q44 and 8p23.1-p23.2 were found to be characteristic of CD5⁺ DLBCL. These findings, in addition to characteristic clinical behavior, indicate that CD5⁺ DLBCL is a distinct entity. It has not been demonstrated yet that genes included in the regions of 10p14-p15.3 and 1q43-q44 are linked to malignancy, but the 19q13.2-q13.43 region includes tumor-related genes such as *BAX*, *PEG3* (34), *CD37*, and *IL4R1*, with the last one having been identified as a gene responsible for primary mediastinal diffuse large B-cell lymphoma (35). Genomic loss of 8p23 has frequently been found in leukemic MCL (36), and this deletion may be linked with leukemic dissemination and poor prognosis for patients with MCL. Given that, in addition to MCL, 8p23 is recurrently lost in CD5⁺ DLBCL, one could speculate that this locus may contain tumor suppressor genes accounting for poor prognosis of patients with both CD5⁺ DLBCL and leukemic MCL.

It was also found that although gain of 13q21.1-q34 and loss of

1p34.3-p36.21 had deleterious impacts on the survival of CD5$^+$ cases, these regions had no such impact on CD5$^-$ cases. We are currently analyzing expressions of genes that are included in the loci specifically gained or lost in CD5$^+$ DLBCL to gain additional insights into their roles in lymphoma development. One possible candidate gene for 13q31-q32 amplification is *C13orf25*, which has been found in the case of gain of 13q31-q32 in B-cell lymphoma. *C13orf25* contains several microRNAs (miR-17, miR-18, miR-19a, miR19b-1, miR-20, and miR-92-1) that might be associated with lymphomagenesis (10, 37). There have been no reports, however, on tumor suppressor gene(s) of 1p34.3-p36.21.

In contrast to the CD5$^+$ cases, we were not able to find genomic regions, either gained or lost, which were characteristic of CD5$^-$ DLBCL, although gain of 5p was found to be associated with a favorable survival. More cases need to be analyzed, however, to clarify the prognostic significance of this gain.

To summarize, we subjected DLBCL cases to array CGH analysis and found genomic regions recurrently altered in DLBCL. By comparing CD5$^+$ and CD5$^-$ cases, we were able to identify genomic alterations specific to the CD5$^+$ DLBCL group. Array CGH analysis can thus be expected to provide new insights into the genetic background of lymphomagenesis.

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