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Chromosomal Gain Aberrations Predominate in Murine Myeloid Leukemia Induced by Continuous Exposure to Low-dose γ-radiation

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How continuous exposure to low doses of radiation affects lifespan and health status is essentially unknown. Leukemia is a type of blood cancer that develops in humans and mice soon after irradiation. We compared chromosomal aberrations between LDR-induced murine myeloid leukemia (ML) and spontaneous ML to clarify the leukemogenic effect of chronic low-dose radiation (LDR). Formalin-fixed, paraffin-embedded spleens from mice with spontaneous ML (n = 11) and ML that developed after 400 days of exposure to 20 mGy/22 h/day of γ-radiation (n = 11) were analyzed using array comparative genomic hybridization (array CGH). We found that gain aberrations predominated among the chromosomal anomalies of LDR-induced ML, whereas loss aberrations were most prevalent in spontaneous ML. The genomic regions with gain aberrations of LDR-induced ML included the *Etv6*, *Ntrk2* and *Rasgrf2* genes that are associated with leukemia or self-renewal. Exposure to chronic LDR induces ML via a mechanism that differs from that of spontaneous ML.

Key words: continuous radiation exposure, low dose-rate irradiation, mice, myeloid leukemia, genomic aberration

1. Introduction

The accidents at the Fukushima nuclear power plant focused attention on the long-term health effects of chronic low-dose radiation (LDR). Myeloid leukemia (ML) is induced by high doses of radiation (HDR). Epidemiological studies have shown that protracted exposure to low doses of radiation significantly increases risk for leukemia[1-3], and chromosomal instabilities are promoted in individuals who work or live in environments with high background radiation such as nuclear workers[4], medical radiologists[5], and residents in areas of China with high background radiation[6], as well those working or living in buildings contaminated with radiation in Taiwan[7]. However, whether LDR-induced chromosomal aberrations contribute to the onset of ML is uncertain. Associations between LDR-induced chromosomal instability and ML that develops in environments with high background radiation must be determined to prevent and treat LDR-induced leukemia.

Several studies have shown that continuous exposure to low levels of radiation above a specific dose rate has malignant effects in mice. Exposing B6C3F1 mice to 20 mGy/day of γ-radiation for 400 days shortened the lifespan of both sexes by about 100 days[8]. However,

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exposure to 1 and 0.05 mGy/day of γ-radiation for 400 days decreased the lifespan of female mice by only 10 days and did not affect the lifespan of either sex, respectively. One of the cancers that shortened the lifespan of male mice exposed to 20 mGy/day of radiation was ML[9]. Although irradiated males with other cancers died sooner than non-irradiated males, the incidence of ML in irradiated males was significantly increased. The incidence of ML in females exposed to 20 mGy/day of γ-radiation did not increase, but the onset was earlier. That is, > 20 mGy/day of LDR has leukemogenic effects in mice.

Here, we investigated genomic aberrations in formalin-fixed, paraffin-embedded (FFPE) tissues of mice with ML induced by 20 mGy/day of γ-radiation. The acute ML induced in mice by HDR usually has a large hemizygous deletion of chromosome 2, whereas mice with spontaneous ML do not[10]. Radiation is thought to cause the chromosome 2 deletion[11, 12], but whether or not mice with LDR-induced ML have the same genomic aberrations is unknown. Innovations in universal linkage systems have allowed genomic DNA analysis in archival FFPE tissues[8] using array CGH[13]. Our results of a CGH analysis showed that genomic aberrations differ between LDR-induced and spontaneous murine ML.

2. Materials and methods

2.1. Animals, radiation exposure, pathology

All ML samples were collected from specific-pathogen-free (SPF) B6C3F1 Jcl mice during a previous study[8]. Eight-week-old SPF B6C3F1 mice housed in a specific pathogen-free animal room under 12-h light/dark cycles were exposed to 8 Gy of ^{137}Cs γ-radiation at a rate of 20 mGy/22 h/day. Tissue samples were also obtained from non-irradiated mice that developed spontaneous ML.

Myeloid leukemia was diagnosed from histopathological findings of Hematoxylin and eosin-stained, FFPE tissues of the spleen, femur, sternum, lymph nodes, liver and kidneys of mice[8]. All experiments proceeded according to Japanese legal regulations governing the humane care of animals and the Guidelines for Animal Experiments at the Institute of Environmental Sciences.

2.2. DNA extraction from formalin-fixed, paraffin-embedded tissues

Genomic DNA was extracted from myeloid leukemic cells in sections (5-μm-thick) of FFPE spleens using QIAamp DNA FFPE Tissue kits (Qiagen, Valencia, CA, USA). The sections were deparaffinized using xylene, lysed under denaturing conditions with proteinase K, and then incubated at 90℃ as described in the manufacturer's protocol supplied with the kit. The lysed samples were washed with buffers AW1 and AW2, and then membrane-bound DNA was eluted from the membrane buffer ATE. Normal genomic DNA was extracted from the ear using DNeasy Blood & Tissue kits (Qiagen).

2.3. Detection of genomic alterations by array CGH

Array CGH analysis proceeded as described in the protocol supplied with the genomic DNA ULS (Universal Linkage System) labeling kit (Agilent Technologies, Palo Alto, CA, USA). The CGH microarray consisted of 60,000 spots that corresponded to 15,000 genetic regions determined from 415 leukemia-related genes[10]. Genomic DNA samples from myeloid leukemic cells and from the ear (0.5 mg) were respectively labeled with Cy3-dCTP and Cy5-dCTP, combined, mixed with mouse *Cot1* DNA, and hybridized with the CGH microarray. The fluorescent intensity of spots on the CGH microarray was analyzed using Genomic Workbench Lite Edition (Agilent). The $\log_2$ ratios of the intensities of Cy3 to those of Cy5 were normalized both by centralization (threshold, 6.0; bin size, 10.0) and fuzzy zero algorithms. The normalized $\log_2$ ratios of triplicate spots were averaged in 15,000 regions. The $\log_2$ ratios were averaged for each 50 Mbp, where the threshold of the Z-score algorithm was set at 2.0.

3. Results

3.1. Myeloid leukemia develops earlier in mice exposed to low-dose radiation

We previously showed that 400 continuous days of exposure to 20 mGy/day of LDR significantly increases the incidence of ML in male but not female mice[8]. Figure 1A and B shows the rates of ML at 200-day intervals in the mice. All mice exposed to 20 mGy/day of γ-radiation including females who developed ML died sooner than non-irradiated (0 mGy/d) mice with ML. Female and male mice with ML that were exposed to 20 mGy/d died 156 and 211 days earlier, respectively, than those with ML who were not irradiated ($P < 0.01$, t-test, Table 1). Thus, 20 mGy/day of γ-radiation promoted ML in mice of both sexes. We analyzed tissue samples from mice with ML that were exposed to 0 and 20 mGy/d of γ-radiation (n = 11 per group) using the array CGH (Table 2).

3.2. Genomic regions with gain and loss aberrations differ between LDR-induced and spontaneous ML

Formaldehyde that is used to prepare FFPE samples is mutagenic[12]. We therefore tested ULS-labeled genomic DNA extracted from the FFPE spleens of two mice with HDR-induced acute ML before analyzing the 20 and 0 mGy/d ML samples by array CGH (Fig. 2). Both rAML-1 (A) and rAML-2 (B) have previously been analyzed[13]. The ULS-labeled genomic DNA tended to have more gain and loss aberrations than the enzymatically labeled genomic DNA (Fig. 2). The rAML-1 findings indicated 10 more

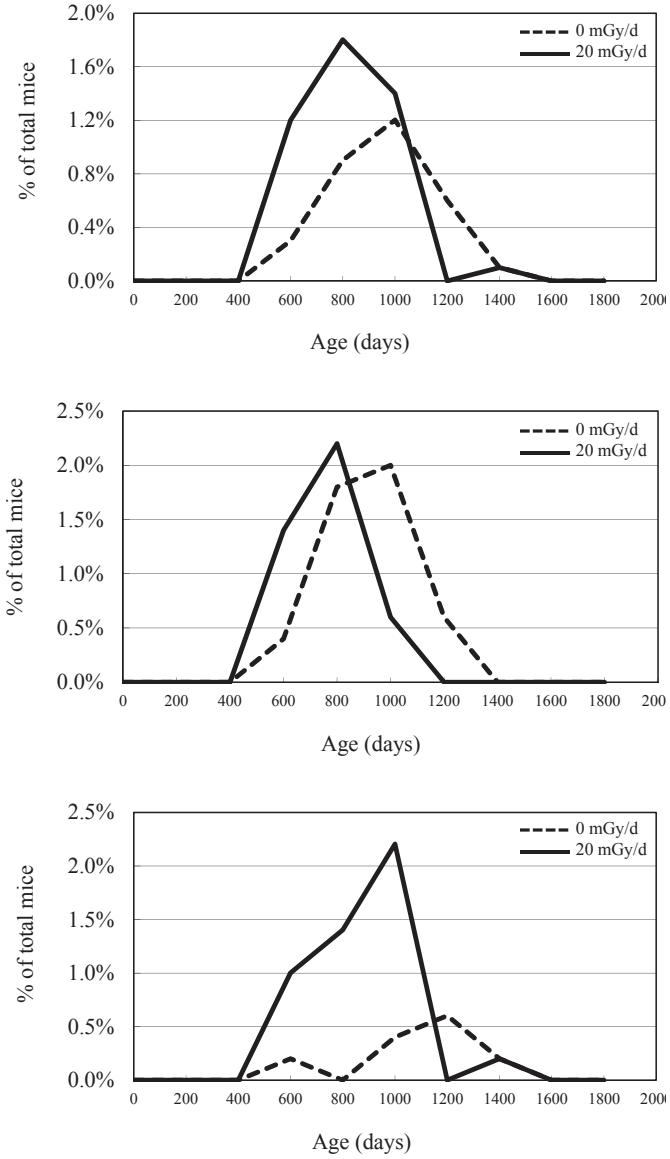

Fig. 1. Mice exposed to low-dose γ-radiation developed myeloid leukemia (ML) earlier than non-irradiated mice.
Rates of ML at 200-day intervals in all mice. Eight-week-old B6C3F1 mice were continuously exposed to 20 mGy/d of γ-radiation for 400 days and then transferred to non-irradiated room, where control non-irradiated B6C3F1 mice (0 mGy/d) were housed. A, B and C, all (n = 31 (0 mGy/d) and n = 45 (20 mGy/d)), female (n = 24 (0 mGy/d) and n = 21 (20 mGy/d)) and male (n = 7 (0 mGy/d) and n = 24 (20 mGy/d)) mice, respectively, with ML.

gain and four more loss aberrations in 13 chromosomes (Fig. 2A). The rAML-2 findings indicated two more loss aberrations in two chromosomes (Fig. 2B). Deletions around qA1-5 (25 Mb), qE2.1-4 (39 Mb) of chromosome 1 and qA1-3 (37 Mb) of chromosome 3 were found in both rAML-1 and 2. Hence, formalin fixation had indeed induced several genomic aberrations.

We compared genomic aberrations between ML in mice exposed to 20 and 0 mGy/d of γ-radiation (Fig. 3). Defining 80% of all ML samples as the threshold, fewer chromosomes had common deletions in ML induced by 20 mGy/d than in ML that spontaneously developed in the absence (0 mGy/d) of radiation (one and eight chromosomes, respectively; Fig. 3A and B). In contrast, 11 and four chromosomes had common amplifications in LDR-induced and spontaneous ML. Chromosomal amplifications in chromosomes 3, 4, 6, 7, 9, 13, 17 and 19 were frequent only in 20 mGy/d ML, and deletions in chromosome 4 and amplifications in chromosomes 11, 15 and 16 appeared in both types of ML. No deletions were specific for the LDR-induced ML.

3.3. Candidate genes associated with LDR-induced ML

The microarrays used herein were designed to focus on leukemia-related genes. The 60,000 probes in the microarrays were generated from four copies of 15,000 oligonucleotides corresponding to genomic DNA sequences within alleles of leukemia-related genes. To improve the reliability of the array CGH results with ULS-labeling, we extracted leukemia-related genes with genomic aberrations by comparing the fluorescence intensity of four copies of oligonucleotides in each allele with those at baseline (Table 3). A few genes with allelic gains and 21 genes with allelic losses were found in 80% of all samples of spontaneous (0 mGy/d) ML, whereas ML induced by 20 mGy/d of radiation had 18 and no genes with allelic gains and losses, respectively. Among the 18 genes with allelic gains in LDR-induced ML, only *Fzd2* and *Cacng2* were identical to those found in spontaneous ML. The following 7 of the 18 genes with allelic gains were located within three of the 11 chromosomes with

Table 1. Comparison of Age of death between LDR radiation-induced and spontaneous leukemia mice[9]

Group	Dose rate of γ-ray (mGy/22hours/day)	Total dose (Gy)	No. of mice	No. of leukemia (% of total death)	Age of death with leukemia (day)	*P*-value
all						
0 mGy/d	non-irradiated	–	998	31 (3.1)	871±173	0.001
20 mGy/d	20	8	999	45 (4.5)	739±159	
female						
0 mGy/d	non-irradiated	–	500	24 (4.8)	832±131	0.0002
20 mGy/d	20	8	500	21 (4.2)	676±122	
male						
0 mGy/d	non-irradiated	–	498	7 (1.4)	1004±239	0.013
20 mGy/d	20	8	499	24 (4.8)	793±169	

Table 2. List of myeloid leukemias used in this study[9)]

strain	Irradiation		No.	gender	Onset after beginning of irradiation (day)	Diagnosis
	Dose rate	Total dose				
B6C3F1	20 mGy/22hr/day	8 Gy	20 mGy/d- 1	female	509	Leukemia, Granulocytic
			20 mGy/d- 2	female	513	Leukemia, Erythroid
			20 mGy/d- 3	female	559	Leukemia, Myelomonocytic
			20 mGy/d- 4	female	588	Leukemia, Granulocytic
			20 mGy/d- 5	female	630	Leukemia, Granulocytic
			20 mGy/d- 6	female	697	Leukemia, Myelomonocytic
			20 mGy/d- 7	female	743	Leukemia, Myelomonocytic
			20 mGy/d- 8	male	588	Leukemia, Myelomonocytic
			20 mGy/d- 9	male	676	Leukemia, Myeloid, NOS
			20 mGy/d-10	male	712	Leukemia, Granulocytic
			20 mGy/d-11	male	875	Leukemia, Myeloid, NOS
	0 mGy/day	0 Gy	0 mGy/d- 1	female	702	Leukemia, Granulocytic
			0 mGy/d- 2	female	734	Leukemia, Granulocytic
			0 mGy/d- 3	female	775	Leukemia, Granulocytic
			0 mGy/d- 4	female	807	Leukemia, Granulocytic
			0 mGy/d- 5	female	830	Leukemia, Granulocytic
			0 mGy/d- 6	female	847	Leukemia, Myelomonocytic
			0 mGy/d- 7	female	918	Leukemia, Granulocytic
			0 mGy/d- 8	female	985	Leukemia, Granulocytic
			0 mGy/d- 9	female	1006	Leukemia, Granulocytic
			0 mGy/d-10	male	1042	Leukemia, Granulocytic
			0 mGy/d-11	male	1127	Leukemia, Granulocytic
C3H	1.0 Gy/min	3 Gy	rAML-1[a]	male	121	Monocytic leukemia
			rAML-2[b]	male	165	Myelomonocytic leukemia

rAML-1[a] and rAML-2[b] correspond to samples in previous study[12)].

gain aberrations in LDR-induced ML (Figure 3): *Etv6* (qG1) and *Dusp16* (qG1) in chromosome 6, *Bmp6* (qA3.3), *Cage1* (qA3.3), *Ntrk2* (qB2) and *Rasgrf2* (qC3) in chromosome 13 and *Tmem10* (qC3) in chromosome 19. The rates of gain aberrations of these genes ranged from 18% to 45% and those in the four genes in chromosome 13 ranged from 18% to 36% in spontaneous ML. Thus, the allelic gains in chromosome 13 were specific to LDR-induced ML.

4. Discussion

This study aimed to elucidate genes that contribute to the LDR-induced onset of murine ML. Myeloid leukemia rarely developed in LDR-irradiated mice and appeared after several hundred days of irradiation (Table 1). Therefore, we used array CGH analysis to analyze FFPE samples of spleens from mice with LDR-induced and confirmed ML[8, 9)]. The results of the array CGH analysis showed that most genomic anomalies were gain aberrations (Table 3). Genomic loss aberrations were found not only in chromosome 4 of irradiated mice with ML, but also in that of mice with spontaneous ML. We found characteristic gains of the *Bmp6*, *Cage1*, *Ntrk2* and *Rasgrf2* genes at the allelic level in chromosome 13 that were rarely identified in spontaneous ML. Other studies of C3H mice that are the paternal progenitors of B6C3F1 showed that large hemizygous deletions of chromosome 2 around the *PU.1* allele comprise a unique chromosomal aberration in murine HDR-induced leukemia, and that these deletions are rare in spontaneous C3H leukemias[10, 14)]. Although the common chromosomal aberrations for LDR radiation-induced ML identified herein involved deletions of chromosome 2, these deletions were also frequent in spontaneous ML (Fig. 3A). Further studies should investigate how differences in murine strains affect ML induced by LDR and HDR. The present results of the array CGH analysis indicated that unique genes with allelic mutations in LDR-induced ML are responsible for the early onset of ML.

Seven of 18 genes with allelic gains were found within frequently amplified chromosomes of LDR ML (Fig. 3 and Table 3). We explored the possible contributions of these genes to leukemogenesis in mice irradiated with LDR. The Ingenuity Knowledge Base (Ingenuity Systems, Redwood City, CA, USA) provides information about biological interactions and functional annotations among genes, proteins, complexes, cells, tissues, drugs and diseases. Based on the assumption that allelic gain is likely to increase gene expression, the Ingenuity Knowledge Base predicted that allelic gains of *Etv6*, *Ntrk2* and *Rasgrf2* play several important roles in leukemogenesis (Table 4)[15-30)]. The development of leukemic stem cells seems to require at least one gene mutation that promotes self-renewal and another that blocks or suppresses cell differentiation[30)]. Gains of *Etv6*, *Ntrk2*

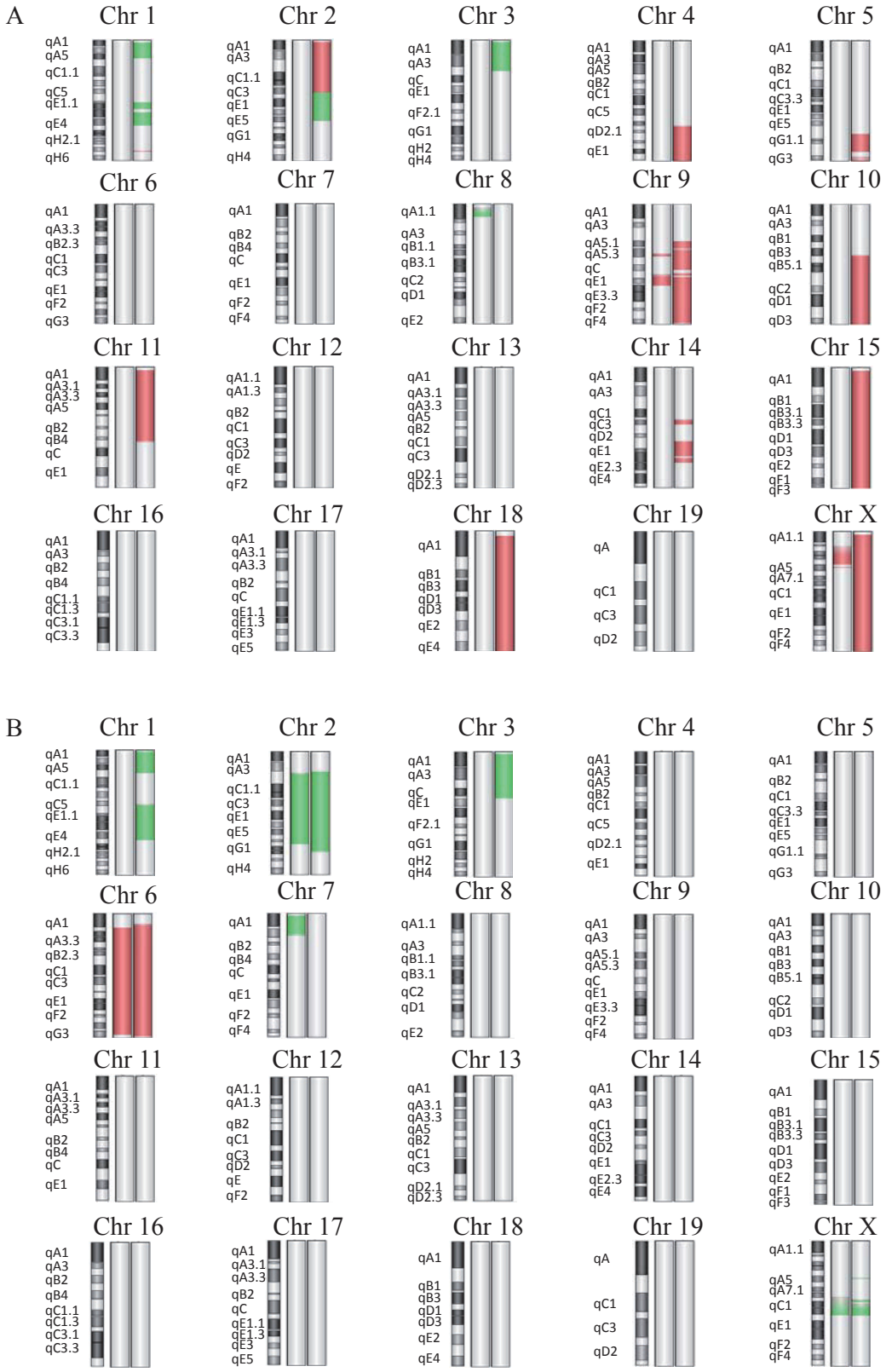

Fig. 2. Comparison of array CGH results of myeloid leukemia (ML) induced by high-dose radiation between Universal Linkage System (ULS) and enzymatic labeling.
Genomic DNA in FFPE tissues derived from ML induced by 3 Gy of γ-radiation (rate, 1.0 Gy/min), rAML-1 (A) and 2 (B) were fluorescently labeled using (ULS). Large hemizygous deletion of chromosome 2 and amplification of chromosome 6 evident in rAML-2 are characteristic of murine ML induced by high-dose radiation. Bars labeled "E" and "U" show array CGH results of enzymatic- and ULS-labeling, respectively. Green and red regions indicate chromosomal losses and gains. Chromosome cytobands are shown on left side of panel.

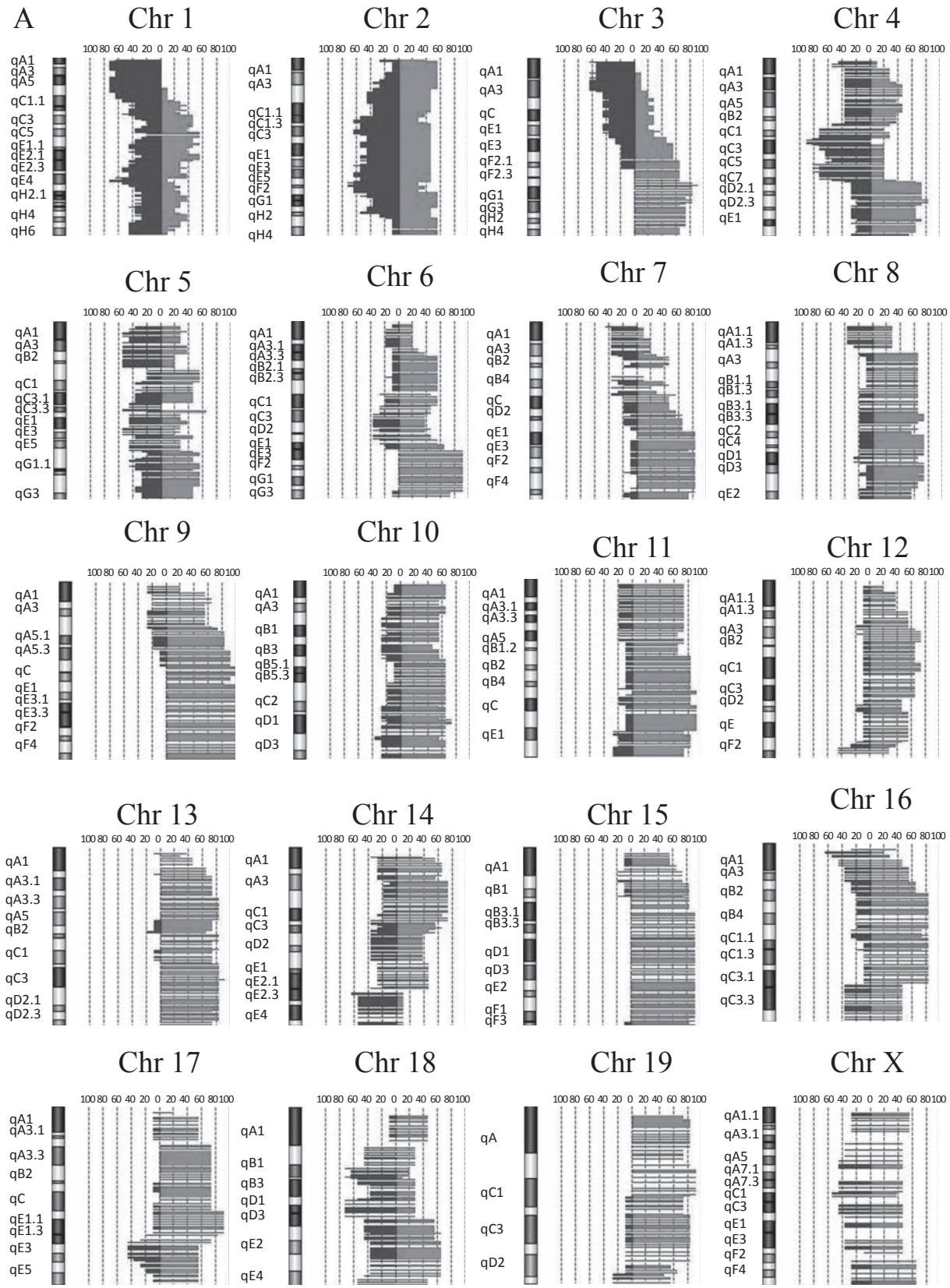

Fig. 3. Genome-wide gains and losses in low-dose radiation (LDR)-induced and spontaneous myeloid leukemia.
Summary of gain and loss aberrations per chromosome in murine LDR-induced (A), and spontaneous (B) MLs (n = 11 each). Genomic DNA extracted from FFPE tissues was labeled with fluorescence using Universal Linkage System (ULS). Plus (gray) and minus (dark gray) indicate frequency of gains and of losses, respectively. Chromosome cytobands are shown on left side of panel.

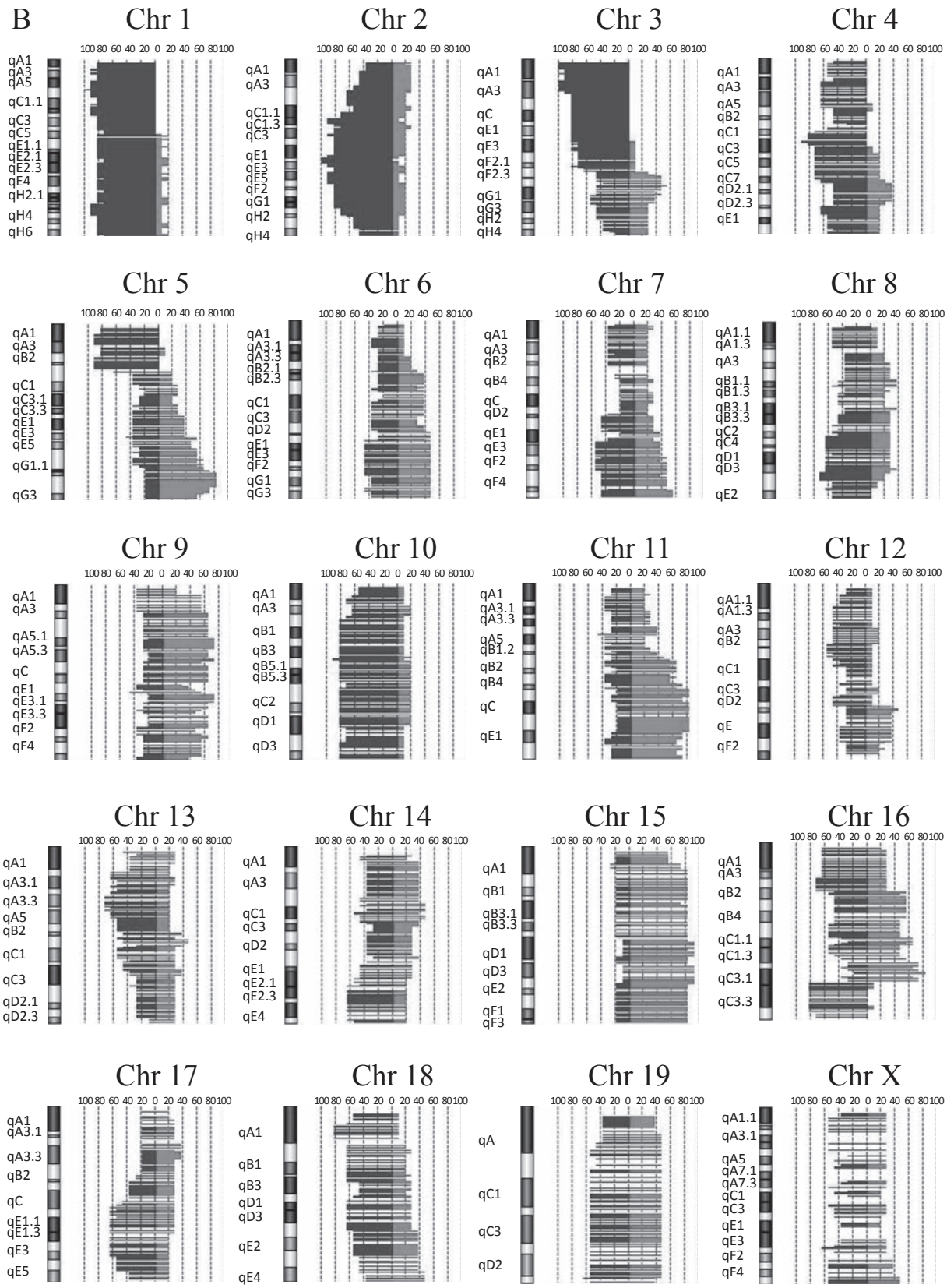
B
Chr 1
Chr 2
Chr 3
Chr 4
Chr 5
Chr 6
Chr 7
Chr 8
Chr 9
Chr 10
Chr 11
Chr 12
Chr 13
Chr 14
Chr 15
Chr 16
Chr 17
Chr 18
Chr 19
Chr X

Table 3. Genes indicated to bear gain/loss alleles with high reliability by calculation of signal intensity of spots on CGH microarray

Location	Cytoband	Genes	20 mGy/d (*n*=11)		0 mGy/d (*n*=11)	
			Gain	Loss	Gain	Loss
Chr 1	qC4	*Speg*	45%	-9%	0%	-73%
	qD	*Hdac4*	45%	-36%	9%	**-82%**
	qG3	*Cacna1e*	36%	-27%	18%	**-82%**
	qH2.1	*Rc3h1*	27%	-27%	9%	**-91%**
		Dnm3	27%	-27%	9%	**-91%**
		Tnr	27%	-27%	9%	**-91~82%**
	qH4	*Akt3*	36%	-27%	9%	**-82%**
		D230039L06Rik	36%	-27%	9%	**-82%**
		Sdccag8	36%	-27%	9%	**-82%**
	qH5	*Tgfb2*	9%	-45%	9%	**-82%**
Chr 2	qA3	*Cacna1b*	55%	-27%	27%	-45%
	qC3	*Zfp385b*	27%	-64%	9%	**-91%**
	qE2	*Cd44*	45%	-45%	0%	**-100%**
	qE5	*Mapkbp1*	45%	-55%	9%	**-91%**
	qF3	*Plcb1*	27%	-64%	18%	**-82%**
		Plcb4	27%	-64%	18%	**-82%**
Chr 3	qA1	*Ralyl*	9%	-55%	0%	**-91%**
	qA3	*Cldn11*	18%	-45%	0%	**-82%**
		Nlgn1	9%	-64%	0%	**-100%**
Chr 4	qA3	*Cngb3*	27%	-36%	0%	-45%
	qC6	*Jak1*	18%	-73%	18%	-73%
	qD1	*Pik3r3*	73%	-27%	36%	-45%
Chr 5	qE5	*Mapk10*	27%	-45%	45%	-36%
Chr 6	qF1	*Cacna1c*	**91%**	0%	45%	-45%
	qF3	*Ntf3*	**91%**	0%	45%	-45%
		Clec2g	**91%**	0%	45%	-45%
	qG1	*Etv6*	**91%**	0%	45%	-36%
		Dusp16	**91%**	0%	45%	-36%
Chr 7	qD1	*Igf1r*	45%	-18%	18%	-18%
Chr 8	qB3.2	*Feb29*	73%	-18%	27%	-45%
	qD3	*Nfat5*	73%	-9%	27%	-64%
	qE1	*Zdhhc7*	64%	-9%	9%	-55%
Chr 9	qC	*Smad3*	**100%**	0%	64%	-27%
Chr 10	qB1	*Frk*	55%	-18%	9%	**-82%**
	qB3	*Ros1*	55%	-27%	9%	**-82%**
	qD1	*Lin7a*	64%	-27%	9%	-73%
	qD2	*Xpot*	64%	-27%	9%	**-82%**
Chr 11	qA3.1	*Meis1*	73%	-18%	18%	-36%
	qE1	*Prkca*	**82%**	-18%	64%	-36%
		Fzd2	**91%**	-9%	**82%**	-18%
Chr 12	qC3	*Daam1*	64%	-9%	18%	-27%
Chr 13	qA3.3	*Bmp6*	**82%**	0%	18%	-73%
		Cage1	**82%**	0%	18%	-73%
	qB2	*Ntrk2*	**82%**	0%	36%	-45%
	qC3	*Rasgrf2*	**82%**	0%	27%	-27%
Chr 14	qA3	*Cacna2d3*	73%	-18%	36%	-36%
	qD1	*Gata4*	36%	-36%	27%	-36%
	qD2	*Gfra2*	36%	-36%	27%	-36%
	qE5	*Fgf14*	9%	-55%	18%	-55%
Chr 15	qE1	*Cacng2*	**91%**	0%	**91%**	-9%
Chr 16	qB2	*Fgf12*	**82%**	-18%	55%	-45%
		Pak2	**82%**	-18%	55%	-45%
	qB5	*Cd96*	**82%**	-18%	45%	-55%
Chr 18	qA1	*Zfp521*	45%	-9%	9%	**-82%**
	qB3	*Ppp2r2b*	27%	-36%	27%	-45%
	qE2	*Dcc*	64%	-36%	36%	-55%
	qE4	*Cd226*	45%	-55%	45%	-36%
Chr 19	qC3	*Tmem10*	**82%**	-9%	45%	-55%
	qD1	*Sorcs1*	**82%**	-9%	45%	-55%
Chr X	qA1.1	*Bcor*	55%	-27%	27%	-55%
	qA3.3	*Il13ra1*	45%	-36%	27%	-55%
	qA5	*Gpc3*	45%	-45～36%	27%	-36%
	qA7.3	*Dusp9*	45%	-45%	27%	-36%
		Tbl1x	45%	-45%	27%	-45%
	qE1	*Rps6ka6*	45%	-36%	18%	-36%
	qF4	*Rps6ka3*	64%	-27%	36%	-55%
	qF5	*Figf*	64%	-27%	45%	-55%
		Mid1	64%	-27%	45%	-55%

Gain and loss frequencies per gene were summarized by integrating results of 4 copies of oligonucleotides whose averaged intensities were significantly different from those of baselines ($P<0.01$).
Bold letters show the allelic gains or losses more than 80% of examined myeloid leukemias

Table 4. Predictions of effects of 3 genes with gain aberration on LDR radiation-induced MLs

Bio-function	Effect on bio-function[a]				Predicted state by gene aberration
	Etv6	*Etv6-fusion gene*[b]	*Ntrk2*	*Rasgrf2*	
accumulation of progenitor cells		Pos (*AML1*)[14]			Increase of progenitors
cancer	Pos[15]	Pos (*Jak2*)[16], Pos (*PDGFRB*)[17]	Pos[18]		Progression of carcinogenesis
proliferation of cells	Pos[19]	Pos (*Ntrk3*)[20, 21]	Pos[18]	Pos[22]	Increase of cells
quantity of cells		Pos (*Jak2*)[16]	Pos[23-25]		Incrase of cells
differentiation of lymphocyte		Neg (*AML1*)[14]			Inhibition of lymphocyte differentiation
differentiation of pro-B lymphocyte		Neg (*AML1*)[14]			Inhibition of pro-B lymphocyte differentiation
hematopoiesis of bone marrow cells	Pos[24]				Inhibition of hematopoiesis in bone marrow
production of B lymphocytes		Neg (*AML1*)[14]			Decrease of B lymphocytes
apoptosis	Neg[25]		Neg[26]		Suppression of apoptosis
apoptosis of granule cells			Neg[26]		Suppression of apoptosis in granule cells
necrosis	Neg[25]		Neg[26]		Suppression of necrosis
cell transformation		Pos (*Ntrk3*)[21], Pos (*Jak2*)[27], Pos (*MN1*)[28]			Promotion of cell transformation
tumorigenesis of malignant tumor	Pos[15]	Pos (*PDGFRB*)[17]			Promotion of malignant tumorigenesis

[a] "Pos" and "Neg" show that each gain aberration of gene is possible to positively and negatively effects on a bio-function. The numbers as superscripts correspond to reference numbers of previous studies yielding the predicted result.
[b] Effects of fusion genes with *Etv6*. Genes in parentheses show fused with *Etv6*.

and *Rasgrf2* imply enhanced cell proliferation[16, 19, 23-26]. In addition, allelic gains of *Ntrk2* also predict the suppression of cell death[27]. Cell proliferation that was enhanced and cell death that was suppressed by LDR-induced gene mutations might have led hematopoietic cells to mutate into leukemic cells and gain a survival advantage in hematopoietic tissues. As a result, the spleens of mice with LDR-induced ML seem to harbor many more cells with these gene mutations. With respect to cell differentiation, the hemizygous allelic deletions of *PU.1* that contribute to blocking myeloid maturation in murine HDR-induced leukemia[31] were rarely found in LDR-induced ML. Our prediction showed that the differentiation of pro-B and B lymphocytes is negatively affected by *Etv6-AML1* fusion genes[15]. However, to accurately detect *Etv6-AML1* fusion genes in FFPE samples was complicated by the fact that the genomic DNA was highly fragmented. Further studies of fresh, unfixed ML samples should confirm these predictions.

Array CGH analysis of FFPE samples tended to indicate false genomic aberrations (Fig. 2). For example, genomic losses were found in rAML-1 and 2 in the qA and qE bands of Chromosome 1, and in the qA band of Chromosome 3. Therefore, we attempted to remove false genomic aberrations by statistically analyzing the fluorescence intensity of four copies of the relevant probes. However, these results should also be confirmed in fresh samples of ML. In addition, further study should clarify genomic aberrations of *Etv6*-fusion genes in LDR-induced MLs in more detail.

It is generally accepted that chromosomal aberrations induced by radiation is supposed to be chromosomal deletion. Therefore, the partial chromosomal gain aberrations in LDR-induced MLs were caused by unknown effects of LDR radiation. We speculated that the partial chromosomal gains aberrations were caused by DNA replication-based rearrangement[32]. Our previous study showed that HDR-radiation caused long-term decreases of hematopoietic stem cell after irradiation[10], and Ban *et al.* suggested a possibility that the hematopoietic stem cells were forced to proliferate several months after irradiation[11]. Those might be also occurred in hematopoietic stem cells in mice exposed to 8 Gy of LDR-radiation, and the excess DNA replication could induce partial chromosomal gain aberrations. However, to evaluate the hypothesis, further study will investigate the cell cycle and genomic instability of hematopoietic stem cell during and after irradiation.

The present findings revealed that murine LDR-induced MLs had eight unique chromosomal regions with gain aberrations that were rare in spontaneous ML. Fusion genes with *Etv6*, and increases in the expression of *Ntrk2* and *Rasgrf2* that were involved in the common chromosomal aberrations appeared to promote the development of leukemic stem cells. In conclusion, the genes that induce ML in mice exposed to LDR differ from those in mice with spontaneous ML that arises in the absence of radiation.

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