Differences of Leukemic Stem Cells of Acute Myeloid Leukemias Induced by High-Dose-Rate and Low-Dose-Rate Gamma-ray Irradiations

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Abstract

It is important to clarify how mice exposed to high-dose γ-rays at a low-dose rate (LDR) have a significantly higher leukemia incidence, although LDR γ-ray irradiation has very few DNA double strand breaks. Then, this study focused on clarifying the leukemic stem cells of LDR radiation-induced leukemias, by comparing them with those of high-dose-rate (HDR) or middle-dose-rate (MDR) γ-ray-induced leukemias. The LDR, MDR or HDR γ-ray-induced leukemias were obtained from mice which were exposed to 8,000 mGy at 20 mGy/22h/day, 4,000 mGy at 400 mGy/22h/day and 3,000 mGy at 890 mGy/min, respectively. Leukemic stem cells from γ-ray-induced leukemias at three dose rates were identified by intravenous transplantation to syngeneic mice of 100 cells each of 7 populations corresponding to different hematopoietic differentiation stages. The leukemic stem cells from HDR and MDR γ-ray-induced leukemias had hemizygous deletions of chromosome 2 around *PU.1* allele and both showed identical CD antigen profiles to those of normal common myeloid progenitor and similar gene expression profiles to those of normal common myeloid progenitor. In contrast, leukemic stem cells from LDR or a part of the MDR γ-ray-induced leukemias with intact *PU.1* allele did not reveal similar profiles for CD-antigen and gene expression to those of normal common myeloid progenitor, but frequently resembled those of common lymphoid progenitor cells, granulocytes and monocytes. These results showed a possibility that murine leukemias induced by LDR γ-ray irradiation might be independent of directly induced DNA damages by radiation and the origin of their leukemic stem cells was in lymphoid lineages cells, which was in contrast to that of HDR γ-ray leukemogenesis.

Table 1 Similarities of gene expression profiles of rAML stem cells with those of HSC, MPP, CMP and CLP

CD antigen profile[a]	AML sample	Population with highest similarity with rAML stem cell[b]	Confidence Measure[c]
LSK-	1.0 Gy/min-2	Common myeloid progenitor	0.63
	1.0 Gy/min-3	Common myeloid progenitor	0.94
	1.0 Gy/min-9	Multipotent progenitor	0.95
LSK+	0 mGy/day-2	Multipotent progenitor	0.95
	1.0 Gy/min-10	Multipotent progenitor	0.83
CMP	1.0 Gy/min-1	Common myeloid progenitor	0.97
	1.0 Gy/min-2	Common myeloid progenitor	0.89
	1.0 Gy/min-3	Common myeloid progenitor	0.98
	1.0 Gy/min-6	Common myeloid progenitor	0.97
	1.0 Gy/min-9	Common myeloid progenitor	0.93
	1.0 Gy/min-10	Common myeloid progenitor	0.91
CLP	0 mGy/day-7	Common lymphoid progenitor	0.66
	20 mGy/day-1	Multipotent progenitor	0.65
	20 mGy/day-3	Multipotent progenitor	0.95
Gr-1^{pos}	400 mGy/day-4	Common lymphoid progenitor	0.91
	1.0 Gy/min-9	Common myeloid progenitor	0.95
	1.0 Gy/min-10	Common myeloid progenitor	0.93
CD45R/$B220^{pos}$	20 mGy/day-1	Common myeloid progenitor	0.83
	20 mGy/day-3	Common lymphoid progenitor	0.97
	400 mGy/day-3	Common lymphoid progenitor	0.88
	1.0 Gy/min-10	Hematopoietic stem cell	0.55

[a]CD antigen profile of rAML stem cell. Each abbreviation represents specific CD antigen profiles as follows: LSK-, $lin^{neg}Sca\text{-}1^{pos}c\text{-}kit^{pos}CD34^{neg}$; LSK+, $lin^{neg}Sca\text{-}1^{pos}c\text{-}kit^{pos}CD34^{pos}$; CMP, $lin^{neg}Sca\text{-}1^{neg}c\text{-}kit^{pos}CD34^{pos}$; CLP, $lin^{neg}Sca\text{-}1^{pos}c\text{-}kit^{neg}CD34^{pos}$; Gr-$1^{pos}$; and CD45R/$B220^{pos}$.

[b]Population with highest similarities with rAML stem cell computed by the Run Prediction algorithm of GeneSpring.

[c]Confidence Measure scored by the Run Prediction algorithm gives reliability of the results.

Table 2 Alterations of expression levels of 23 genes by allelic copy number aberration had potentials to contribute to cell proliferation and anti-apoptosis in rAML stem cells.

Chromosome	Cytoband	Gene	Chr. aberration[a]		Ratio to normal CMP[b] (p value)	Effect of mutated gene on CMP-like rAML stem cell[c]	Enhancement of *Myc* expression[d]
			Amp (%)	Del (%)			
chr2	qH2	*Rbl1*	0%	33%	0.5 (0.16)	Cell proliferation (TGF-β)	Up-regulation
chr6	qA2	*Cav2*	33%	0%	3.0 (0.21)	Anti-apoptosis (Focal adhesion)	
		Cav1	33%	0%	3.0 (0.30)	Anti-apoptosis (Focal adhesion)	
		Met	33%	0%	2.3 (0.12)	Cell proliferation (Focal adhesion), Anti-apoptosis (Focal adhesion)	
		Wnt2	33%	0%	3.8 (0.18)	Cell proliferation (WNT)	Up-regulation
	qA3.1	*Wnt16*	33%	0%	2.2 (0.38)	Cell proliferation (WNT)	Up-regulation
	qA3.3	*Lep*	33%	0%	2.8 (0.26)	Cell proliferation (JAK-STAT), Anti-apoptosis (JAK-STAT)	Up-regulation
	qB2.3	*Cul1*	33%	0%	1.1 (0.83)	Cell proliferation (Cell cycle, TGF-β), Anti-apoptosis (TGF-β)	Up-regulation
	qC1	*Il12rb2*	33%	0%	1.8 (0.55)	Cell proliferation (JAK-STAT), Anti-apoptosis (JAK-STAT)	Up-regulation
		Il23r	33%	0%	3.0 (0.23)	Cell proliferation (JAK-STAT), Anti-apoptosis (JAK-STAT)	Up-regulation
	qD1	*Tgfa*	33%	0%	2.5 (0.32)	Cell proliferation (ErbB), Anti-apoptosis (ErbB)	
		Wnt7a	33%	0%	1.8 (0.54)	Cell proliferation (WNT)	Up-regulation
	qE1	*Cav3*	33%	0%	1.5 (0.71)	Anti-apoptosis (Focal adhesion)	
	qE3	*Raf1*	33%	0%	1.4 (0.40)	Cell proliferation (MAPK, ErbB, Focal adhesion, VEGF), Anti-apoptosis (ErbB, Focal adhesion)	
	qF1	*Cacna2d4*	33%	0%	2.2 (0.41)	Cell proliferation (MAPK)	
		Wnt5b	33%	0%	1.8 (0.27)	Cell proliferation (WNT)	Up-regulation
	qF3	*Ntf3*	33%	0%	2.5 (0.29)	Cell proliferation (MAPK)	
		Fgf6	33%	0%	2.1 (0.43)	Cell proliferation (MAPK)	
		Fgf23	33%	0%	2.4 (0.39)	Cell proliferation (MAPK)	
		Csda	33%	0%	1.3 (0.53)	Cell proliferation (Tight junction), Anti-apoptosis (Tight junction)	
	qG1	*Lrp6*	33%	0%	1.9 (0.06)	Cell proliferation (WNT)	Up-regulation
		Cdkn1b	33%	0%	1.8 (0.32)	Cell proliferation (ErbB, Cell cycle)	

[a]The frequency of chromosomal copy number aberration of the gene in 6 rAMLs (rAML-1, 2, 3, 6, 9, and 10). rAMLs: radiation-induced acute myeloid leukamias

[b]The ratio of the averaged expression level of the gene in the 6 CMP-like rAMLs to that in 5 CMPs. P values are shown in parentheses (Student's t-test).

[c]Effect of the gene on CMP-like *PU.1*$^{del/mut}$ rAML logically predicted by referring to the KEGG pathway database (http://www.genome.jp/kegg/pathway.html). The pathway through which the gene affected the CMP-like *PU.1*$^{del/mut}$ rAML is abbreviated within parentheses as follows: JAK-STAT (Jak-Stat signaling pathway); MAPK (MAPK signaling pathway); TGF-β (TGF-beta signaling pathway); focal adhesion (focal adhesion); WNT (Wnt signaling pathway); cell cycle (cell cycle); ErbB (ErbB signaling pathway); and VEGF (VEGF signaling pathway)

[d]Prediction of the effect of the gene on enhancement of Myc expression by referring to the KEGG pathway database.