7.3.2 高線量率及び低線量率γ線照射マウスに生じた白血病の幹細胞の違い

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

廣内 篤久，田中 聡，田中 イグナシア III ブラガ，一戸 一晃，
小木曽 洋一，田中 公夫
生物影響研究部
Tokuhisa HIROUCHI, Satoshi TANAKA, Ignacia Braga-TANAKA, Kazuaki ICHINOHE, Yoichi OGHISO, Kimio TANAKA
Department of Radiobiology

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.

1. 目的

これまでの調査研究により、高線量率γ線照射のみでなく、20 mGy/22h/day の低線量率γ線を長期連続照射し、総線量が高線量に達したマウスでも白血病が非照射群に比べて有意に高頻度で出現することが明らかになった（Tanaka *et al.* 2003, Tanaka *et al.* 2007）。本調査研究では、異なる線量率の放射線が白血病誘発に及ぼす影響を明らかにするために、高線量率、中線量率、低線量率のγ線照射によってそれぞれ誘発された白血病と非照射マウスに自然発生した白血病についてゲノム異常、白血病細胞の細胞分化段階と白血病幹細胞の起源となる細胞を比較した。これらの結果をまとめ、異なる線量率の放射線によって誘発される白血病のメカニズムの違い等について考察した。

2. 方法

高線量率放射線誘発白血病の好発系であるC3H/He Nrs 雌雄マウス（Hayata *et al.* 1979）に、低線量率（20 mGy/22 hr/day; 以下 20 mGy/day）γ線を約 400 日間連続照射（集積線量 8 Gy)、あるいは中線量率（400 mGy/22 hr/day; 以下 400 mGy/day）γ線を 10 日間連続照射（集積線量 4 Gy）した。また比較のために、C3H/He Nrs 雄マウスに高線量率（0.9 Gy/min）γ線を約 3 分間照射（集積線量 3 Gy）した。また非照射で自然発症したマウスも用いた。各実験群で白血病を発生したマウスの脾臓と大腿骨から白血病細胞を採取してアレイ CGH 法によってゲノム異常を検出した。また、CD 抗体で特異的細胞表面抗原を標識し細胞分化段階を FACS にて解析した。FACS 解析と同時に 7 つの細胞分化段階の細胞を分取し、その 100 細胞ずつを同系（C3H/He Nrs）マウスに尾静脈から移植して経過を観察し、白血病幹細胞を特定した。白血病の発症が認められた移植した細胞集団から、RNA を抽出し発現アレイ法によって全遺伝子の発現解析を行った。末梢血のスメア標本をメイ・グリュンワルド・ギムザ染色し、顕微鏡下で白血病の診断を行った。さらに、心臓、肺、肝臓、腎臓をホルマリン固定後、組織標本を作製して顕微鏡下で病理組織学的検索を行った。

3. 成果の概要

移植実験によって特定された高線量率照射群の白血病幹細胞と、低線量率照射群及び非照射群の白血病幹細胞は、遺伝子発現プロファイルの比較から、それぞれ正常マウス由来の骨髄球系共通前駆細胞とリンパ球系共通前駆細胞に近い特性を有していることが分かった（Table 1)。また、これら高線量率照射群の骨髄球系共通前駆細胞様白血病幹細胞と、低線量率照射群・非照射群のリンパ球系共通前駆細胞様白血病幹細胞の遺伝子発現プロファイルをそれぞれ健常なマウス由来の骨髄球系共通前駆細胞、リンパ球系共通前駆細胞の遺伝子発現プロファイルと比較したところ、細胞増殖を促進する働きを持つ *Myc* 遺伝子の発現上昇がそれぞれ認められた。また、この *Myc* 遺伝子の発現上昇は、高線量率照射群の白血病幹細胞では 2 番染色体以外の領域に含まれる複数の遺伝子によって引き起こされている可能性がアレイ CGH による染色体異常の結果と遺伝子発現プロファイルによって示された（Table 2)。

引用文献

Hayata, I. *et al.* (1979) *J Natl Cancer Inst.* **63**, 843-848.

Tanaka, S. *et al.* (2003) *Radiat Res.* **160**, 376-379.

Tanaka. I.B. 3rd *et al.* (2007) *Radiat Res.* **167**, 417-437.

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-1^{pos}c-$kit^{pos}CD34^{neg}$; LSK+, lin^{neg}Sca-1^{pos}c-$kit^{pos}CD34^{pos}$; CMP, lin^{neg}Sca-1^{neg}c-$kit^{pos}CD34^{pos}$; CLP, lin^{neg}Sca-1^{pos}c-$kit^{neg}CD34^{pos}$; Gr-1^{pos}; and CD45R/$B220^{pos}$.

rAML: radiation-induced acute myeloid leukamia

[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.