6.3.4 低線量率ガンマ線連続照射マウスの造血幹細胞の遺伝子発現プロファイルの解析

Analysis of Gene Expression Profiles of Hematopoietic Stem Cell of Mice Exposed to Continuous Low Dose-rate Gamma-rays

廣内 篤久，田中 聡，田中 イグナシャ，小村 潤一郎
生物影響研究部
Tokuhisa HIROUCHI, Satoshi TANAKA, Ignacia TANAKA, Jun-ichiro KOMURA
Department of Radiobiology

Abstract

We have previously shown that continuous exposure to low dose-rate (LDR) radiation is leukemogenic, and that hematopoietic stem cells (HSCs) in mice irradiated with LDR γ-rays (20mGy/day) were significantly decreased at day 400 of irradiation when compared with age-matched non-irradiated mice. In this study, we investigated the changes in gene expressions that may be related to the observed decrease in HSCs of mice exposed to LDR radiation. RNA extracted from HSCs of irradiated and non-irradiated mice was subjected to gene expression microarray, and the gene expression profiles were analyzed by bioinformatic methods with the Ingenuity Pathway Analysis software. We observed that less than 1% of the total analyzed genes were differently expressed in HSCs in LDR-irradiated versus non-irradiated mice. The pathway analysis suggested a possibility that bio-functions of “cell viability”, “cell survival”, and “maturation of cells” were promoted in irradiated HSCs. Surprisingly, contrary to high-dose-rate-irradiated HSCs, no bio-function associated with cell death, such as apoptosis, was altered in LDR-irradiated HSCs. These findings suggest that the decreases in HSCs observed in LDR-irradiated mice might be a result of cell differentiation.

1. 目的

これまでの調査によって、低線量率放射線の長期照射は白血病の発症率を上昇させることが明らかになった。前年度の調査では 20 mGy/day の低線量率γ線を 400 日間照射したマウスの造血幹細胞と 2 種類の前駆細胞の遺伝子発現解析を行った結果、それぞれの遺伝子発現プロファイルから予測される生物学的機能が異なることが分かった。今年度調査では、20 mGy/day のγ線に対して最も影響の大きかった造血幹細胞の照射中および照射終了後の遺伝子応答を明快にする事を目的として、照射開始 200 日、300 日、500 日の遺伝子発現解析を行った。

2. 方法

B6C3F1 のオスマウスに SPF 環境下で 8 週齢から 20 mGy/22 h/day の低線量率γ線を 400 日間照射した。照射終了後は非照射区域で 100 日間飼育を行った。照射開始より 200 日、300 日、500 日で、それぞれ 4 匹のマウスの上腕と大腿の骨随から造血幹細胞を採取し、-80℃に保存した。これらの細胞から抽出した微量の RNA を EPICENTRE 社の TargetAmp 2-round amplification kit により増幅、蛍光ラベル（Cy3-UTP の取り込み反応）し、Agilent 社の SurePrint G3 マイクロアレイを用いて遺伝子発現プロファイルを得た。非照射同週齢の 4 匹のマウスの造血幹細胞について

も同様の解析を行い、照射群と比較した。遺伝子発現プロファイルの意味付け作業（Annotation）は、Ingenuity Systems 社の Ingenuity Pathway Analysis (IPA)を使用した。

3. 成果の概要

造血幹細胞は連続照射 200 日、300 日、400 日で非照射群に比べて細胞数が有意に少なく、照射後非照射区域で 100 日飼育した後も回復しなかった。これらの細胞の遺伝子発現解析を行った結果、照射開始 200 日では細胞増殖の促進、細胞死の抑制、300 日目では細胞増殖の抑制、400 日目では細胞死の抑制が起きている事が予測された（Table 1）。400 日目と 500 日目では有意ではないが細胞分化の促進が示された。また、高線量率放射線照射で観察されるアポトーシスなどによる細胞死の促進は観察されなかった。

平成 26 年度は、造血幹細胞と同様に、20 mGy/day 照射群の造血前駆細胞の遺伝子発現解析を行い、今回の造血幹細胞の解析で得られた細胞分化の促進が造血幹細胞の下流にあたる前駆細胞でも起きているかを確認する。これまでの 5 年間の成果をまとめることで、低線量率放射線が造血細胞の細胞数に与える影響を継続的に捉え、感受性を示した分化段階の細胞の遺伝子発現解析を行い、最終的には白血病発生につながるストレスを解明し、低線量率放射線の白血病誘発機構の全貌の解明につながる知見が得られることが期待される。

Table 1 Bio-functions predicted to be altered in irradiated hematopoietic stem cells

Irradiation period (days)	Category	Diseases/Functions	Predictions of activation state
200	Cell Death and Survival	cell viability	activated (significant)
		cell viability of tumor cell lines	activated (significant)
		cell survival	activated (significant)
		cell viability of cervical cancer cell lines	activated (significant)
		cell death of connective tissue cells	inactivated
		apoptosis of bone cancer cell lines	inactivated
		cell death of fibroblast cell lines	inactivated
		cell death of kidney cell lines	inactivated
		cell death of bone cancer cell lines	inactivated
		apoptosis	inactivated
		cell death	inactivated (significant)
		necrosis	inactivated (significant)
		cell death of tumor cell lines	inactivated (significant)
		apoptosis of tumor cell lines	inactivated (significant)
	Cellular Growth and Proliferation	proliferation of tumor cell lines	activated (significant)
		proliferation of cells	activated (significant)
		proliferation of endothelial cell lines	activated (significant)
	Cellular Development	proliferation of tumor cell lines	activated (significant)
		proliferation of endothelial cell lines	activated (significant)
	Cellular Function and Maintenance	endocytosis	activated (significant)
		Clathrin mediated endocytosis	activated (significant)
	Hematological System Development and Function	quantity of blood platelets	inactivated
	Tissue Morphology	quantity of blood platelets	inactivated
	Hematopoiesis	differentiation of bone marrow cells	inactivated
300	Hematological System Development and Function	cell movement of antigen presenting cells	inactivated
		quantity of blood cells	inactivated
		quantity of leukocytes	inactivated
	Cellular Development	proliferation of melanoma cell lines	activated
		proliferation of tumor cell lines	inactivated
		proliferation of breast cancer cell lines	inactivated
	Cellular Growth and Proliferation	proliferation of melanoma cell lines	activated
		proliferation of tumor cell lines	inactivated
		proliferation of breast cancer cell lines	inactivated
	Tissue Morphology	quantity of blood cells	inactivated
		quantity of leukocytes	inactivated
	Cell Death and Survival	necrosis	activated
		cell viability of breast cancer cell lines	inactivated
	Organismal Injury and Abnormalities	bleeding of tissue	activated
	Metabolic Disease	glucose metabolism disorder	activated
400	Hematological System Development and Function	aggregation of blood cells	activated
		quantity of red blood cells	activated
		hematocrit	activated
		bleeding time	inactivated (significant)
	Cancer	quantity of tumor	activated (significant)
	Cancer	metastasis of lung	activated
	Cell Death and Survival	apoptosis of breast cancer cell lines	activated (significant)
		apoptosis of tumor cell lines	activated
	Tissue Morphology	permeability of blood vessel	activated
	Tissue Morphology	quantity of red blood cells	activated
	Cellular Development	differentiation of tumor cell lines	inactivated (significant)
	Cardiovascular System Development and Function	permeability of blood vessel	activated
	Tissue Development	aggregation of blood cells	activated
	Hematopoiesis	quantity of red blood cells	activated
500	Cellular Growth and Proliferation	proliferation of connective tissue cells	activated (significant)
		proliferation of fibroblasts	activated (significant)
		proliferation of cancer cells	activated
		proliferation of tumor cells	activated
	Cellular Development	proliferation of fibroblasts	activated (significant)
		proliferation of cancer cells	activated
		proliferation of tumor cells	activated
	Tissue Morphology	quantity of cells	activated
		proliferation of cancer cells	activated
		proliferation of tumor cells	activated
	Cell Cycle	senescence of tumor cell lines	inactivated
		G1 phase	inactivated
	Cancer	cell transformation	activated
	Cell Morphology	morphology of leukocytes	inactivated
		morphology of blood cells	inactivated
		morphology of cells	inactivated
	Cell-mediated Immune Response	differentiation of thymocytes	activated