Expression analysis of radiation-responsive genes in human hematopoietic stem/progenitor cells

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ABSTRACT

To clarify the nature of the genes that contribute to the radiosensitivity of human hematopoietic stem/progenitor cells (HSPCs), we analyzed the gene expression profiles detected in HSPCs irradiated with 2 Gy X-rays after culture with or without an optimal combination of hematopoietic cytokines. Highly purified CD34$^+$ cells from human placental/umbilical cord blood were used as HSPCs. The cells were exposed to 2 Gy X-irradiation and treated in serum-free medium under five different sets of conditions for 6 h. The gene expression levels were analyzed by cDNA microarray, and then the network of responsive genes was investigated. A comprehensive genetic analysis to search for genes associated with cellular radiosensitivity was undertaken, and we found that expression of the genes downstream of *MYC* oncogene increased after X-irradiation. In fact, the activation of *MYC* was observed immediately after X-irradiation, and *MYC* was the only gene still showing activation at 6 h after irradiation. Furthermore, *MYC* had a significant impact on the biological response, particularly on the tumorigenesis of cells and the cell cycle control. The activated gene regulator function of *MYC* resulting from irradiation was suppressed by culturing the HSPCs with combinations of cytokines (recombinant human thrombopoietin + interleukin 3 + stem cell factor), which exerted radioprotective effects. *MYC* was strongly associated with the radiosensitivity of HSPCs, and further study and clarification of the genetic mechanisms that control the cell cycle following X-irradiation are required.

KEYWORDS: umbilical cord blood, radiosensitivity, gene expression, hematopoietic stem and progenitor cells

INTRODUCTION

It has been suggested that the radiosensitivity of hematopoietic organs [1] is attributable to the high cell division rates of hematopoietic cells [2]. Hematopoietic cells are maintained by self-renewal and the differentiation of hematopoietic stem/progenitor cells (HSPCs). Our previous study showed that Tie2, a cell surface antigen related to the quiescence of hematopoietic stem cells [3], was associated with the radiosensitivity of CD34$^+$ human placental/umbilical cord blood cells [4]. Tie2$^+$ hematopoietic stem cells that adhere to osteoblasts are quiescent and anti-apoptotic [3]. While these results suggest that

the radiosensitivities of hematopoietic cells positively correlate with the cell division rates, it is unknown whether the anti-apoptotic activity of quiescent hematopoietic stem cells is affected by radiation.

In addition, hematopoietic cells exhibit an increased risk of becoming malignant after irradiation. The incidence of acute myeloid leukemia in atomic bomb survivors peaked earlier than that of solid cancers, i.e. 3–5 years after irradiation [5]. It is also possible that radioresistant hematopoietic cells are mutated in response to radiation exposure and exhibit anti-apoptotic responses. Although the mutations in leukemic cells originate from unrepaired radiation-induced DNA damage, it is

poorly understood how the DNA repair system in HSPCs responds to radiation. In murine hematopoietic stem cells, DNA repair is associated with the cell cycle and exerts its strongest effects at cell cycle–activated points [6, 7]. Desai *et al.* proposed that *Exonuclease 1* was essential for DNA damage processing in active hematopoietic stem cells [6], and Yulin *et al.* reported that Gadd45a regulated the response to radiation-induced DNA damage in hematopoietic cells [8]. However, it is unknown how these genes interact with each other, and whether there are upstream regulators for these genes. There remains a lack of information on how HSPCs respond to radiation at the level of gene expression in humans.

To understand the critical response of the human hematopoietic system to radiation, the present study analyzed the gene expression profiles of irradiated CD34+-enriched HSPCs derived from placental/umbilical cord blood (CB). Long-term *ex vivo* cultivation of HSPCs derived from CB requires the inclusion of several cytokines, but these molecules stimulate HSPCs and alter their gene expression. By reducing the effects of these cytokines (by employing no-cytokine control groups), we found that *MYC* may act as an important upstream regulator in human HSPCs in the early stages of the post-irradiation response.

MATERIALS AND METHODS
Human CD34+-enriched HSPCs in placental/umbilical cord blood

After informed consent was obtained from five mothers participating in this study, free flowing CB was collected into sterile collection bags containing citrate phosphate dextrose anticoagulant (NIPRO, Osaka, Japan). Within 24 h of collection, individual CB samples were centrifuged for 30 min at 250g in Lymphosepar I, 1.077 ± 0.001 g/ml of Ficoll–Conray solution (Immuno-Biological Laboratories, Takasaki, Japan). Cells from the light fraction of the centrifuged CB were washed three times with phosphate-buffered saline containing 5 mM ethylenediaminetetraacetic acid. Subsequently, the CD34+ cells in the processed CB were enriched using an auto-MACS human CD34 selection kit (Miltenyi Biotech, Bergisch-Gladbach, Germany) according to the manufacturer's instructions. The isolated CD34+ cells were subjected to the experiments described below.

Analysis of the cell surface antigen profiles of CD34+ cells

Purified CD34+ cells have been reported to be enriched in HSPCs [9]. The enrichment of HSPCs in CD34+ cells has previously been investigated by profiling cell surface antigens using flow cytometry. In the present study, the CD34+ cells in CB were incubated for 30 min at 4°C with FITC-conjugated anti-human CD34 (Beckman Coulter Immunotech, Marseille, France) and each of the following fluorescent monoclonal antibodies: PE-conjugated anti-human CD38, PC5-conjugated anti-human CD45, PE-conjugated anti-human CD41, PC5-conjugated anti-human CD45RA (Beckman Coulter, Brea, USA), PE-conjugated anti-human Tie-2 (R&D Systems, Minneapolis, MN), PE-conjugated anti-human CD110, PC5-conjugated anti-human CD117 and PC5-conjugated anti-human CD123 (Becton Dickinson Biosciences, San Jose, CA). After incubation, the CD34+ cells were washed and analyzed using a Cytomics FC500 flow cytometer (Beckman Coulter Immunotech, Marseille, France). A combination of isotype-matched monoclonal antibodies, mouse IgG1-FITC, IgG1-PE and IgG1-PC5 (Beckman Coulter Immunotech, Marseille, France) was used as a negative control.

X-irradiation and culture of CD34+ cells

CD34+ cells isolated from CB were cultured *in vitro* after being exposed to X-rays. Thereafter, 5.0–7.5×10^5 CD34+ cells were suspended in 2.5 ml of serum-free IMDM (Life Technologies, Carlsbad, CA, USA) supplemented with BIT9500 (Stem Cell Technologies, Vancouver, Canada). Cell suspensions with cell densities of 2.0–3.0×10^5 cells/ml were equally divided into five wells of a 24-well plate, and each cell fraction was subjected to different conditions as follows: two of the cell fractions were irradiated with 2 Gy X-rays (150 kVp, 20 mA) using a MBR-1520R-3 device (Hitachi Medical, Tokyo, Japan) with a dose rate of ~1 Gy/min. Then, one of these cell fractions was cultured for 6 h with recombinant human thrombopoietin (TPO, PeproTech, Rocky Hill, NJ), interleukin 3 (IL-3, PeproTech, Rocky Hill, NJ) and stem cell factor (SCF, PeproTech, Rocky Hill, NJ), and the other cell fraction was cultured without cytokines. If there is no cytokine stimulation, the proliferative capacity of hematopoietic stem cells will rapidly disappear [10]. This combination has been reported to be optimal for HSPC survival and proliferation [11, 12]. The next two cell fractions were not irradiated, and were cultured with or without TPO, IL-3 and SCF. The remaining cell fraction was directly used for the next stage of analysis.

Colony-forming units assay

The radiosensitivity of lineage-committed HPCs in CD34+ cells irradiated and/or treated with TPO, IL-3 and SCF was determined by a colony-forming units assay. Five mixtures of hundreds of CD34+ cells were suspended in 1 ml of methylcellulose medium (MethoCult H4230, Stem Cell Technologies, Vancouver, Canada), consisting of a concentration of 50 ng/ml of TPO, 100 ng/ml of IL-3, 100 ng/ml of SCF, 10 ng/ml of granulocyte colony-stimulating factor (G-CSF), 10 ng/ml of granulocyte/macrophage colony-stimulating factor (GM-CSF) (PeproTech, Rocky Hill, NJ) and 4 U/ml of recombinant human erythropoietin (EPO) (Kyowa Hakko Kirin, Tokyo, Japan). The mixtures were transferred to 24-well culture plates at 0.3 ml/well, and then incubated at 37°C for 14 days in a humidified atmosphere containing 95% air/5% CO_2. Three types of colony-forming cells—colony-forming unit granulocyte-macrophages (CFU-GM), erythroid burst-forming units (BFU-Es) and granulocyte-erythroid-megakaryocyte-macrophage colony-forming units (CFU-Mix)—were counted. Colonies consisting of more than 50 cells were counted using an inverted microscope.

Gene expression analysis of CD34+ cells after irradiation and/or treatment with cytokines

The alterations of gene expression in CD34+ cells irradiated and/or treated with cytokines were determined using a gene expression microarray. The total RNA was extracted from each of the five above-mentioned cell fractions (see 'X-irradiation and cell culture of CD34+ cells') using an RNeasy isolation kit (Qiagen, Hilden, Germany), and was checked with a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, USA). Using the extracted RNA as a template, complementary RNA was synthesized with the incorporation of Cy3-labeled UTP according to the Affymetrix GeneChip Expression technical manual (Affymetrix, Santa Clara, USA). The Cy3-labeled complementary RNA was hybridized with the Affymetrix Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, USA). Annotation of the gene

expression profiles was performed using Agilent GeneSpringGX (Agilent Technologies, Santa Clara, USA) and the Ingenuity Pathways Analysis software program (Qiagen, Hilden, Germany). The effects of radiation and cytokine treatment were determined by comparing the results of the various cell fractions.

RESULTS
Sensitivity of the colony-formation units produced from human CD34+ cells in response to X-irradiation

It is well known that human CD34+ cells are enriched in hematopoietic stem cells and various progenitors that produce blood cells, but the effects of radiation on their multipotency remains largely unexplored. Figure 1 shows that expression of the early hematopoiesis-related cell-surface antigens, including CD34, CD38, CD45RA, CD110, CD123 and Tie-2, on the harvested cells was analyzed by flow cytometry according to methods based on previous studies [4, 13]. CD34+ cells are heterogeneous populations that contain various functional cells, such as lineage-committed progenitors, early progenitors [14, 15] and some stromal cells [16]. CD45RA antigen, a member of the CD45 antigen family, is expressed in all cells of hematopoietic origin, except for granulocytes and monocytes [17, 18]. CD110 is the receptor for thrombopoietin, and is expressed on HSPCs and on the cells of the megakaryocytic lineage and platelets [19]. CD123 antigen, which is also known as interleukin-3 receptor alpha chain, is expressed at high levels only on plasmacytoid dendritic cells and basophils, but also at lower levels on monocytes, eosinophils, myeloid dendritic cells, and subsets of hematopoietic

progenitor cells (multipotent and myeloid precursors, but not lymphoid precursors). A tyrosine kinase with immunoglobulin and the epidermal growth factor homology domain 2 (Tie-2) is a receptor for angiopoietin-1, and is expressed in hematopoietic stem cells [3, 20]. Furthermore, the CD34+/CD38− population is well known to be expressed in primitive hematopoietic cells. The rate for CD34+/CD38− cells and CD34+/CD38+ cells was 92.2 ± 4.3% and 3.7 ± 2.6%, respectively (Fig. 1A). So, HSPCs are certainly contained in the cells used in our study.

Therefore, we first analyzed the radiosensitivity of human CD34+ cells in terms of the differentiation status for each cell type by performing colony-forming unit assays. The human CD34+ cells in the CB that were enriched in HSPCs were irradiated with 2 Gy of X-rays, and were colonized in CFU-GM, BFU-E and CFU-Mix in the presence of IL-3, SCF and TPO (Table 1). The mean number of colony-forming cells (CFCs), which were composed of CFU-GM, BFU-E and CFU-Mix, was 266 ± 10 per 10^3 cells (data not shown). The irradiated CD34+ cells exhibited significantly decreased colony numbers in all of the colony-formation assays (the CFU-GM, BFU-E and CFU-Mix in irradiated CD34+ cells were 20.8%, 36.2% and 46.8%, respectively, compared with those in non-irradiated CD34+ cells). Although there were differences in the colony numbers, no significant differences were found among the colony-formation units.

Gene expression analysis

The changes in gene expression associated with the radiosensitivity of the HSPCs were determined by gene expression microarrays. We first
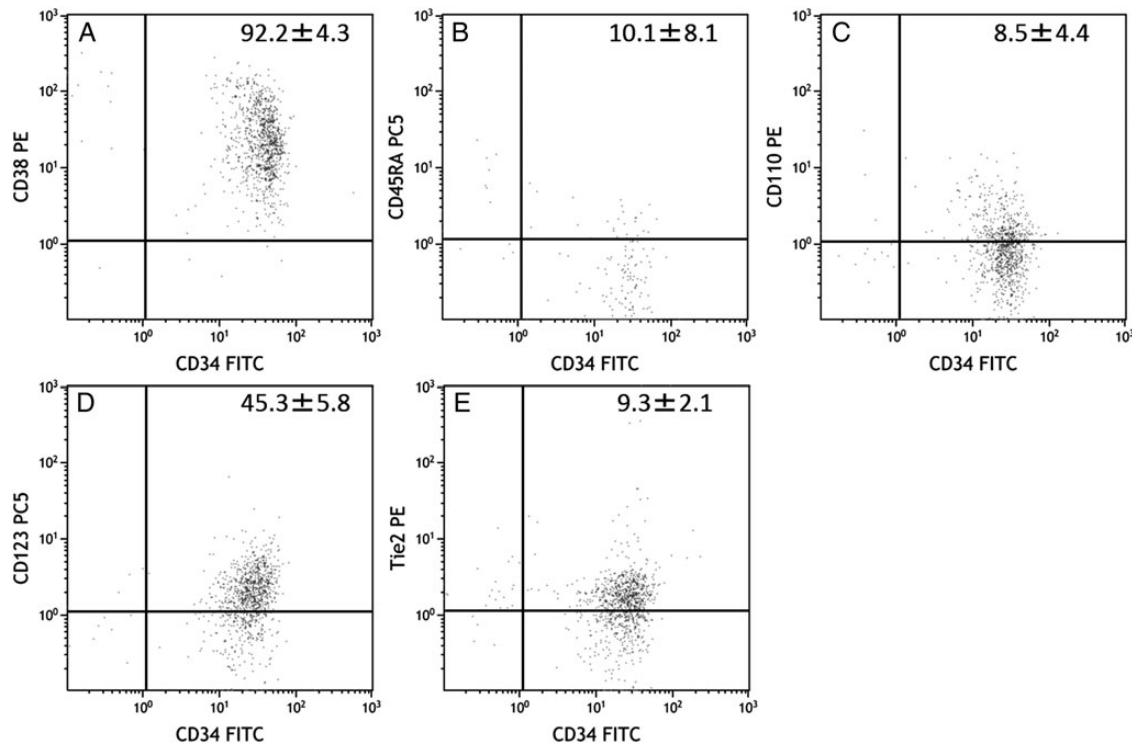

Fig. 1. Representative cell surface antigen profiles of CD34+ cells just after purification from placental/umbilical cord blood. The purified CD34+ cells were counter-stained for the following eight antigens: CD38 [A], CD45RA [B], CD110 [C], CD123 [D] and Tie-2 [E]. These panels indicated that HSPCs were enriched in the purified CD34+ cells.

tried to extract the genes that responded to radiation by comparing the gene expression profiles of irradiated and non-irradiated HSPC groups (Fig. 2). Figure 2A shows the data for three types of radiation-responsive genes whose expression levels were significantly different between the irradiated (IR (+)) and non-irradiated (IR (−)) HSPCs cultured with or without the three cytokines (Cyt(+) or Cyt(−)). The irradiated HSPCs cultured without cytokines (IR(+)Cyt(−)) were also compared with the non-irradiated HSPCs before cultivation (0 h). In the three comparisons, each radiation-responsive gene comprised ~1% of the total 20 108 genes with the lowest P-values. The numbers of radiation-responsive genes were 243 genes in the IR(+)Cyt(+) vs IR(−)Cyt(+) ($P < 0.472$) cells, 237 genes in the IR(+)Cyt(−) vs IR(−)Cyt(−) ($P < 0.0791$) cells and 237 genes in the IR(+)Cyt(−) vs 0 h ($P < 0.00409$) cells. There were 17 radiation-responsive genes common to all groups (Table 2A).

Similarly, the radiation and cytokine-responsive genes were extracted by comparisons between IR(+)Cyt(+) vs IR(−)Cyt(−) and IR(+)Cyt(+) vs 0 h cells (Fig. 2B). There were 215 radiation- and cytokine-responsive genes between the IR(+)Cyt(+) vs 0 h ($P < 0.00308$) cells, and 231 radiation- and cytokine-responsive genes between the IR(+)Cyt(+) vs IR(−)Cyt(−) ($P < 0.0571$) cells. There were 36 common genes between these comparisons (Table 2B).

The Ingenuity pathway analysis software program used for the annotation of the gene expression profiles in the present study, can predict several biological features showing differences between two experimental groups. We therefore extracted the upstream genes affected by radiation based on these annotation findings (Table 3). In all two-group comparisons, *MYC* was activated with z-scores of more

Table 1. The surviving fractions of progenitor cells in irradiated HSPCs

CFU-GM	BFU-E	CFU-Mix	CFCs
0.208 ± 0.018	0.362 ± 0.025	0.468 ± 0.028	0.284 ± 0.016

The numbers show the average and standard deviation of the ratio of the number of colonies in irradiated HSPCs to those in non-irradiated HSPCs of each progenitor type.
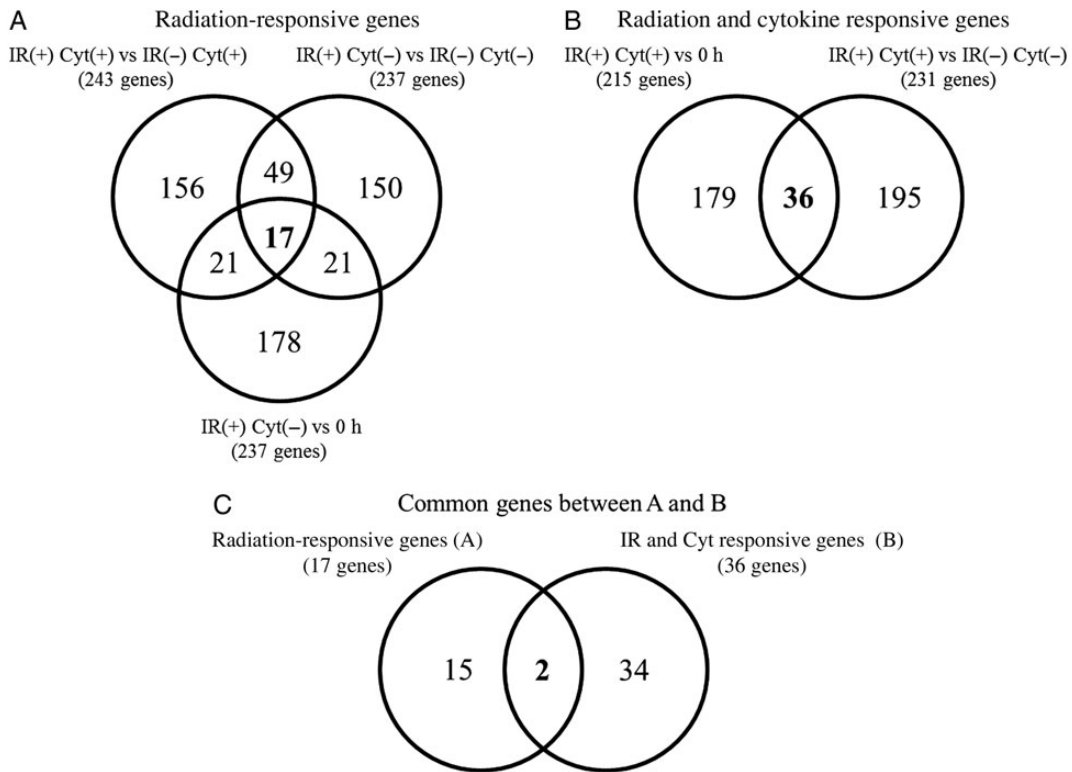

Fig. 2. The numbers of radiation-responsive and radiation- and cytokine-responsive genes obtained by comparing the gene expression levels between two experimental groups. Five experimental groups were classified according to the irradiation condition (IR(+) or IR(−)), cytokine treatment (Cyt(+) or Cyt(−)) and cultivation (6 h or not). The group named '0 h' were non-irradiated HSPCs without cultivation. (A) Venn diagrams showing the numbers of three types of radiation-responsive genes; those differently expressed between IR(+)Cyt(+) vs IR(−)Cyt(+) (243 genes, $P < 0.472$), IR(+)Cyt(−) vs IR(−)Cyt(−) (237 genes, $P < 0.0791$) and IR(+)Cyt(−) vs 0 h (237 genes, $P < 0.00479$). (B) Venn diagrams showing the numbers of the two types of radiation- and cytokine-responsive genes. The expression levels of 215 and 231 genes were significantly different between IR(+)Cyt(+) vs 0 h ($P < 0.00308$), and IR(+)Cyt(+) vs IR(−)Cyt(−) ($P < 0.00571$) cells. (C) Venn diagrams of 17 radiation-responsive genes and 36 radiation- and cytokine-responsive genes.

Table 2. The genes showing changes in expression in response to treatment

(A) Radiation-responsive genes

Symbol	Entrez gene name	Location
BNIP3L	BCL2/adenovirus E1B 19 kDa interacting protein 3-like	Cytoplasm
DCAF13	DDB1 and CUL4-associated factor 13	Nucleus
EIF4G1	eukaryotic translation initiation factor 4 gamma, 1	Cytoplasm
GAL	galanin/GMAP prepropeptide	Extracellular space
GART	phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase	Cytoplasm
MLLT4	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, *Drosophila*); translocated to, 4	Nucleus
NDFIP1	Nedd4 family interacting protein 1	Cytoplasm
NSUN2	NOP2/Sun RNA methyltransferase family, member 2	Nucleus
PMM2	phosphomannomutase 2	Cytoplasm
PNO1	partner of NOB1 homolog (*S. cerevisiae*)	Nucleus
PSMA3	proteasome (prosome, macropain) subunit, alpha type, 3	Cytoplasm
SEH1L	SEH1-like (*S. cerevisiae*)	Cytoplasm
TM4SF1	transmembrane 4 L six family member 1	Plasma membrane
WWC2	WW and C2 domain containing 2	Other
YPEL2	yippee-like 2 (*Drosophila*)	Nucleus
YPEL5	yippee-like 5 (*Drosophila*)	Other
ZNF277	zinc finger protein 277	Nucleus

(B) Radiation- and cytokine-responsive genes

Symbol	Entrez gene name	Location
BNIP3L	BCL2/adenovirus E1B 19 kDa interacting protein 3-like	Cytoplasm
BTBD10	BTB (POZ) domain containing 10	Other
CD70	CD70 molecule	Extracellular space
CERS5	ceramide synthase 5	Cytoplasm
CLEC2B	C-type lectin domain family 2, member B	Plasma membrane
CREBRF	CREB3 regulatory factor	Other
CXCL11		Other
EIF4G1	eukaryotic translation initiation factor 4 gamma, 1	Cytoplasm
Gm6749	predicted pseudogene 6749	Nucleus
HLA-E		Other
LEPROT	leptin receptor overlapping transcript	Plasma membrane
LOC286052	uncharacterized LOC286052	Other
MAK16	MAK16 homolog (*S. cerevisiae*)	Nucleus
MDM2	MDM2 oncogene, E3 ubiquitin protein ligase	Nucleus

Table 2. Continued

(B) Radiation-and cytokine-esponsive genes

Symbol	Entrez gene name	Location
MLLT4	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, *Drosophila*); translocated to, 4	Nucleus
NDFIP1	Nedd4 family interacting protein 1	Cytoplasm
NSUN2	NOP2/Sun RNA methyltransferase family, member 2	Nucleus
OPA3		Other
PI4KB	phosphatidylinositol 4-kinase, catalytic, beta	Cytoplasm
PIK3IP1	phosphoinositide-3-kinase interacting protein 1	Other
PLXND1	plexin D1	Plasma membrane
PNO1	partner of NOB1 homolog (*S. cerevisiae*)	Nucleus
PRKCB	protein kinase C, beta	Cytoplasm
PSMA3	proteasome (prosome, macropain) subunit, alpha type, 3	Cytoplasm
PWP2	PWP2 periodic tryptophan protein homolog (yeast)	Nucleus
RBM28	RNA binding motif protein 28	Nucleus
RIN3	Ras and Rab interactor 3	Cytoplasm
SEH1L	SEH1-like (*S. cerevisiae*)	Cytoplasm
SNX3		Other
TIMP2	TIMP metallopeptidase inhibitor 2	Extracellular space
TM4SF1	transmembrane 4 L six family member 1	Plasma membrane
TMEM68	transmembrane protein 68	Other
UBE2R2	ubiquitin-conjugating enzyme E2R 2	Other
WWC2	WW and C2 domain containing 2	Other
YPEL5	yippee-like 5 (*Drosophila*)	Other
ZBTB11-AS1	ZBTB11 antisense RNA 1	Other

than 2.0. In particular, the predicted alteration of *MYC* regulation from IR(−) Cyt(−) to IR(+) Cyt(−) showed the highest z-score (4.81) of all upstream regulatory genes. The degrees of the changes in *MYC* expression between the IR(+) and IR(−) and Cyt(+) and Cyt(−) cells are summarized in Table 4A. As a result, although the difference in *MYC* expression levels were not statistically significant, it appears that the gene regulator function of *MYC* was enhanced by irradiation when comparing IR(+) and IR(−) cells.

To clarify whether the induced *MYC* actually affected its downstream genes, we investigated the relationships of the 17 radiation-responsive and 36 radiation- and cytokine-responsive genes shown in Fig. 2A and B with *MYC* (Table 4B). As shown in Table 4B, three radiation-responsive genes, *EIF4G1*, *GART* and *PNO1*, and five radiation- and cytokine-responsive genes, *EIF4G1*, *HLA-E*, *MDM2*, *PNO1* and *TIMP2*, are all downstream genes of *MYC*, and *EIF4G1* and *PNO1* were also common genes found in the radiation-responsive and cytokine-responsive genes (Fig. 2C).

DISCUSSION

In the present study, the characteristics of the gene expression detected in highly purified human CD34$^+$ HSPCs exposed to 2 Gy X-irradiation were analyzed. The CD34$^+$ cells were treated under four different experimental conditions after X-irradiation for 6 h as follows: non-irradiated cells treated without cytokines (IR(−) Cyt(−)), non-irradiated cells treated with cytokines (IR(−) Cyt(+)), 2 Gy-irradiated cells treated without cytokines (IR(+) Cyt(−)), 2 Gy-irradiated cells treated with cytokines (IR(+) Cyt(+)). Non-irradiated cells without any treatment were used as a control in this analysis.

In a comparison of the gene expression obtained from cells cultured under the five different conditions, *MYC* was found in all comparisons as shown in Table 3. *MYC* is an oncogene, and it is widely known that increased *MYC* expression is involved in chromosomal translocation [21, 22]. In addition, *MYC* plays an important role in starting the cell cycle, and is associated with the G1/S phase and the G0 phase; the *MYC* expression level is abnormally elevated in many

Table 3. Prediction of the upstream regulators whose activation states were significantly affected by X-ray irradiation

Treatment (A)	Treatment (B)	Upstream regulator	Predicted activation State (A to B)	z-score[a]
0 h	IR(−) Cyt(−)		No data	
0 h	IR(+) Cyt(−)	MYC	Activated	3.07
		MYCN	Activated	2.19
		NFKB1	Activated	2.19
		MGEA5	Inhibited	−2.00
0 h	IR(+) Cyt(+)	MYC	Activated	2.74
		MYCN	Activated	2.23
		OSM	Activated	2.04
		STAT5B	Inhibited	−2.00
		PGR	Inhibited	−2.00
		STAT5A	Inhibited	−2.00
IR(−) Cyt(−)	IR(+) Cyt(−)	MYC	Activated	4.81
		CD28	Inhibited	−2.00
IR(−) Cyt(−)	IR(+) Cyt(+)	MYC	Activated	2.81
		TP53	Activated	2.50
		IL3	Activated	2.24
		FOS	Activated	2.20
		IL2	Activated	2.17
		TP73	Activated	2.00
		TREM1	Activated	2.00
IR(−) Cyt(+)	IR(+) Cyt(+)	MYC	Activated	3.54
		IKBKB	Activated	2.45
		TGFB1	Activated	2.35
		IL13	Activated	2.17
		BRD4	Activated	2.00
		CD28	Inhibited	−3.00

[a]The z-scores were calculated by the 'z-score algorithm' of the IPA software program on the basis of the expression profiles of each downstream gene. Upstream regulators with z-scores higher than 2.0 or lower than −2.0 were predicted to be activated or inhibited, respectively.

human cancers [23–25]. Table 4A shows that the gene regulator function of *MYC* was accelerated by irradiation. Lin *et al.* reported that elevated expression of c-Myc occurs frequently in human cancers and is associated with tumor aggressiveness and a remarkable range of cellular phenotypes, but the effect of high levels of c-Myc on global gene regulation in tumor cells is poorly understood [26]. They showed that the increase in c-Myc occupancy leads to increased transcription elongation by RNA polymerase II and increased levels of transcripts per cell. However, since this experiment was performed in a single under the conditions of only performed 6 h after X-irradiation by using human CD34+ HSPCs, the exact role of *MYC* in CD34+ cells cannot be entirely explained by the presented data.

In addition to the above analyses, the number of altered genes was narrowed down, based on the *P*-values of the IPA statistical program. Finally, two *MYC*-related downstream genes, *EIF4G1* and *PNO1*, were identified as being differentially expressed in response to irradiation (Table 4B). *EIF4G1* encodes human protein eukaryotic translation initiation factor 4 gamma 1, and *PNO1* encodes human protein RNA-binding protein PNO1, i.e. both genes are involved in the functions of RNA.

Another paper has discussed the radiation responses of various genes [27]. In that review, Chiarugi *et al.* described a variety of the conflicting results reported in the literature concerning the effects of dominant oncogenes on sensitivity to irradiation and to anticancer agents in a number of cell lines of human and animal origin. They speculated that the tumor suppressor gene, *TP53*, and the apoptosis suppressor gene, *BCL2*, modulate the effects of dominant oncogenes, and that the effects of dominant oncogenes on the resistance or sensitivity are dependent on the balance between *TP53* and *BCL2* expression [28]. Another study showed that *N-ras* oncogene expression in embryonic fibroblast NIH/3T3 cells increased their radioresistance *in vitro*, and these results have been confirmed and extended to human cell lines expressing the *RAF1* oncogene [29].

FitzGerald *et al.* reported that a clonal hematopoietic progenitor cell line, 32Dcl3, transfected and expressing the *v-myc* oncogene, demonstrated increased radioresistance to both 5 cGy/min and 116 cGy/min [30]. The data on this cell line may correlate with the radioresistance of ABL1-expressing human hematopoietic cell malignancies treated by radiation therapy. Tanaka *et al.* demonstrated that preleukemic, disease-

Table 4. The changes in the expression of *MYC*

(A) The effects of X-irradiation and cytokine treatment on *MYC* expression

Group A	Group B	Upstream regulator		Expression change	
		Predicted activation state	z-score[a]	Fold Change	P-value
0 h	IR(+) Cyt(−)	Activated	3.07	2.13	0.28
0 h	IR(+) Cyt(+)	Activated	2.74	3.29	0.11
IR(−) Cyt(−)	IR(+) Cyt(−)	Activated	4.81	1.48	0.62
IR(−) Cyt(−)	IR(+) Cyt(+)	Activated	2.81	1.18	0.69
IR(−) Cyt(+)	IR(+) Cyt(+)	Activated	3.54	1.32	0.85

[a]The z-scores were calculated by the 'z-score algorithm' using the IPA software program. Upstream regulators with z-scores higher than 2.0 were considered to be in significantly activated states; those with z-scores of less than −2.0 were considered to be significantly inhibited.

(B) The relationship between radiation-responsive and radiation- and cytokine-responsive genes and genes downstream of *MYC*

Symbol	Downstream of *MYC*	Entrez gene name	Groups
EIF4G1	Positive	eukaryotic translation initiation factor 4 gamma, 1	A[a] and B[b]
GART	Positive	phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase	A
HLA-E	Positive	major histocompatibility complex, class I, E	B
MDM2	Positive	MDM2 oncogene, E3 ubiquitin protein ligase	B
PNO1	Positive	partner of NOB1 homolog	A and B
TIMP2	Positive	TIMP metallopeptidase inhibitor 2	B

[a]A: Group of radiation-responsive genes [b]B: Group of radiation- and cytokine-responsive genes.

free Spa-1(−/−) mice exhibited reduced steady-state hematopoiesis and attenuated resistance to whole-body γ-irradiation, which was attributable to a sustained p53 response in HPCs [31]. Preleukemic Spa-1(−/−) HPCs show *c-Myc* overexpression with increased p19Arf, as well as enhanced γH2AX expression with activation of the Atm/Chk pathway. The authors of that study suggested that increased *c-Myc* expression and DNA damage in HPCs precedes myeloproliferative disease (MPD) development in Spa-1(−/−) mice, and the resulting *TP53* response functions as a barrier for the onset of MPD and blast crisis progression. However, little information has been reported regarding the radiosensitivity of HSPCs and the importance of cytokines. The present findings show the possibility that *MYC* is a useful indicator of the radiosensitivity of human hematopoietic cells.

In conclusion, the present study suggests that *MYC* plays a key role in the radiosensitivity of HSPCs, and that *MYC*-regulated genes, particularly *EIF4G1* and *PNO1*, have important functions in the radiosensitivity of human hematopoietic cells. However, more precise experimental approaches are required to confirm and build on our results.

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