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 I was essential for DNA damage processing in active hematopoietic stem cells [6], and Yulin et al. reported that Gadd45α 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 Lymphoprep 1, 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 ethylene-diaminetetraacetic 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 monochromatic 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 × 105 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 × 104 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% CO2. Three types of colony-forming cells—colony-forming unit granulocyte-macrophages (CFU-GM), erythroid burst-forming units (BFU-E) 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 RNase 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 10³ 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
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 < 0.00479) 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 | phosphoribosylglycinamidase formyltransferase, phosphoribosylglycinamidase synthetase, phosphoribosylaminomimidazole 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 |
| YPRL2 | yippee-like 2 (Drosophila) | Nucleus |
| YPELS | 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 |
| CERS | 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 |
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).

**Table 2. Continued**

| 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            |

**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-scorea |
|--------------|--------------|--------------------|----------------------------------|--------|
| 0 h          | IR(−) Cyt(−) | No data            |                                  | −2.00  |
| 0 h          | IR(+) Cyt(−) | MYC                | Activated                        | 3.07   |
|              |              | MYCN               | Activated                        | 2.19   |
|              |              | NFKB1              | Activated                        | 2.19   |
| 0 h          | IR(+) Cyt(+) | MYC                | Activated                        | 2.74   |
|              |              | MYCN               | Activated                        | 2.23   |
|              |              | OSM                | Activated                        | 2.04   |
|              |              | STAT5B             | Inhibited                        | −2.00  |
|              |              | PGR                | Inhibited                        | −2.00  |
|              |              | STAT3A             | 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   |
|              |              | TGFβ1              | Activated                        | 2.35   |
|              |              | IL13               | Activated                        | 2.17   |
|              |              | BRD4               | Activated                        | 2.00   |
|              |              | CD28               | Inhibited                        | −3.00  |

*aThe 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.

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 | Predicted activation state | z-scorea | Expression 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             |

*aThe 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<sup>a</sup> and B<sup>b</sup> |
| GART    | Positive          | phosphoribosylglycinamidase formyltransferase, phosphoribosylglycinamidase synthetase, phosphoribosylaminimidazole 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      |

<sup>a</sup>A: Group of radiation-responsive genes  <sup>b</sup>B: Group of radiation- and cytokine-responsive genes.

free Spa-1<sup>(−/−)</sup> 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<sup>(−/−)</sup> 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<sup>(−/−)</sup> 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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