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Novel Biomarkers in Human Blood for Radiation Exposure

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Abstract

Biomarkers to indicate past exposure to radiation have not yet been entirely satisfactory. Using cDNA microarray hybridization to find new potential biomarkers, we identified highly expressed gene in human peripheral blood lymphocytes (PBL) after *ex vivo* 1Gy irradiation. The present set of radiation markers in PBL was identified 12 h of radiation. Total 44 genes were identified. However, when RT-PCR was performed with mRNA of 5 individual PBL, only 4 genes, including TRAIL receptor 2, DRAL, cyclin G and cyclin protein gene, showed greater than 50% agreement between gene induction detected by microarray and by RT-PCR. When more than 32 donors were tested for the above 4 genes, greater than 85% agreement were obtained between gene induction measured by microarray and by RT-PCR. Furthermore, there was a linear dose-response relationship between 0.5 and 4Gy at 12 hr after irradiation, however, there was less linearity at later times. These results suggested that relative expression levels of genes such as TRAIL receptor 2, DRAL, cyclin G and cyclin protein gene protein in PBL may provide estimates of radiation exposures.

INTRODUCTION

Although, some changes in mRNA levels have been observed at doses below 1 Gy (1,2), and there are a limited number of reports using doses as low as 0.1Gy (2,3), most published studies of radiation-inducible mammalian genes have

applied historically extremely high, even supralethal, doses to ensure strong gene activation, and extrapolation of the results obtained from such studies to physiologically relevant doses can be difficult with fraught. The surveillance mechanisms for DNA double-strand breaks, one of the major lesions produced by ionizing radiation, can be very sensitive (4-6), and mRNA of human myeloid cancer cell line has been carefully quantified to define stress-response genes such as CIP1/WAF1 and GADD45 at doses between 2 and 50 cGy g-rays (7).

As nuclear waste and other sources of man-made radiation rise, it is of increasing interest to develop biological markers that can be used to identify exposed individuals in human populations. The methods used in the past to estimate exposures after nuclear accidents include scoring chromosome aberrations and micronuclei in peripheral blood lymphocytes (PBL), glycophorin A-based somatic mutation in erythrocytes, and electron spin resonance of tooth enamel. With the recent developments in high throughput gene expression screening, it may be possible to develop gene expression profiles in human peripheral blood lymphocytes that correlate with the timing and dose of radiation exposures. The identification of such a gene set would enable more rapid and noninvasive testing of potentially exposed populations.

In this study, we identified 4 genes which were well correlated to individual exposure and exposed doses, therefore, we suggest these genes as possible candidate biomarkers for ionizing radiation exposure in peripheral blood lymphocytes.

MATERIALS AND METHODS

Separation and irradiation of human peripheral blood lymphocytes

Human blood from normal healthy donors who work in Korea Cancer Center Hospital was obtained, and lymphocytes were isolated by centrifugation on a Ficoll-Hypacq (GIBCO) density gradient method.

Total RNA isolation

Isolation of total RNA from cells was carried out by using Trizol agent according to the instruction manual provided by Life Technologies, Inc. (Gaithersburg, MD). Ten million cells were used for total RNA isolation.

Irradiation

Cells were exposed to g-rays with 137Cs g-ray source (Atomic Energy of Canada, Ltd., Ontario, Canada) with dose rate of 3.81 Gy/min.

Microarray analysis

There are three steps (labeling, hybridization, and washing) involved in cDNA microarray. Microarray scanning and data analysis: for complete gene description and grid orientation on NEN MICROMAX cDNA microarrays.

Reverse transcriptase PCR (RT-PCR)

To quantitate mRNA of each gene, total RNA was isolated with TRITM reagent (MRC, Cincinnati, OH) according to manufacturers instruction. Quantitation was carried out using an Image analyzer with MCID software program (Image Research Inc., Ontario Canada).

RESULTS

Genes responsive to ionizing radiation in PBL

To determine which genes expression was altered by radiation, gene expression profiles were obtained using DNA microarray analysis that included 2,400 known human genes. Actually, we did microarray analysis with 2 time points, 6 h and 12 h after radiation. The numbers of altered genes after radiation were much more at 12 hr than at 6 hr. Table 1 shows genes whose expressions were increased in irradiated PBL 12 h of 1 Gy irradiation. More than 2-fold upregulation of 44 genes were noted in the irradiated PBL than the unirradiated PBL.

Confirming the radiation-induced elevated gene expression using RT-PCR

The increase of 44 genes expression in the irradiated PBL was confirmed by RT-PCR analysis. The primer sequences for 44 genes are listed in Table 2. When PBL from 5 individuals after 1 Gy irradiation were tested, only 4 genes (TRAIL receptor 2, DRAL, cyclin protein gene and cyclin G) showed more than 60% correlation with the microarray data (Fig. 1). When we performed RT-PCR analysis of the above 4 genes with PBL from more than 32 additional persons after 1 Gy irradiation, more than 85% of the PBL revealed indication of response to the radiation (Fig. 2 and Table 1). The functions of these genes are: TRAIL receptor 2 is apoptosis inducing gene, DRAL is small GTPase family, and cyclin G and cyclin protein gene are cell cycle regulatory genes.

Dose and time dependency

The next step was to examine time and dose responsiveness of each gene. Thus, we selected a person whose 4 genes showed the highest increased response to radiation. As shown in Fig. 3, when harvested at 12h, all 4 genes showed dose-responsiveness between 0.5Gy and 4Gy, however, their dose responsiveness was disappeared after 24h.

Individual variation in basal level expression

To estimate interindividual variation in the levels of these genes in unirradiated PBL, we tested transcript expression of 12 individuals using RT-PCR. As shown in Fig. 4, when mean levels of TRAIL-receptor 2, DRAL, Cyclin G and Cyclin protein genes in unirradiated PBL after normalization to the levels in the first donor, there was less than 2 fold interindividual variation in the levels of these genes expression in unirradiated PBL, indicating that distinct ranges of uninduced transcript levels, might be defined for these genes.

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Fig. 1

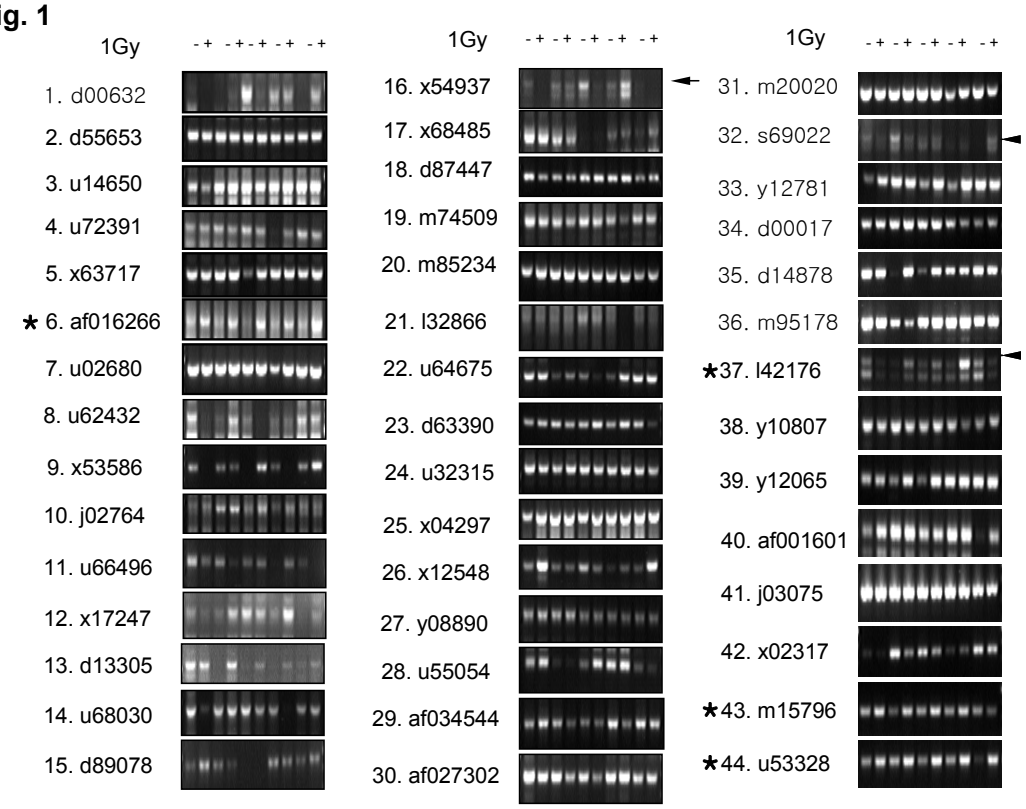

Fig. 1. RT-PCR analysis of 44 genes which were up-regulated in microarray analysis, in peripheral blood lymphocytes (PBL) of 5 donors. PBL was irradiated 1 Gy and RT-PCR analysis was performed at 12 h after irradiation. Relative up-regulation expression ratio by radiation in 5 donors. *Positive response by radiation more than 3 donors.

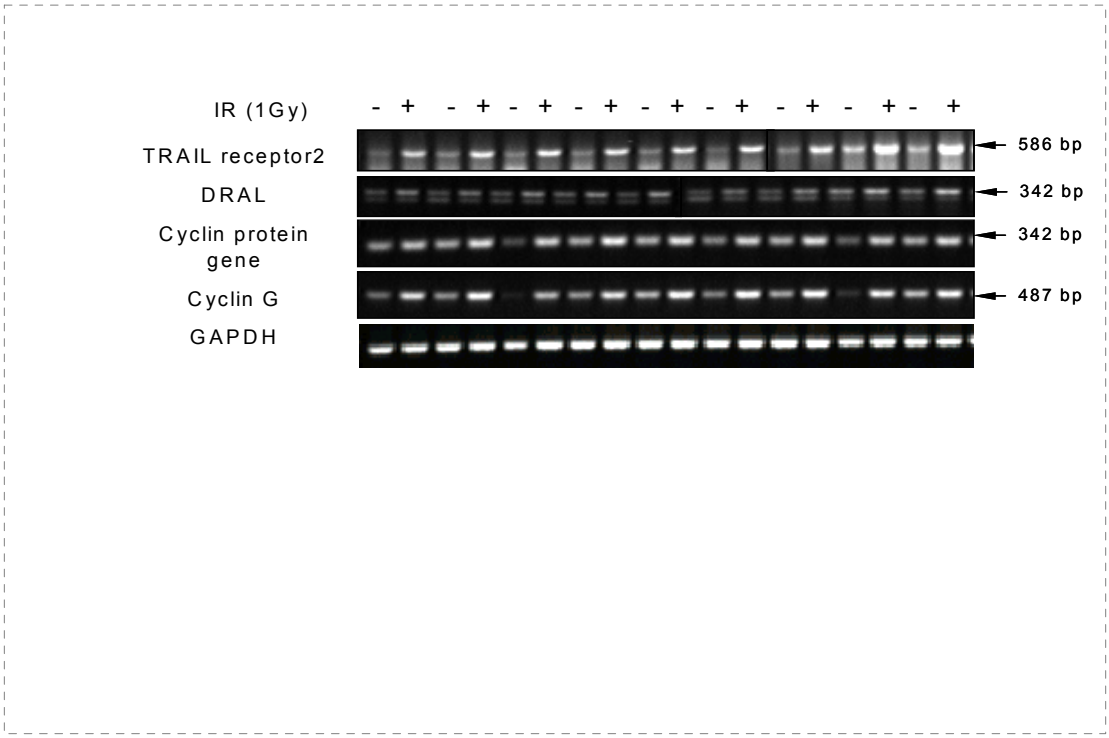

Fig. 2. RT-PCR analysis of TRAIL-receptor 2, DRAL, Cyclin G and Cyclin protein genes in peripheral blood lymphocytes (PBL) of extended number of donors. *A. Upper panel*, RT-PCR analysis data at 12 h after 1 Gy radiation of TRAIL-receptor 2, DRAL, Cyclin G and Cyclin protein gene in representative 9 donors. *Lower panel*, Relative band intensity of TRAIL-receptor 2, DRAL, Cyclin G and Cyclin protein genes at 12 h after 1 Gy radiation. *B*. Summary of RT-PCR analysis of TRAIL-receptor 2, DRAL, Cyclin G and Cyclin protein genes in PBL of extended number of donors. Bar represent the mean fold induction of each untreated control levels. Error bars are standard deviations.

Fig. 3

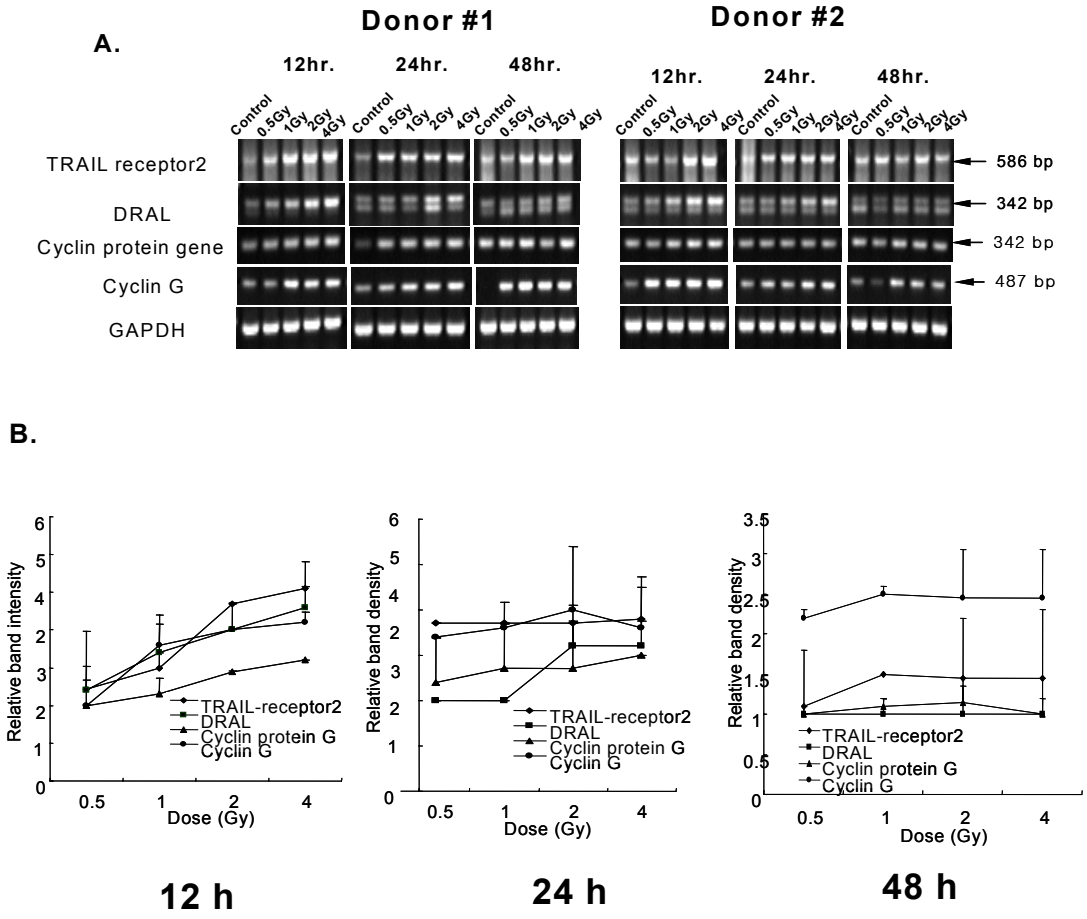

Fig. 3. Radiation dose and time dependency in RT-PCR analysis of TRAIL-receptor 2, DRAL, Cyclin G and Cyclin protein genes in peripheral blood lymphocytes (PBL) of 2 individual donors. RT-PCR analysis data at 12, 24 and 48 h after 0.5, 1, 2 and 4 Gy radiation(A). Relative band intensity of TRAIL-receptor 2, DRAL, Cyclin G and Cyclin protein genes. The bars are the mean of measurement of in two independent donors and the mean fold induction of each untreated control levels. Error bars are standard deviations (B).

Fig. 4

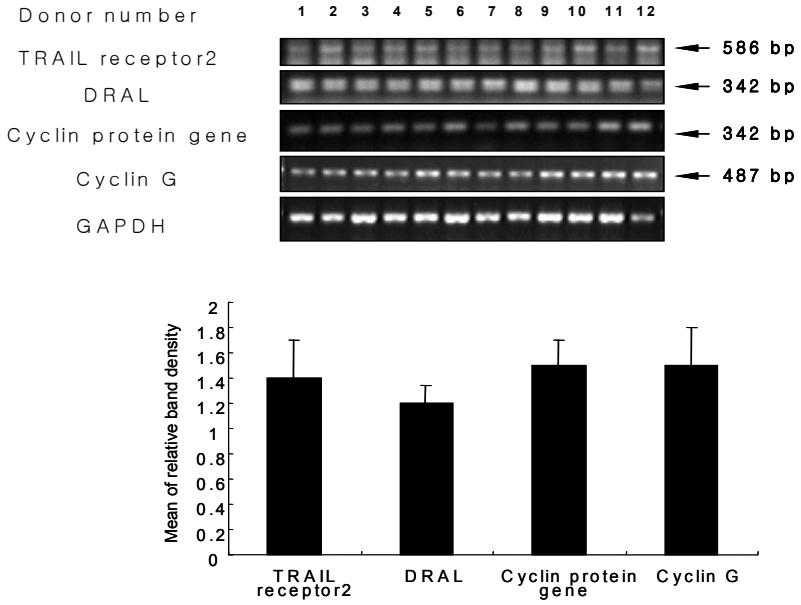

Fig. 4. Individual variation in basal level expression in RT-PCR analysis of TRAIL-receptor 2, DRAL, Cyclin G and Cyclin protein genes in peripheral blood lymphocytes (PBL) of 12 individual donors. *Upper panel*, RT-PCR analysis data of unirradiated PBL. *Lower panel*, Mean levels of TRAIL-receptor 2, DRAL, Cyclin G and cyclin protein genes in unirradiated PBL after normalization to the levels in the first donor. The bars are the mean of measurements of in 12 independent donors, and error bars are standard deviations.

Table 1. RT-PCR analysis of TRAIL receptor 2, DRAL, cyclin protein gene, and cyclin G in PBL.

Gene bank name	Description	Increase	Decrease	No change	Total	Putative function
AF016266	TRAIL receptor2	29 (91%)	3 (9%)		32	**Apoptosis**
L42176	DRAL	39 (89%)	3 (7%)	2 (4%)	44	**Small GTPase superfamily**
M15796	Cyclin protein gene	34 (92%)	3 (8%)		37	**Cell cycle regulation**
U53328	Cyclin G	34 (92%)	3 (8%)		37	**Cell cycle regulation**