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Possible Biomarker Gene for Radiation Workers in Hospital

Young-Woo Jin[1], Meeseon Jeong[1], Kien Moon[1], Chee Young Lee[3], Sangwoo Bae[2], Soo Yong Choi[2] & Yun-Sil Lee[2]

[1]Radiation Health Research Institute, Korea Hydro and Nuclear Power Co., Ltd, Seoul 132-703, Korea
[2]Division of Radiation Effects, Korea Institute of Radiological and Medical Sciences, Seoul 139-706, Korea
[3]Hanil General Hospital, Seoul 132-703, Korea
Correspondence and requests for materials should be addressed to Y. S. Lee (yslee@kcch.re.kr)

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Abstract

Biomarkers indicating past exposure to radiation have not yet been entirely satisfactory. In this study, we validated several genes reported as radiation response genes, as biomarkers to detect past exposure to radiation in occupationally exposed workers, especially workers in the medical field. A total of 54 radiation workers in hospital were investigated for radiation exposure dose. Their average radiation dose of recent one year was 1.09 mSv (±1.63) with a 10.63 mSv (±12.91) cumulative dose. The results of the multiple regression analysis for the various variables indicate that the Hsc70 (P=0.0292) and DRAL (P=0.0045) may be candidate biomarkers for the recent 1 year radiation exposure in radiation workers, whereas AEN (P=0.0334) and PGAMI (P=0.0003) might be for cumulative exposure.

Keywords: Candidate biomarkers, Blood lymphocytes, mRNA

The effects of low-level exposure to ionizing radiation are a concern to a large number of people, including workers occupationally exposed to radiation. Medical radiation workers are employees of hospitals, clinics and private offices where radiation is used in the process of delivering health care to humans. It is very important to estimate the absorbed doses from individuals occupationally exposed to ionizing radiation in order to carry out radioprotection procedures and restrict the hazards to human health[1]; however the extent of the health hazard is difficult to assess. Therefore, the development of procedures which can be used as part of effective programs for disease prevention to precisely identify health hazards in exposed populations are important to establish[2].

Several methodologies have been developed in the biomonitoring of radiation effects; however, their sensitivity and specificity are often too low to detect genetic effects (considering the annual occupational limit of 20 mSv). Since it is well known that exposure to ionizing radiation may induce malignant diseases, the International Commission on Radiation Protection (ICRP) has decreased the dose limit for the occupationally exposed workers and for the general population[3]. Consequently, there is a need for a reliable, quick and accurate method for the measurement of radiation dose, not only for use in a restricted number of individuals, but also for public health purpose. The assessment of the frequencies of dicentrics and chromosome translocations by Giemsa staining[4] or by chromosome specific fluorescence in situ hybridization (FISH) painting in general, are currently considered the most sensitive methods[5]. Moreover, one of the advantages of cytogenetic dosimetry is that the biological dosimeter can be assessed at any moment, whereas physical dosimeters are not always present on the subject[1]. However, the techniques are time consuming and demand a high level of expertise. The scoring of ~1,000 metaphases would need to be scored for conventional Giemsa analysis of dicentrics or ~10,000 metaphases by the FISH technique using a cocktail of chromosomes (e.g., 2, 4, and 8 whole-chromosome painting probes) for the detection of 0.25 Gy[6,7].

Complex molecular systems at the cellular level have evolved in response to many stresses including wounding, nutrient depletion, changes in oxygen tension, oxidative stress, metabolic toxins and DNA-damaging agents including ionizing radiation and chemical mutagens. Responses to such a diversity of stresses may give rise to different outcomes. With the recent developments in high throughput gene expression screening, it may be possible to develop gene expression profiles in human peripheral blood lymphocytes which correlate with the timing and dose of radiation exposure. The identification of such a gene set would enable more rapid and noninvasive testing of poten-

tially exposed populations. Our previous study identified genes of cyclin G1, DRAL, and Trail receptor 2[8], AEN and PIGAMI[9] as low dose response genes. Some of these genes were already published as radiation responsive genes *in vitro* or *in vivo* system[10,11]. In addition, since mRNA of Hsp27 and Hsp70 were elevated by low dose radiation *in vivo* system[12], in this study, we added these genes for developing possible candidate biomarkers for ionizing radiation exposure in the blood of occupationally exposed workers. To our knowledge, no previous studies reported an expression of the genes and mRNA after adjustment for confounding factors in workers occupationally exposed to radiation. Therefore we examined other mRNA levels of Hsps such as Hsp60 and Hsc70 and, gene expression status when adjusted for confounding factors.

Table 1. General characteristics of study subjects.

Variables	Number (%)*	Mean	Std. deviation
Sex			
Male	37 (68.52%)	–	–
Female	17 (31.48%)	–	–
Age (years)		33.77	7.50
Stress index		52.59	6.54
Job period (years)		7.08	5.18
Smoking (Packs-years)		4.59	6.32
Alcohol drinking (times/week)		1.83	0.82
Exposure dose			
Recent 1 year (mSv)		1.09	1.63
Cumulative dose (mSv)		10.63	12.91

*Number of subjects: 54

Table 2. Relative mRNA expressions in study subjects.

Variables	Mean	Std. Deviation
Cyclin G1	1.12	0.94
Hsp27	0.56	0.30
Hsp70	0.39	0.29
Hsc70	0.84	0.32
Hsp90	1.24	0.63
Trail-receptor 2	0.41	0.19
DRAL	1.02	1.01
AEN	1.51	1.52
PGAMI	2.43	2.43

Number of subjects: 54
Relative band density of mRNA or serum protein levels. Band density was calculated as the ratio of β-actin

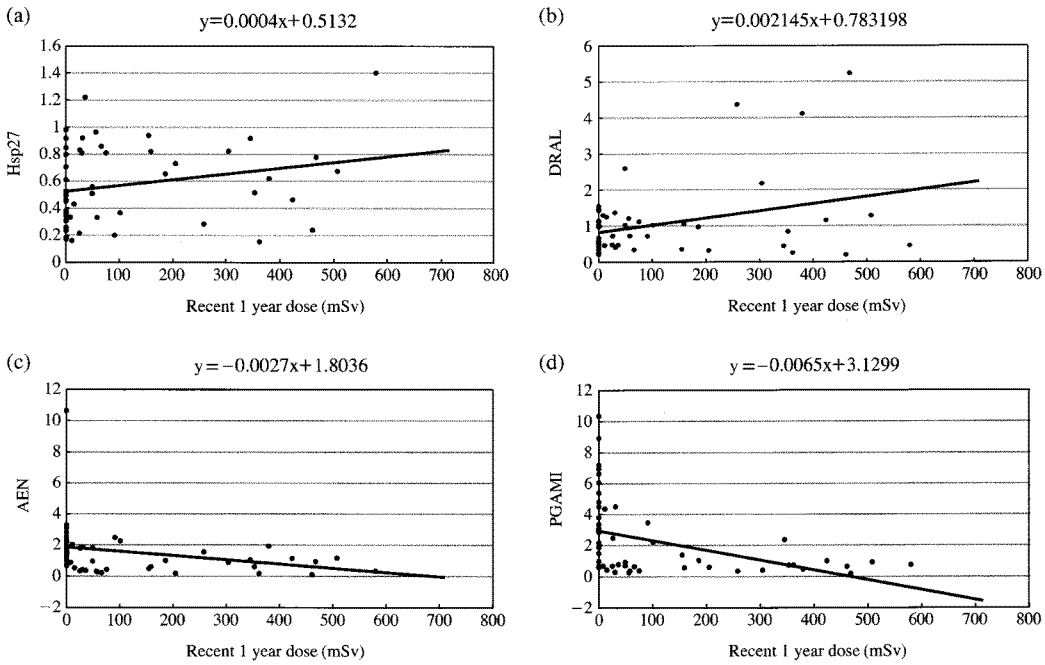

Figure 1. Scatter plots of exposure dose during the recent 1 year versus expression values for significant mRNA or serum protein levels. (a) Hsp27 (R^2=0.06, P=0.0749), (b) DRAL (R^2=0.12, P=0.0105), (c) AEN (R^2=0.09, P=0.0348), (d) PGAMI (R^2=0.19, P=0.0010).

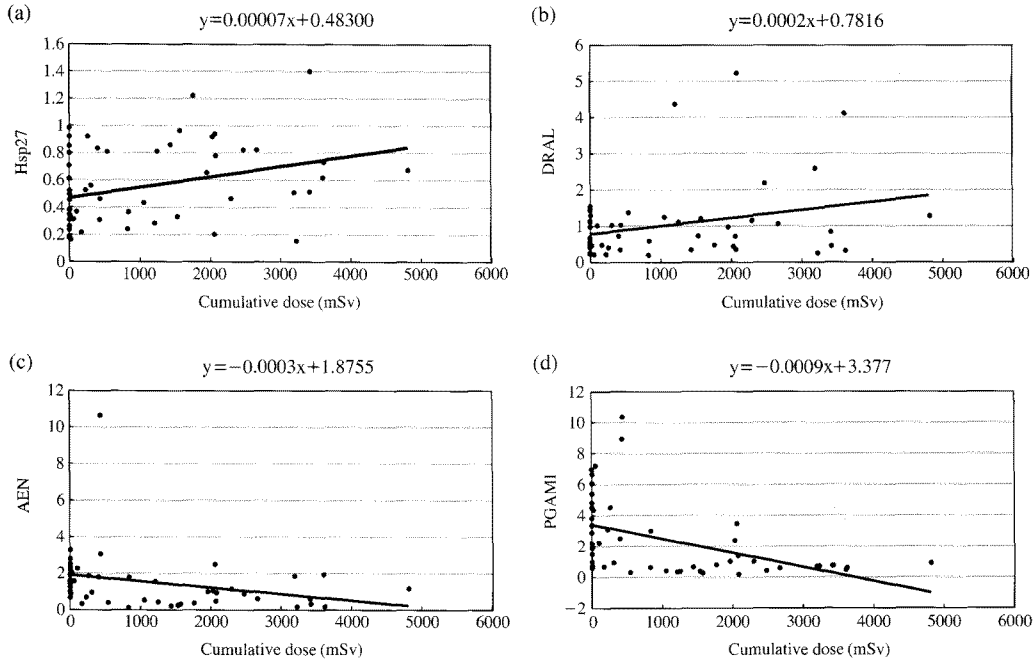

Figure 2. Scatter plot of cumulative exposure dose versus expression values for significant mRNA or serum protein levels. (a) Hsp27 (R^2=0.11, P=0.0177), (b) DRAL (R^2=0.08, P=0.0394), (c) AEN (R^2=0.09, P=0.0334), (d) PGAMI (R^2=0.23, P=0.0003).

General Characteristics and Gene Expression Profiles of Study Subjects

Table 1 shows the general characteristics of the study population with respect to age, stress index, exposure dose, smoking and alcohol drinking. We selected a total of 37 males and 17 females with an average age of 33.77 yrs (±7.50). The stress index showed a 52.59 (±6.54) and average job period (or years of occupational exposure) for all subject of 7.08 yrs (±5.18) and range of 0.03-24.01 yrs. The smoking amount was 4.59 (±6.32) packs-years. Frequency of drinking was 1.83 (±0.82) times per week. The radiation exposure dose was 1.09 mSv (±1.63) in the recent 1 year dose and 10.63 mSv (±12.91) for the cumulative dose.

The relative expression levels of mRNA for cyclin G1, Hsp27, Hsp70, Hsc70, Hsp90, Trail receptor 2, DRAL, AEN and PGAMI in blood lymphocytes are shown in Table 2. The averages of mRNAs of Hsp27, DRAL, AEN and PGAMI in subjects were 0.56 (± 0.30), 1.02 (±1.01), 1.51 (±1.52) and 2.43 (±2.43), respectively.

Scatter Plots of Gene Expression and Exposed Dose

The scatter plots of significant mRNA and exposed dose are shown in Figure 1, Figure 2. In the recent 1 year exposure, there were significant correlations with DRAL (R^2=0.12, P=0.0105), AEN (R^2=0.09, P= 0.0348) and PGAMI (R^2=0.19, P=0.0010), whereas it was marginally significant for mRNAs of Hsp27 (R^2= 0.06, P=0.0749) (Figure 1). The mRNAs of Hsp27 (R^2=0.11, P=0.0177), DRAL (R^2=0.08, P=0.0394), AEN (R^2=0.09, P=0.0334) and PGAMI (R^2=0.23, P=0.0003), in cumulative exposure (Figure 2).

Multiple Regression Analysis by Stepwise Method

The results of the multiple regression analysis by the stepwise method are included in Table 3. The assessment of cyclin G1 as a response variable found that cumulative dose was not significant covariate in the model and the estimate of the regression coefficient was 1.73052 (P=0.0821). The model for Hsp27 selected sex as significant covariates with an estimated regression coefficient of −0.19190 (P=0.0227). In the model for Hsp70, sex and stress index were selected as covariates with R^2=18.18%. The estimate of the regression coefficient was −0.19934 (P=0.0174) in sex, 0.01645 (P=0.0066) in stress index. Hsc70 was significantly correlated with recent 1 year dose; the

Table 3. Results of multiple regression analysis by stepwise method.

Gene or protein levels (R^2/P-value for F-test)	Selected covariates	β	SE	P-value
Cyclin G1 (0.0570/0.0821)	Cumulative dose	1.73052	0.97611	0.0821
Hsp27 (0.0.1645/0.0102)	Sex	−0.19190	0.08164	0.0227
	Job period	0.01278	0.00739	0.0898
Hsp70 (0.1818/0.0060)	Sex	−0.19934	0.08107	0.0174
	Stress index	0.01645	0.00581	0.0066
Hsc70 (0.0882/0.0292)	Recent 1 year dose	−5.87027	2.61666	0.0292
Trail-receptor 2 (0.1528/0.0389)	Stress index	0.00737	0.00403	0.0734
	Smoking pack-years	0.00866	0.00413	0.0410
	Alcohol drinking	−0.04546	0.03092	0.1477
DRAL (0.2162/<0.0064)	Age	0.03520	0.01697	0.0432
	Stress index	0.02950	0.01992	0.1450
	Recent 1 year dose	23.68626	7.96586	0.0045
AEN (0.0841/0.0334)	Cumulative dose	−3.42319	1.56655	0.0334
PGAMI (0.2261/<0.0003)	Cumulative dose	−8.94616	2.29502	0.0003

Sex: reference=male

estimates of the regression coefficients were −5.87027 (P=0.0292) and the Trail receptor 2, the stepwise regression found stress index, smoking pack-year, and alcohol drinking to be the controlling variables; the estimates of the regression coefficients were 0.00866 (P=0.0410) for smoking pack-year however, stress index and alcohol drinking were not significant at α= 0.05. The model for DRAL was fitted on age, stress index, recent 1 year dose with R^2=21.62%. The estimates of the regression coefficients were 0.03520 (P =0.0432) for age and 23.68626 (P=0.0045) for the recent 1 year dose. Stress index was not a significant factor. For both AEN and PGAMI, the cumulative dose was the only significant covariate and their estimates of regression coefficients were −3.42319 (P= 0.0334) and −8.94616 (P=0.0003) in the model with R^2=8.41 and 22.61%, respectively.

Discussion

The monitoring of gene expression profiles in blood according to various variables, provides a molecular approach for identifying candidate biomarkers after accidental or occupational exposure to radiation. In this study, we compiled a list of 9 genes as possible biomarker genes for ionizing radiation exposure in the blood lymphocytes of radiation workers. The results of the multiple regression analysis for the various variables revealed that mRNA's of DRAL and Hsc70 for recent 1 year radiation exposure, and mRNA's of AEN and PGAMI for cumulative exposure may be candidate biomarkers in radiation workers.

The strength and novelty of the present study is the investigation of biological markers of effect and susceptibility on the same subjects exposed to chronic low level of ionizing radiation. To the best of our knowledge, the present subjects study is the first in vivo study of radiation response by gene expression in exposed hospital workers.

The conventional approach to estimate radiation exposure has been to integrate physical and clinical measurements to optimize dose assessment; however, this approach has practical limitations. Many attempts have been made to improve dosimeters using hematological, biochemical, immunological, and cytogenetic end points[13,14]. The lymphocyte count is a sensitive indicator of exposure to IR that declines rapidly with dose[15,16]; however, because of the large variation in lymphocyte counts among normal individuals[17-19] it necessitates repeated measurements over a prolonged period of time. Moreover, lymphocyte count is hardly affected by chronic low doses of irradiation. Cytogenetic measurements of the abnormalities of chromosome structure in metaphases of cultured lymphocytes[20,21] have played an important role in retrospective estimates of radiation dose received by A-bomb survivors and individuals exposed during the Chernobyl nuclear accident[22]. Although chromosomal analyses are considered the gold standard for assessing radiation dose, the experimental methods require skilled personnel and it requires several days to a week to be completed.

An effective biomarker requires validation of individuals in the general human population regardless of sex, age, health status and so on[23,24]. Also important

is the specificity of the response to ionizing radiation versus other biological, chemical or physical exposures[25]. Large variations among individuals or a generalized response to other types of stresses in relation to the amount of change induced by radiation exposure would limit the use of an otherwise sensitive biomarker. Yet, there is very little information in the literature on inter-individual variation in gene or protein responses to radiation. Moreover, almost nothing is known of inter-individual variability of dose- and time-response.

In the present study, the mRNA expression level of DRAL and Hsc70 of PBL, when examined using semiquantitative RT-PCR analysis, are well correlated to radiation exposure dose during the recent year, suggesting that mRNA of DRAL and Hsc70 may be candidates biomarkers for radiation exposed occupational workers. DRAL (downregulated in the rhabdomyosarcoma LIM) is a component of the small GTPase family[26] and is involved in protein-protein interaction and transcriptional regulation. The detailed functions of DRAL in response to radiation were not well identified. And as a possible candidate for radiation exposure markers, it was never evaluated. Hsc70, an Hsp70 family protein, which is related to radioprotection, may be altered by low dose irradiation, even though the underlying mechanisms of radiation-induced gene expression are unknown. Induction of HSP was also shown after treatment of stress or chemicals[27,28]. Therefore, we hypothesized that low dose radiation may participate in the cytoprotection via gene induction of Hsc70. In the case of biomarkers for cumulative exposure, the mRNAs of AEN and PGAMI were selected in our system. AEN (a nuclease) and PGAMI (a phosphoglycerate mutase) were isolated from low dose exposed cells by the subtraction hybridized method[9] and in this study, they were also identified as candidate biomarkers for cumulative radiation exposure. We do not know how these genes were accumulated in the blood lymphocytes by consistent low dose radiation, therefore, more detailed experiments might be necessary.

It was found in the present study that the genes did show significant correlated with these factors after adjusted for confounding factors, such as smoking and alcohol drinking and sex. Although this study had the limitations of a small number of subjects, the importance of this result is to get the result more precise as adjusting confounding factors.

In summary, we identified possible gene candidates of DRAL and Hsc70 in recent 1 year radiation exposure and AEN and PGAMI for cumulative radiation exposure. The importance of this paper is the identification of genes, showing a persistent response after IR exposure.

Materials & Methods

Study Population

A gene expression analysis was performed on a total of 54 radiation workers from Department of Nuclear Medicine of University Hospital in Korea. All the radiation workers were provided with written informed consent forms and questionnaires on demographic factors, job period, socio-psychological examinations (22 items), cigarette smoking and alcohol drinking as well as the measurement of height and weight. In addition, the smoking amount was calculated from multiplication of the total years and the average pack per day smoked.

We calculated the stress index using a Job Content Questionnaires, which measures the job stress of Karasek for the socio-psychological examination and compared their level[29]. Dosimetric data were obtained from the National Dose Registry of Korea, which was maintained by the Korea Radioisotope Association entrusted by the Ministry of Science and Technology and from the dose record from Korea Hydro and Nuclear Power Company.

Isolation of Lymphocytes

Blood samples were aseptically collected in haparinized sterile glass tubes from median cubital vein of radiation workers. Blood was kept at 4°C until lymphocyte isolation and lymphocyte isolation was performed within 24 hrs after blood collection. Lymphocytes were isolated using Ficoll-Histopague (Sigma, USA). Blood was diluted 1 : 1 with phosphate buffered saline (PBS) and layered onto Histopague with ratio of blood+PBS: Histopague maintained at 4 : 3. The blood was centrifuged at 1,340 rpm for 35 min at room temperature. The lymphocyte layer was removed and washed twice in PBS at 1,200 rpm for 10 min each.

Total RNA Isolation

RNA was extracted from lymphocytes with two rounds of Trizol reagent (Invitrogen, Carlsbad, CA) and subsequent purification on Rneasy columns (Qiagen, Valencia, CA) in accordance with the instruction of the suppliers.

Reverse Transcriptase PCR (RT-PCR)

Aliquots of 0.5 μg of total RNA were used. The reaction mixture contained 2 μL of 10x RT buffer (QIAGEN), 2 μL of 5 mM dNTPs (QIAGEN), 1 μL of 10 U/ μL RNAsin (Promega), 2 μL of 10 μM oligo (dT)-15 primer, 1.25 μg of total RNA and 1 μL of

Table 4. Primers for RT-PCR.

Gene Name	Forward	Reverse
Cyclin G1	AGCCAAAGGTCTGTGGTTG	TGACATGCCTTCAGTTGAGC
DRAL	GAAGTGCTCCCTCTCACTGG	ATTTGGGTGTGCCTTACTCG
Trail receptor 2	GCATTGTGTGTTTTGTTCCG	GAGTTCGAGACCAGCCTGAC
AEN	CGCAAGGCTGTCCCCTTCCA	CTGCGGGCCTCCTGCTGTTC
PGAMI	GTGCAGAAGAGAGCGATCCG	CGGTTAGACCCCCATAGTGC
Hsp27	ATGGCGTGGTGGAGATCACC	CAAAAGAACACACAGGTGGC
Hsp70	TTCCGTTTCCAGCCCCCAATC	CGTTGAGCCCCGCGATGTACA
Hsc70	AAGTTGCAATGAACCCCACC	TTGCGCTTAAACTCAGCAA
Hsp90	AAAAGTTGAAAAGGTGGTTG	TATCACAGCATCACTTAGTA

Omniscript reverse transcriptase (QIAGEN) in a final volume of 20 μL. The mixture was incubated at 37°C for 1 h and the transcription reaction was terminated by heating the mixture at 95°C for 5 min, annealing for 30 s, extension at 72°C for 30 s, which was followed by 32-35 cycles of denaturation at 95°C for 15s. The quality of the isolated RNA was assayed by agarose gel electrophoresis by checking 18S and 28S RNA. The negative controls, lacking either RNA or RT, were routinely included in the assay. Respective pairs of specific primers were designed and synthesized as previously described (Table 4). Moreover, the overexpressed genes in peripheral blood lymphocytes (PBL) after 1Gy radiation were included in the analysed genes[10]. The PCR products were analyzed by 2% agarose gel electrophoresis and stained with ethidium bromide. The quantification was carried out using an image analyzer with the MCID software program (Image Research Inc., Ontario, Canada). Relative band density was calculated as the ratio of β-actin.

Statistical Analysis

Before the detailed analysis, we determined descriptive statistics using frequencies, percents as categorical variables, means and standard deviation for continuous variables. The Pearson's correlation analyses were used for associations of between gene and effective dose. A stepwise multiple regression with an a priori significance level of $\alpha=0.05$ for entry into model was then performed to select the significant covariates on the model for each gene or protein expression. We used age, sex, stress index, job period, smoking, recent 1 year dose, cumulative dose, and alcohol drinking as the covariates in the model. All of the statistical analyses were performed using SAS version 8.2 (SAS Institute Inc., Cary, NC, USA).

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