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Time-Dependent Induction of the Nucleotide Excision Repair Gene XPA and RAD51 in Homologous Recombination in Human Lymphocytes Exposed to Low Doses of Ionizing Radiation

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ABSTRACT

Introduction: The aim of the present study was to understand the effect of low-doses of ionizing radiation (LDIR) on repair genes expression in blood samples that were taken from healthy donors. The next purpose was to examine the time-effect on the modified gene expression caused by low-doses of ionizing radiation.

Material and Methods: The RNA of peripheral blood lymphocytes (PBLs) taken from four healthy donors was isolated at different time points after exposure including 4, 24, 48, 72, and 168 hours and then cDNA was synthesized. Modification of XPA and RAD51 expression levels due to LDIR (2, 5, 10 cGy) were evaluated by relative quantitative reverse transcription-polymerase chain reaction.

Results: Significant up-regulation of both repair genes was observed at the 4 and 168 h following to 10 cGy. Also, this dose could increase expression levels of RAD51 at 48 and 72 h after radiation. For lower doses at 5 cGy, only XPA levels were significantly up-regulated after 168 h. A significant regression was found between the XPA levels and the dose, at 168 h after irradiation to PBLs that can represent a new potential biomarker for biological dosimetry purposes.

Conclusion: The results of this study could support the hypothetical role of the different DNA repair pathways in response to LDIR. This led us to propose a molecular biodosimetry method for ionizing radiation in the range of LDIR.

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Introduction

Ionizing radiation can be introducing multi-kind DNA lesions such as mismatch, Damaged base, DNA-protein cross-links (DPC), Single-strand breaks (SSB), and double-strand breaks (DSBs) that, left unrepaired or misrepaired, can lead to mutation, serious genomic instability, and cell death. Activation of multiple signaling pathways in mammalian cells by DNA damaging agents results in the modified expression of numerous target genes. Finally, the response of mammalian cells to ionizing radiation involves three main processes that they are respectively; cell cycle arrest, DNA repair, and apoptosis[3].

Based on the scientific literature, exact DNA repair mechanisms in cells are activated (after cell cycle arrest) to restore the structural integrity of DNA. Besides the fact, that activation of DNA-repair pathways in order to maintain the survival of the organism is vital and defects in these pathways can result in human syndromes. DNA-repair pathways have been used widely as an end-point of radiation effects in many studies[4-6]. Xeroderma pigmentosum, complementation group A (XPA) protein is an essential factor for nucleotide excision repair (NER), that in humans is encoded by the XPA gene. It is believed that NER proteins are involved in the detection and excision mechanism of DNA damage induced by UV radiation[7], and ionizing radiation[8-11].

DNA Double-Strand Breaks (which are one of the most cytotoxic DNA lesions) are repaired by Homologous recombination (HR) and Nonhomologous end joining (NHEJ) pathways [12, 13]. Several proteins are involved in these pathways including ATM, RAD51, RPA, Ku, DNA-PK$_{cs}$, MRN complex, etc. RAD51 as one of the most important proteins plays a central role in HR and catalyzes a strand-exchange reaction with the complementary strand[14, 15].

The dose-response relationship is an important issue in gene expression alteration and the results

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obtained in this case are multifactorial and generally complex. One of the most important factors in a dose-response relationship is the time-effect due to a change in gene expression[16]. Although, time-pattern of the dose-response curve in human PBLs has been limitedly studied in previous studies [17, 18]; but many ambiguities remain in this regard. In this study, we have used quantitative real-time PCR to quantify XPA and RAD51 genes expression at various time points after irradiation in low doses of γ-irradiated PBLs.

Materials and Methods

Gamma irradiation

Blood samples were collected from four healthy nonsmoking individuals (aged 24–26 years old) with no history of previous exposure to radiation or chemicals. Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll gradient (Cedarlane, Canada) according to the recommendations of the manufacturer. The cells were counted and 1×10^6 cells/ml were suspended in a 10 ml RPMI 1640 (Gibco, USA) into a T-25 flask (SPL, Korea) with 20% FBS (Biosera, France), 100 Iu/ml penicillin, 0.1µg/ml Streptomycin and 1% of 200 mM L-glutamine (Biosera, France). Finally, one percent of PhytoHaemAgglutinin (PHA) (Gibco, USA) was added following to irradiation.

Sets of flasks were exposed to deliver 20, 50, and 100 mGy from a Cobalt 60 (Phoenix Theratron, Canada), the gantry angle was set to 180 and the field size was 18×8 cm^2, source-skin distance (SSD) = 0.8 m,and dose rate 13.7 mGy/min.Six centimeter of lead was used to reduce the output dose rate of the source.

RNA preparation and cDNA synthesis

The PBMCs RNA was extracted using Tripure Reagent (Roche, Germany) according to the protocol recommended by the manufacturer. The integrity of isolated total RNA in all samples was confirmed by agarose gel electrophoresis of 1 µg of total RNA. Complementary DNA (cDNA) was synthesized using a commercial kit (Fermentas, Lithuania).

The resulting cDNA was amplified by Astec gradient thermocycler (Tokyo, Japan) using primers for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Prime Taq DNA polymerase, Genet Bio, South Korea).

Gene Expression Analyses by real-time PCR

Real-time RT-PCR reactions were carried out as duplicates with SYBR® Premix Ex Taq™ (Takara, Japan). Gene expression assessments were performed on a StepOne Real-Time PCR system by a relative quantitative method. All Primers were purchased from Metabion (Martinsried, Germany) and gene-specific amplification of all genes was confirmed by sequencing of the PCR product. The primer sequences are represented in Table 1.

Table 1. Primer Sequences

Primers	Sequences(5'-3')
XPA	Forward: CTGGAGGCATGGCTAATG
	Reverse: CAAATTCCATAACAGGTC
RAD51	Forward: GTGGCTGAGAGGTATGGTC
	Reverse:CGAGTAGTCTGTTCTGTAAAGG
Beta-2 microglobulin	Forward: GTATGCCTGCCGTGTGAAC Reverse: ACCTCCATGATGCTGCTTAC

Statistical analysis

To analyze the correlation of gene expression data pairs, Pearson correlation coefficient was used and *P-value* ≤ 0.05 was considered as statistically significant. Unpaired t-test was performed to compare the average of groups. These analyses were performed using the GraphPad Prism, version 8.3.0.

Results

The results were expressed as the means and standard error of the means (S.E.).

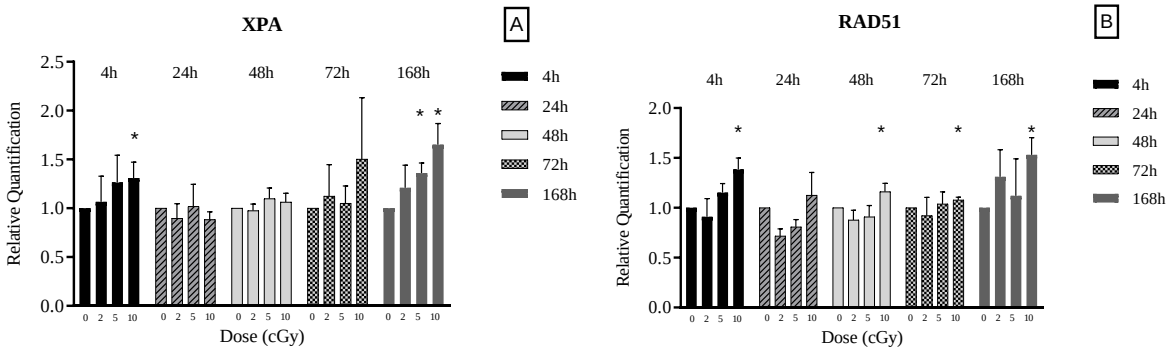

Figure 1- Effect of 0–10 cGy irradiation on XPA (A) and Rad51 (B) expression patterns revealed by relative quantitative in PBLs 4h,24h ,48h, 72h, and168h post-irradiation. Each bar represents mean value for the 4 donors and Error bars show standard Error of mean. *represent P-value<0.05

Dose-response curve for DNA repair genes

As shown in figure 1.A significant up-regulation of XPA was observed following 10 cGy at the 4 and 168 h. Also, this dose could increase expression levels of RAD51 at four different time points: 4, 48, 72, and 168 h after irradiation (Figure 1.B). For lower doses at 5 cGy, only XPA level was significantly up-regulated after 168 h. Interestingly, linear correlations between the XPA levels and the dose as shown in figure 2 were observed. Although, significant regression (*P-value* = 0.008, R^2=0.98) was found only at 168 h after irradiation to PBLs.

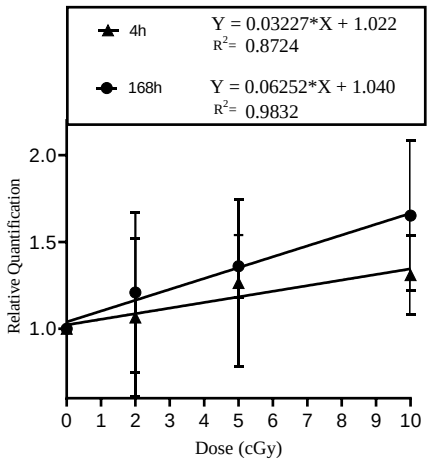

Figure 2- Simple linear regression between RQ of XPA and Dose (cGy) 4h and 168h post irradiation. Equation and R square listed in the box.

Discussion

In this study expression levels of two repair genes (via a different signaling pathway) were examined in different intervals following irradiation by low doses of gamma radiation. The results are expected to help understand the DNA repair mechanism induced by LDIR. Moreover, the repair mechanism's role in radio-adaptive response induced by LDIR can be evaluated. Most studies have been shown that the effects of biological factors are various at LDIR and time effects can change these factors, on the other hand, information about it, is very limited, unclear and the subject of intense debates in recent years [19-21]. The results of the present study showed that XPA protein as an essential factor for NER and RAD51 that catalyzes a strand-exchange reaction with the complementary strand in HR mechanism (as shown in fig 3) can be stimulated due to LDIR. This enhancement of DNA damage repair potential could support the hypothesis proposed by Cheng al. [22] that the LDIR adaptive-response is induced by repair mechanisms.

In a recently published article [23] the authors revealed the activity of DNA repair genes is essential for adaptive response and with mutations in these genes, the radio-adaptive response will be absent. However, Goldberg et al. [24] assessment of repair gene expression at LDIR has revealed the response of the repair system is completely gene-dependent. In Table 2 we have shown an overview of the previous studies carried out on the LDIR by evaluation of repair genes expression. Previously, Grudzenski et al. [25] showed that efficient removal of γ-H2AX and 53BP1 foci (as markers of DSB damage) was observed 24 h after irradiation to 10 cGy and was not after 1 cGy. These data suggest that the IR response is substantially different even in the LDIR region.

Table2. Evaluation of repair gene expression in the current study and the previous studies carried out on the LDIR.

It should be noted any specific changes in the repair mechanisms induced by radiation exposure (such as alteration of gene expression and protein synthesis) can be used for estimation of received dose (biodosimetry) in the event of accidental radiation exposure [26].

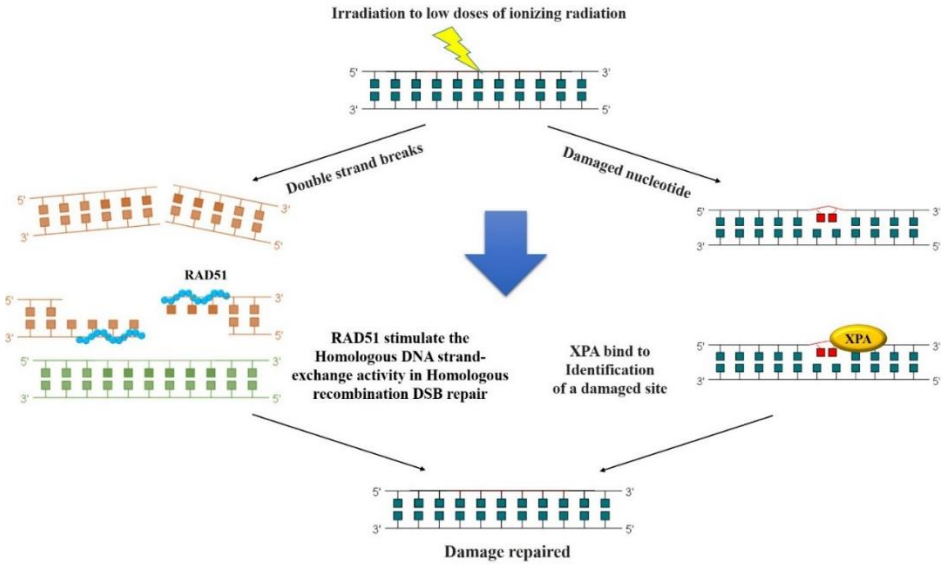

Figure 3. An outline of the role of XPA in Nucleotide Excision Repair (NER) and RAD51 in Homologues recombination pathways

Table 2. Comparison between the current study and the previous studies carried out on the LDIR by evaluation of repair gene expression.

Reference	Cell Type	Method	Gene(s) Investigated	Conclusion
Vosoughi et al. [30]	Human Peripheral Blood Lymphocytes	RT-PCR	FDXR and XPA	Increased expression of FDXR and decreased expression of XPA at 24 h after TC-99 MIBI injection following to 10 cGy.
Goldberg et al. [31]	Human Skin Biopsies	RT-PCR	P21, Tp53, and GADD45A	Reduced expression of TP53 and P21 and increased expression of GADD45A at 24 h following to 10 cGy.
Bladen et al. [32]	Zebrafish Embryonic Blastomeres	RT-PCR	Ku70 subunit (XRCC6)	Ku70 protein (a component of the NHEJ pathway) plays a crucial role in protecting from IR-induced DNA damage during embryogenesis.
Tilton et al. [33]	Human Skin Tissue	RT-PCR	GDF15, FDXR, TP53INP1, and P21	Reduced expression of GDF15, FDXR, and TP53INP1 and increased expression of P21 at dose 10 cGy and two post-irradiation time points (3 and 8 h).
Knops et al. [26]	Human Peripheral Blood Lymphocytes	RT-PCR	FDXR	A significant rise of gene expression 24 and 48 h after irradiation to 10 cGy.
Sudprasert et al. [34]	Human Peripheral Blood Lymphocytes	RT-PCR	hOGG1 and XRCC1	Reduced expression of hOGG1 and XRCC1 in gamma-irradiated lymphocytes, that has not been reported elsewhere.
Current study	Human Peripheral Blood Lymphocytes	real-time PCR	XPA RAD51	Up-regulation of XPA and RAD51 at the 4 and 168 h following to 10 cGy and significant regression between the XPA levels and the dose.

The specific purposes of biodosimetry are determining individuals undergoing radiation exposure that need to go into the healthcare system initially, guiding treatment by dose estimates, and finally long-term risks estimation [27]. Significant regression between the XPA levels and the doses in the LDIR range presented in this study can represent a potential molecular biomarker for biological dosimetry purposes. In conformity with the present results, previous studies have shown biological monitoring of exposure to IR by induction of gene expression in PBLs [28, 29]. However, to our knowledge only Knops et al. [26] study has been done to assess this possibility for LDIR biodosimetry and their result shows A significant rise of FDXR gene expression that increases with increasing dose. There are several limitations in our study. Result that presented in the article was not supported by other techniques such as flowcytometry or western blot. Genes that has been evaluated in this article are limited to certain repair pathways and may not represent all repair capabilities.

Conclusion

Our results showed that XPA and RAD51 gene expression levels can be stimulated due to LDIR. On the other hand, significant regression between the XPA levels and the doses that presented in this study can be led to propose a molecular biomarker for ionizing radiation in the range of LDIR. Finally, the results of this study could support the hypothetical role of the different DNA repair pathways for LDIR adaptive-response induction.

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